

Research



Cite this article: Ruf I, Meng J, Fostowicz-Frelik Ł. 2023 Auditory region circulation in Lagomorpha: the internal carotid artery pattern revisited. *Phil. Trans. R. Soc. B* **378**: 20220088. <https://doi.org/10.1098/rstb.2022.0088>

Received: 28 November 2022

Accepted: 4 April 2023

One contribution of 13 to a theme issue ‘The mammalian skull: development, structure and function’.

Subject Areas:

palaeontology, evolution

Keywords:

internal carotid artery, Euarchontoglires, Leporidae, Ochotonidae, µCT scanning

Authors for correspondence:

Irina Ruf

e-mail: irina.ruf@senckenberg.de

Łucja Fostowicz-Frelik

e-mail: lfost@twarda.pan.pl

Electronic supplementary material is available online at <https://doi.org/10.6084/m9.figshare.c.6602952>.

Auditory region circulation in Lagomorpha: the internal carotid artery pattern revisited

Irina Ruf^{1,2}, Jin Meng³ and Łucja Fostowicz-Frelik^{4,5}

¹Abteilung Messelforschung und Mammalogie, Senckenberg Forschungsinstitut und Naturmuseum Frankfurt, 60325 Frankfurt am Main, Germany

²Institut für Geowissenschaften, Goethe-Universität Frankfurt am Main, 60438 Frankfurt am Main, Germany

³Division of Paleontology, American Museum of Natural History, New York, NY 10024, USA

⁴Department of Organismal Biology and Anatomy, University of Chicago, Chicago, IL 60637, USA

⁵Department of Evolutionary Paleobiology, Institute of Paleobiology, Polish Academy of Sciences, 00-818 Warsaw, Poland

IR, 0000-0002-9728-1210; JM, 0000-0002-3385-8383; LF-F, 0000-0002-1266-1178

The internal carotid artery (ICA) is one of the major vessels in the cranial circulation. Characters concerning the ICA, such as its course in the auditory region, have been employed frequently in phylogenetic analyses of mammals, including extinct taxa. In lagomorphs, however, our knowledge on vascular features of the auditory region has been based predominantly on living species, mostly on the European rabbit. We present the first survey on 11 out of 12 extant genera and key fossil taxa such as stem lagomorphs and early crown representatives (*Archaeolagus* and *Prolagus*). The ICA pattern shows a modified transpromontorial course in stem taxa (*Litolagus*, *Megalagus* and *Palaelolagus*) and *Archaeolagus*, which we propose as the ancestral character state for Lagomorpha, similar to that for the earliest rodents, plesiadapids and scandentians. The ICA pattern in leporids is perbullar, but shows structural similarities to stem taxa, whereas the extrabullar ICA course in *Ochotona* is apparently a highly derived condition. *Prolagus* shows a mixed character state between leporids and *Ochotona* in its ICA route. The persistence of the transpromontorial ICA course and similarities in the carotid canal structure among stem taxa and crown leporids support morphological conservatism in Lagomorpha, in contrast to their sister clade Rodentia.

This article is part of the theme issue ‘The mammalian skull: development, structure and function’.

1. Introduction

The paired carotid artery is the most important blood vessel supplying the head. It arises from the aortic arch as the common carotid artery, which branches into the external carotid artery (ECA), supplying most of the face and neck, and the internal carotid artery (ICA), which passing through the auditory region supplies the brain, orbit and nasal cavity through its offshoots: the anterior, middle and posterior cerebral arteries, ophthalmic artery, and ethmoidal artery, respectively [1–5]. However, the ICA itself does not contribute substantially to the blood supply of the ear region.

Characters concerning the organization and course of the internal carotid artery have been employed frequently in phylogenetic analyses of mammals, including extinct taxa [6–11], because the ICA foramina, canals or grooves are often preserved in skulls of fossil specimens.

The ancestral course of the ICA for Mammalia is accepted as medial to the promontorium of the petrosal [7,12], whereas for the Eutheria as lateral to the promontorium and in the tympanic cavity (see e.g. [12–14]). Interestingly, the reconstructed position of the ICA in the Late Cretaceous stem placentals *Asioryctes*, *Kennalestes* and *Zalambdalestes* is medial [10,12]. As currently understood, Matthew’s [15] hypothesis suggesting the presence of two ICA branches

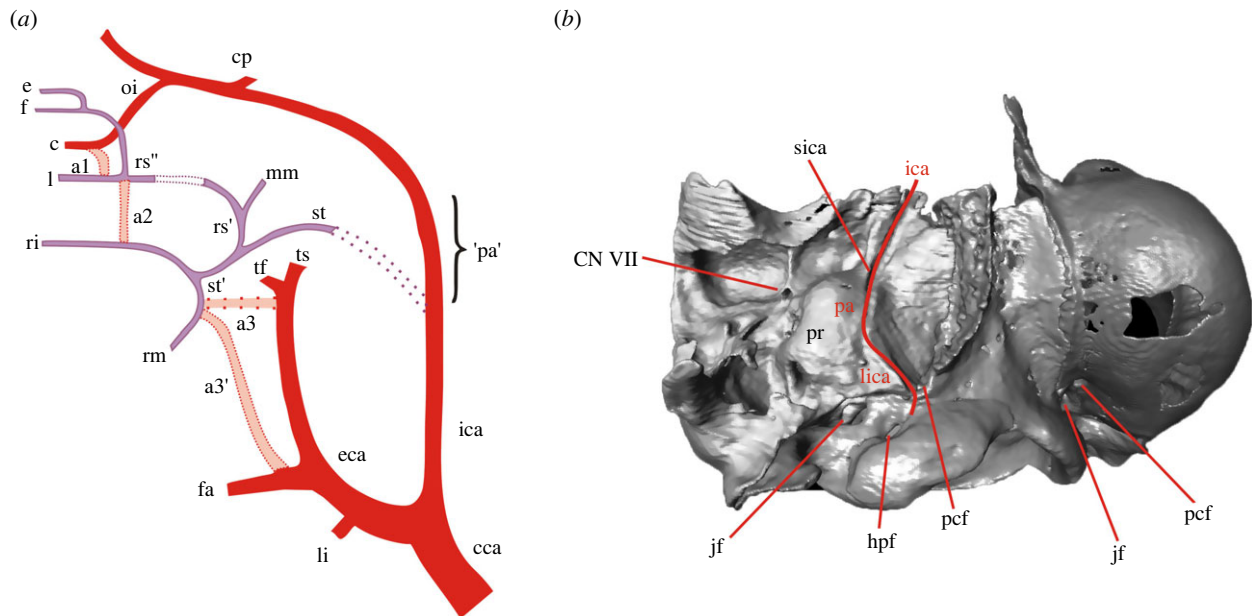


Figure 1. General circulation pattern of the carotid artery in extant Lagomorpha auditory region (a) and the ICA-related anatomic features in the fossil lagomorph *Palaeolagus* (b). a1, a2 (internal ophthalmic artery), a3, and a3' (first part of mandibular artery), anastomoses; c, ciliary artery; cca, common carotid artery; cp, posterior communicating artery; e, ethmoidal artery; eca, external carotid artery; f, frontal artery; fa, facial artery; hpf, hypoglossal foramen; ica, internal carotid artery; jf, jugular foramen; l, lacrimal artery; li, lingual artery; lica, lateral internal artery; mm, medial meningeal artery; oi, ophthalmic artery; 'pa', promontorial artery; pcf, posterior carotid foramen; pr, promontorium; ri, infraorbital artery; rm, mandibular artery; rs' and rs'', distal parts of the supraorbital artery; sica, sulcus of internal artery; st, stapedial artery; st', distal part of stapedial artery; tf, transverse facial artery; ts, temporal superficial artery; CN VII, entrance of CN VII (facial nerve). Carotid artery branches marked in red; stapedial artery and its offshoots marked in purple; anastomoses between the branches of the carotid artery and distal part of the stapedial artery marked in pink; the parts of the blood vessels partly obliterated are marked with dashed lines.

(medial and lateral as postulated for several early eutherian taxa) is not valid, as the ICA at the auditory capsule's level originates from a single embryonic dorsal aorta independent from its later course [16].

In Eutheria, three main types of the ICA arrangement are observed, in its relation to the auditory region: (1) transpromontorial, where the ICA goes through the tympanic cavity and crosses the ventral surface of the promontorium of the petrosal; (2) perbullar, with the ICA contained within a canal in the medial wall of the auditory bulla; and (3) extrabullar, with the ICA passing the ear region externally, medial to the auditory bulla and the tympanic cavity [7]. The transpromontorial route of the ICA is recognized as an ancestral character state for Eutheria [14], whereas the perbullar and extrabullar courses of the ICA are considered derived conditions [7,10,11].

Thus far, our knowledge on vascular features of the auditory region in lagomorphs (figure 1), such as the ICA organization, has been based almost exclusively on living species and mostly on the European rabbit (*Oryctolagus cuniculus*; see [1,3,17,18]). Bugge [17,18] provided a detailed description of the cephalic arterial system, i.e. internal and external carotid arteries and stapedial artery including anastomoses, of extant Lagomorpha (three genera). While the internal carotid artery is relatively well developed in *O. cuniculus*, European hare (*Lepus europaeus*) and Afghan pika (*Ochotona rufescens*), the stapedial artery is substantially reduced (figure 1a). Its tympanic (proximal) part is obliterated and mostly lost, whereas the distal portion and its branches are supplied by the ECA via anastomoses (see [17;18, fig. 15D]). The two living lagomorph families, Ochotonidae and Leporidae, exhibit different character states of the ICA: the former, the extrabullar, while the latter, the perbullar condition [7,18].

The skull anatomy of several fossil lagomorph species has been described in detail (e.g. [19–22]), but only Wolniewicz & Fostowicz-Freluk [23] devoted more attention to the ICA-related features. Wu [24] marks the external opening of the carotid canal 'external carotid foramen' *sensu* [24] in the fossil ochotonid *Alloptox*, similar to the opening in *Prolagus sardus* shown in Dawson [21]; Meng *et al.* [25] describe the tympanic–petrosal complex in another fossil pika, tentatively assigned to *Sinolagomys*, in which the spongy bulla shows an external opening for the ICA. Other contributions concerning the auditory region in lagomorphs are scarce and focused mostly on the organs of hearing (see [26–28]). Thus, our knowledge on the cranial circulation in the ear region of extinct (and most extant) lagomorphs is extremely limited.

Here we provide the first comprehensive and μ CT data-based morphological survey of the ICA-related structures traceable in the skull of Lagomorpha. Our study includes all but one of the extant lagomorph genera and a few fossil taxa for which the course of the internal carotid artery through the ear region could be ascertained from osteological features. This paper furthers the view of lagomorphs as an emergent robust system (see [29]) in which we can study macro- and micro-scale patterns of morphological change.

2. Material and methods

The auditory region of seven fossil and 29 extant lagomorph specimens has been studied, using the μ CT scans and virtual three-dimensional reconstructions performed in Avizo 9.0.1 (Thermo Fisher Scientific 1995–2019) and VG Studio MAX2.2 (Volume Graphics, Heidelberg, Germany) software. The sample of fossil Lagomorpha comprises North American stem taxa: *Megalagus turgidus* (FMNH UC/PM 1642, Field Museum of Natural History, Chicago, IL) from the early Oligocene of

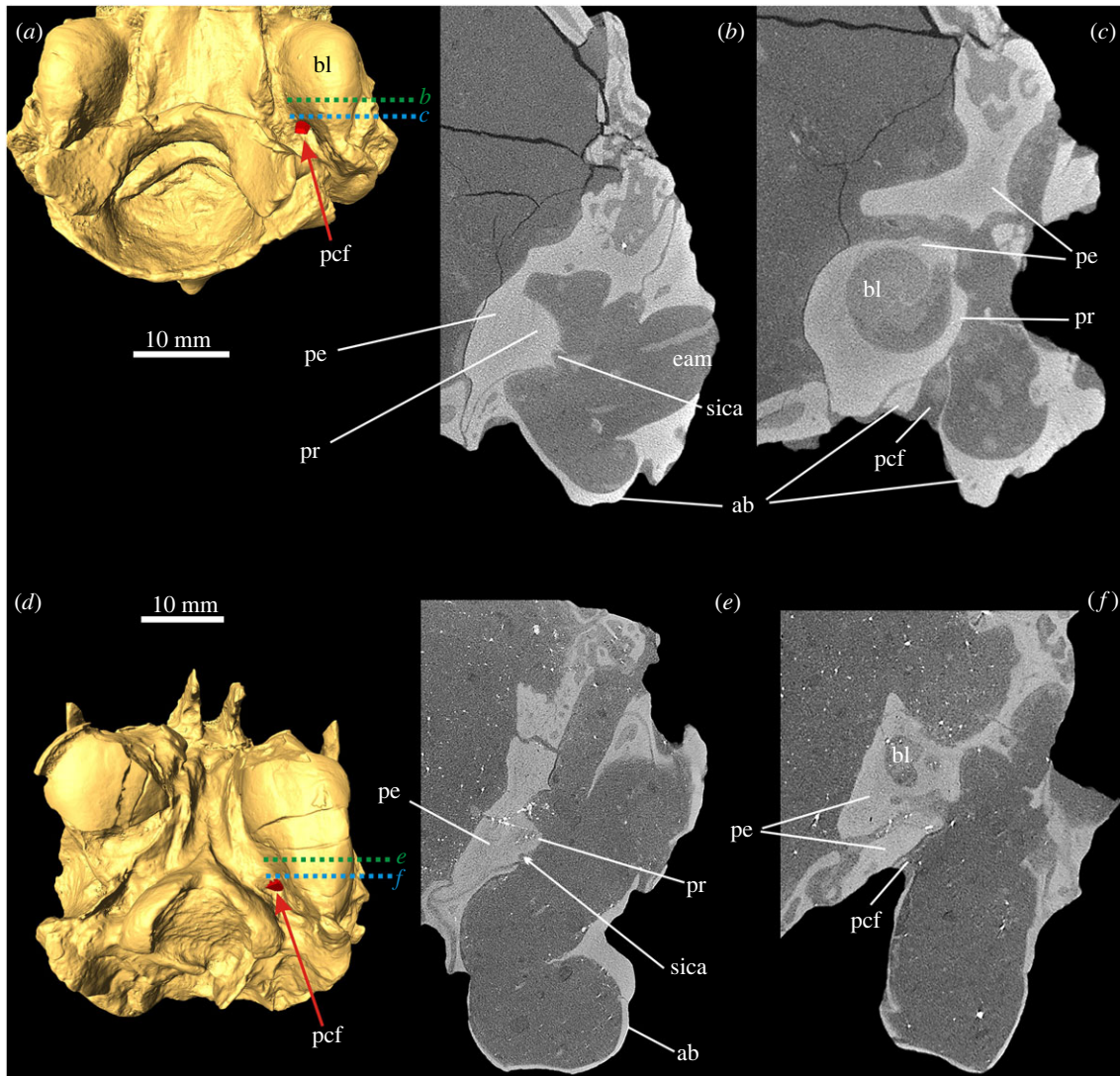


Figure 2. Auditory region of lagomorphs from the Eocene–Oligocene transition period. (a–c) *Megalagus turgidus* (FMNH UC 1642), Brule Formation, Grime’s Ranch, Sioux County, Nebraska, USA. (d–f) *Litolagus molidens* (AMNH FM 143955), Herman Wulff Ranch, Converse County, Wyoming, USA. (a,d) Three-dimensional models of the ear region in ventral view, internal carotid artery (ica) marked in red (see arrow); the sections shown in (b), (c), (e) and (f) are marked as dotted lines. (b,c) transversal μ CT images of the right ear (mirror image to enable comparisons with (e,f)) from anterior (b) (green section line in (a)) to posterior (c) (blue section line in (a)). (e,f) Transversal μ CT images of the left ear from anterior (e) (green section line in (d)) to posterior (f) (blue section line in (d)). ab, auditory bulla (ectotympanic); bl, bony labyrinth; eam, external auditory meatus; pcf, posterior carotid foramen; pe, petrosal; pr, promontorium; sica, sulcus for internal carotid artery.

Nebraska, *Litolagus molidens* (AMNH FM 143955, American Museum of Natural History, New York, NY) from the earliest Oligocene of Wyoming and two *Palaeolagus* species (Oligocene *Palaeolagus burkei* AMNH 8709 and early Oligocene *Palaeolagus haydeni* FMNH PM 9476), as well as the leporid *Archaeolagus ennisianus* (AMNH FM 7190) from the earliest Miocene of Oregon, and the extinct Pleistocene/Holocene ochotonid *Prolagus sardus* (AMNH 116812) from Sardinia, Italy. The osteological material of all specimens apart from *Prolagus* and *Archaeolagus* is represented by almost complete skulls (for details, see [22,23,30]). The holotype skull of *A. ennisianus* is partially preserved with one auditory bulla present, whereas the studied specimen of *P. sardus* is an isolated petrosal associated with the ectotympanic bulla.

Extant Leporidae are represented by cleaned skulls of 24 species comprising all genera except for the monospecific *Bunolagus*. Ochotonidae include cleaned skulls of five extant *Ochotona* species (*Ochotona alpina*, *Ochotona collaris*, *Ochotona dauurica*, *Ochotona rutila* and *Ochotona thibetana*). We studied both ear regions (at the left and right sides of a skull) in each specimen if available. Detailed information on the

species sample and scan parameters are given in electronic supplementary material, table S1. The visual data including three-dimensional models of the region of interest are provided in the Dryad Digital Repository [31].

3. Comparative morphology

3.1. Stem Lagomorpha

In *Megalagus turgidus*, the posterior carotid foramen (PCF, the external opening into the carotid canal) enters the bulla in its posteromedial corner, directly anteromedial to the jugular foramen (figure 2a–c). The entrance is completely formed by the ectotympanic. In its course, the short proximal (or posterior) carotid canal is formed by the ectotympanic and petrosal and continues as a distinct and deep sulcus crossing the medioventral surface of the promontorium in a lateral curve. Anteriorly, it leaves the tympanic cavity in the medial direction; this area is not well preserved in the specimen.

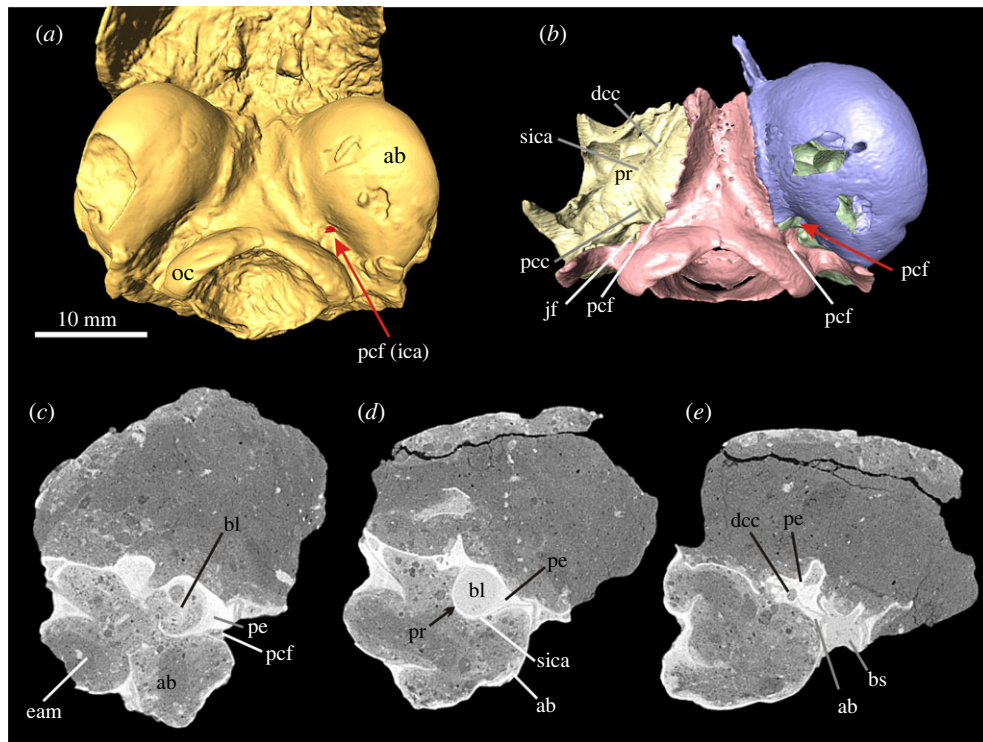


Figure 3. Early lagomorph *Palaeolagus haydeni* (FMNH PM 9476), early Oligocene of the Brule Formation, Nebraska, USA. (a) Three-dimensional model of ear region in ventral view, entrance of the internal carotid artery marked in red (see arrow); (b) ear region in ventral view, right auditory bulla removed ([23], modified). (c–e) *Palaeolagus burkei* (AMNH 8709), early Oligocene, Colorado, USA; transversal μ CT images of the left ear from posterior (c) to anterior (e). For anatomical abbreviations, see captions to figures 1 and 2. bs, basisphenoid; dcc, distal carotid canal; oc, occipital condyle; pcc, proximal carotid canal.

In *Palaeolagus haydeni* and *Palaeolagus burkei*, the ICA enters the basicranium via the PCF, which lies between the ectotympanic and petrosal right in front of the jugular foramen (figure 3). The foramen opens into a relatively short proximal carotid canal that runs anterodorsally between these two bones and opens into the tympanic cavity. The ICA continues in a shallow sulcus on the medial side of the promontorium (figure 1b) and then becomes enclosed again, between the bulla and petrosal bone into the distal (anterior) carotid canal. The ICA then bends medially and enters the brain cavity next to the sella turcica. The anterior course of the ICA from the distal carotid canal into the brain cavity could not be fully traced in *P. burkei* owing to poor preservation in that area. The promontorium of both specimens is smooth and no additional sulci were detected.

In *Litolagus molidensis* (figure 2d–f), the PCF leads into a short proximal carotid canal between the ectotympanic and petrosal. The posterior rim of the foramen is embraced by a small lamella of the bulla. The course of the ICA across the promontorium cannot be traced completely. Posteriorly, no sulcus is present, though it might be obscured by scan artefacts. Anteriorly, the sulcus for the ICA continues into a distal carotid canal formed by the petrosal and ectotympanic.

The whole course of the ICA through the ear region in stem Lagomorpha can be therefore divided into three distinct sections: (1) the posterior carotid foramen leading into a short proximal carotid canal (housing the posterior ICA section) between the ectotympanic and petrosal, (2) the middle (promontorial) section of the ICA, which runs in a sulcus at the ventromedial side of the promontorium, and (3) a long anterior section of the ICA, which is completely enclosed by the surrounding bones (especially the petrosal and ectotympanic) forming a distal (or anterior) carotid canal, before it enters

the cranial cavity. *Megalagus* and *Litolagus* depart in certain respects from this scheme; in both species the posterior carotid foramen is totally or at least mostly located in the tympanic bulla, and in *Litolagus* the posterior part of the ICA promontorial section is not represented by a sulcus. In all cases, there is no evidence of the stapedia artery as a branch of the ICA, a known fact for the extant taxa [18]. However, it should be noted that the absence of specific osteological characters (e.g. sulci) may not, strictly speaking, attest to the absence of the respective blood vessels (see [8]).

3.2. Crown Lagomorpha

The early archaeolagine leporid *Archaeolagus ennisianus* shows a huge PCF located in front of the jugular foramen between the ectotympanic and petrosal (figure 4). The carotid canal and the course of the ICA across the promontorium resemble the pattern observed in the stem lagomorphs under study, i.e. there is a shallow sulcus formed in the tympanic surface of the petrosal. The skull of *A. ennisianus* is only partially preserved; thus, the anteriormost course of the ICA into the cranial cavity is not fully recognizable.

All studied extant leporid specimens have a similar pattern of the osteological features related to the perbullar course of the ICA in the ear region. The auditory bulla shows a distinct PCF (for the ICA entrance) that is entirely formed by the ectotympanic and located posteromedially to medially in the bulla (figure 5), in the vicinity of the jugular foramen. The PCF opens into a wide carotid canal for the ICA. The proximal (posterior) part of the canal is made up by the ectotympanic only (apart from *Nesolagus timminsi* and *Pentalagus furnessi*), whereas the distal (anterior) part runs anterodorsally between the ectotympanic and the petrosal. Along its course, the

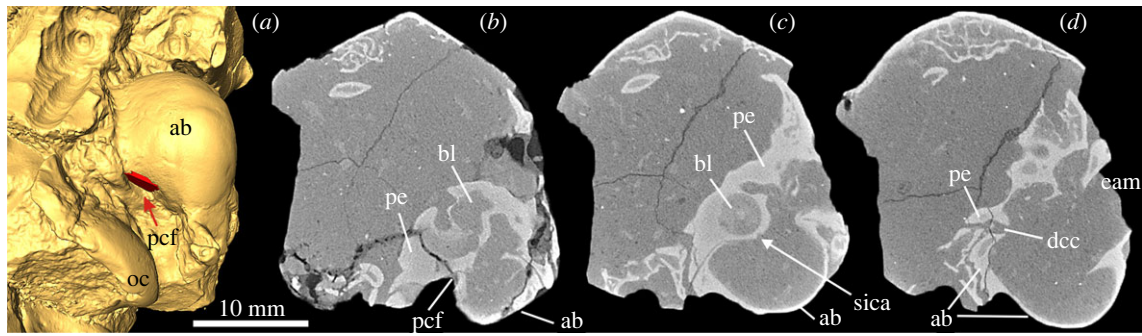


Figure 4. Auditory region of the archaeolagine *Archaeolagus ennisianus* (AMNH FM 7190), Miocene of the John Day Formation, north fork of John Day River, Oregon, USA. (a) Three-dimensional model of the left ear region in ventral view, entrance of internal carotid artery (ica) marked in red (see arrow); (b–d) transversal µCT images of the left ear from posterior (b) to anterior (d). For anatomical abbreviations, see captions to figures 1–3.

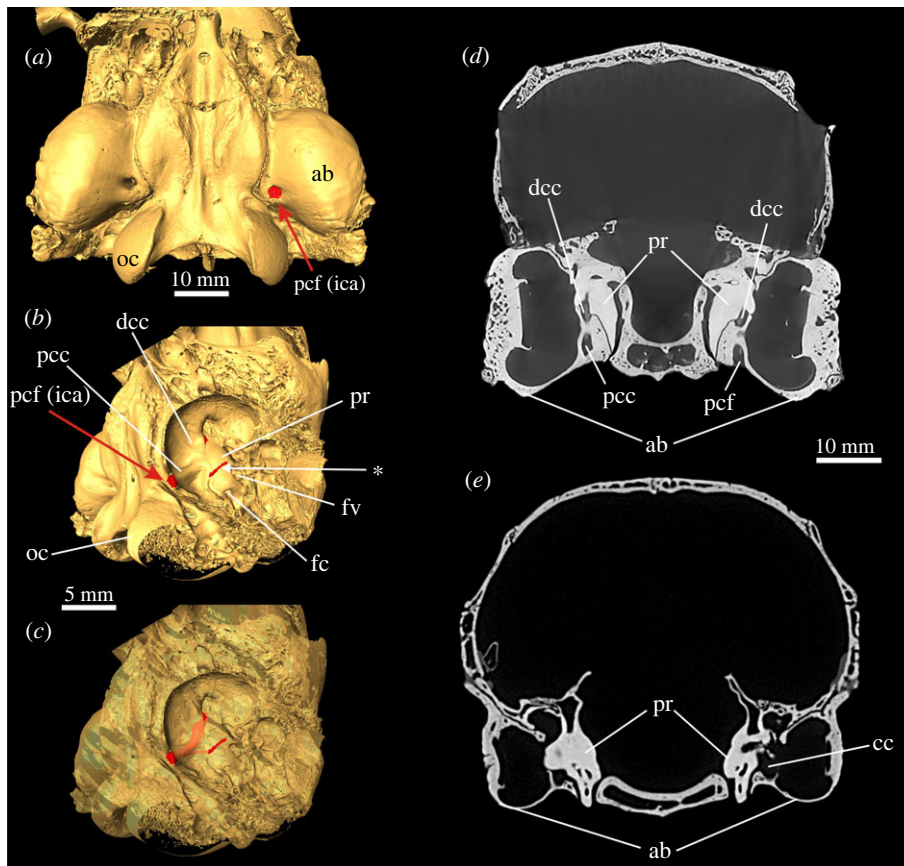


Figure 5. Auditory region and position of the entrance of the internal carotid artery in extant leporids. (a–d) *Oryctolagus cuniculus* (M4860). (a) Three-dimensional model of the ear region in ventral view, anterior to the top, entrance of internal carotid artery in red (see arrow); (b) the same specimen in left lateral view with auditory bulla removed, asterisk (*) indicates branch of the internal carotid nerves (?) running in a shallow sulcus of the promontorium; (c) as in (b), but bones made transparent; (d) transversal µCT image of the ear region of the same specimen; (e) transversal µCT image of the ear region of *Pentalagus furnessi* (M12940), the carotid canal is entirely situated between the ectotympanic and petrosal. For abbreviations, see captions to figures 1–3. cc, carotid canal; fc, fenestra cochleae; fv, fenestra vestibuli.

carotid canal does not enter the tympanic cavity, in contrast to the fossil taxa under study. However, before it bends medially toward the brain cavity (area of the sella turcica), its lateral wall can be incomplete and, thus, the canal lumen becomes confluent with the anteriormost part of the tympanic cavity in the area of the foramen lacerum medium. Most studied specimens have no distinct sulci on the promontorium for e.g. branches of the tympanic plexus; however, some species show certain specific characters due to the exact position of the PCF and the course of the carotid canal, which can vary even within a genus. In *Caprolagus hispidus*, *Lepus* spp., *Pronolagus* spp. and *Sylvilagus* spp., the carotid canal is situated

anterior to the bony labyrinth (and the promontorium) and thus shows a steeper course. *Pronolagus* cf. *saundersiae* has a smaller foramen (possibly for the internal carotid nerves) directly in front of a large foramen; we interpret the latter as the PCF. Both canals are confluent inside the ectotympanic. In *Oryctolagus cuniculus* and *C. hispidus*, a very small canal branches off the proximal part of the carotid canal and runs anterodorsally between the ectotympanic and petrosal; more anteriorly, it enters the tympanic cavity and continues into a shallow sulcus on the promontorium that runs anteriorly and medially to the fenestra vestibuli (figure 5). This canal and sulcus may house a branch of the internal carotid nerves

(internal carotid plexus). Krause [1] described a distinct tympanic sulcus on the promontorium of *O. cuniculus* that houses the tympanic nerve (branch of CN IX); however, our structure seems to be located more anteriorly and thus does not refer to the tympanic nerve as it is clearly associated with the carotid canal. *Lepus townsendii*, *Pronolagus rupestris* and *Sylvilagus nuttallii* also show the small additional canal but no sulcus on the promontorium. In *Lepus sinensis* and *Sylvilagus brasiliensis*, a tiny separate canal is present posterior to the carotid canal that shows the same course (including a shallow sulcus on the promontorium) and corresponds to the small canal observed in *Oryctolagus* and *Caprolagus*. *Sylvilagus floridanus* has two small canals branching off from the carotid canal; the anterior one leads into a sulcus on the anterior tip of the promontorium.

Pentalagus furnessi and *Nesolagus* spp. have very small auditory bullae compared with those of most other leporids; they also differ in the PCF location and morphology. In *Pentalagus*, the PCF lies just at the posteromedial rim of the ectotympanic. The foramen is not fully formed by the ectotympanic but in part also by the petrosal. The canal is quite short and is entirely made up by the ectotympanic and petrosal. *Nesolagus netscheri* and *N. timminsi* show two foramina in the region of interest. A large foramen, most probably the PCF, enters the auditory region right in front of the jugular foramen. In *N. netscheri*, this foramen lies within the ectotympanic; in *N. timminsi*, contrary to other leporids, it is clearly situated between the auditory bulla and petrosal. In both species, the foramen opens into a short carotid canal that runs entirely between the two bones (ectotympanic and petrosal), similar to *Pentalagus*. However, a smaller canal that is confluent with the former is totally enclosed by the ectotympanic and its entrance is situated anteromedially to the large foramen. The promontorium in both *Nesolagus* species shows no sulci connected to the carotid canal.

In extant ochotonids, the ICA enters the cranial cavity (figure 6*a*) via the foramen lacerum medium (carotid notch of the piriform fenestra according to [32]). According to Bugge [18], the ICA runs below the medial tympanic bulla in an anterior direction before entering the basicranium. Absence of a distinct intraosseous course of the ICA in the auditory region, and thus an extrabullar course of the ICA, is confirmed in all the pika species in our study.

Dawson [21] described the external cranial anatomy of the fossil ochotonid *Prolagus sardus* and observed the PCF located anteromedially in the bulla (see her fig. 4). This is confirmed by our studied specimen of *P. sardus* (AMNH 116812). A short carotid canal is traceable in the anterior auditory region that runs from ventral to dorsal into the cranial cavity (figure 6*b–d*). The PCF is formed by the ectotympanic and opens into the anteromedial part of the auditory bulla. Anteriorly, the carotid canal is formed by the ectotympanic and petrosal. The canal does not enter the tympanic cavity. Unfortunately, our specimen shows an incomplete promontorium and posterior auditory bulla; thus, no further information on additional small canals and sulci is available.

4. Discussion

In a broader phylogenetic scheme of the Euarchontoglires clade, lagomorphs noticeably differ in the course of the ICA from rodents, their sister clade within the Glires cohort [8].

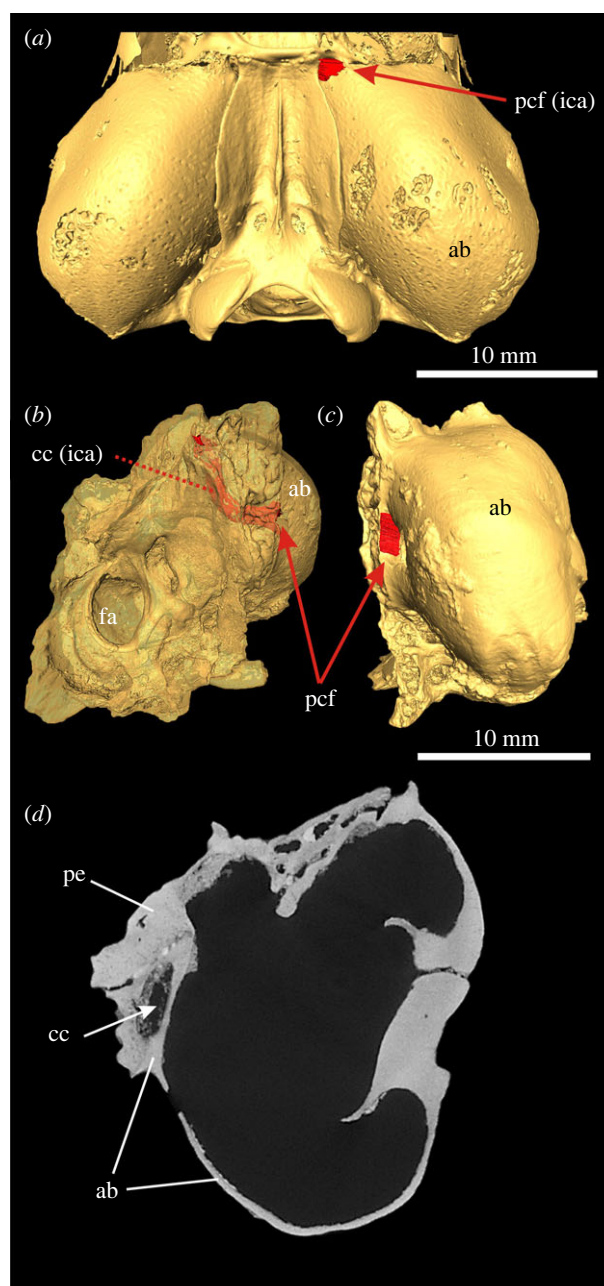


Figure 6. Position of internal carotid artery in Ochotonidae. (a) Three-dimensional model of the ear region of *Ochotona alpina* (M100488) in ventral view, position of the entrance of internal carotid artery in red (see arrow). (b–d) *Prolagus sardus* (AMNH116812). (c) Three-dimensional model of the right ear region (mirror image) in ventral view, anterior to the top, entrance of internal carotid artery in red (see arrow); (b) transparent three-dimensional model of the same specimen in oblique anteromedial view (mirror view), anterior to the left; the course of internal carotid artery in carotid canal indicated in red (see arrow and dotted line); (d) transversal μ CT image of the same specimen (mirrored). For anatomical abbreviations, see captions to figures 1 and 2.

Rodents show a great diversity of the ICA course patterns and of related structures (see [18,33]); they display all three types of the ICA route—transpromontorial, perbullar and extrabullar [12]—early in their evolution. Some most basal taxa, such as *Cocomys*, *Sciuravus* and *Paramys* show a transpromontorial course of the ICA [8], although *Reithroparamys*, a more specialized ischyromyid rodent already displays complete lack of the ICA (see [8,34]). The ancestral state for Glires was presumably the transpromontorial course of the ICA as this character state is shared by stem Lagomorpha

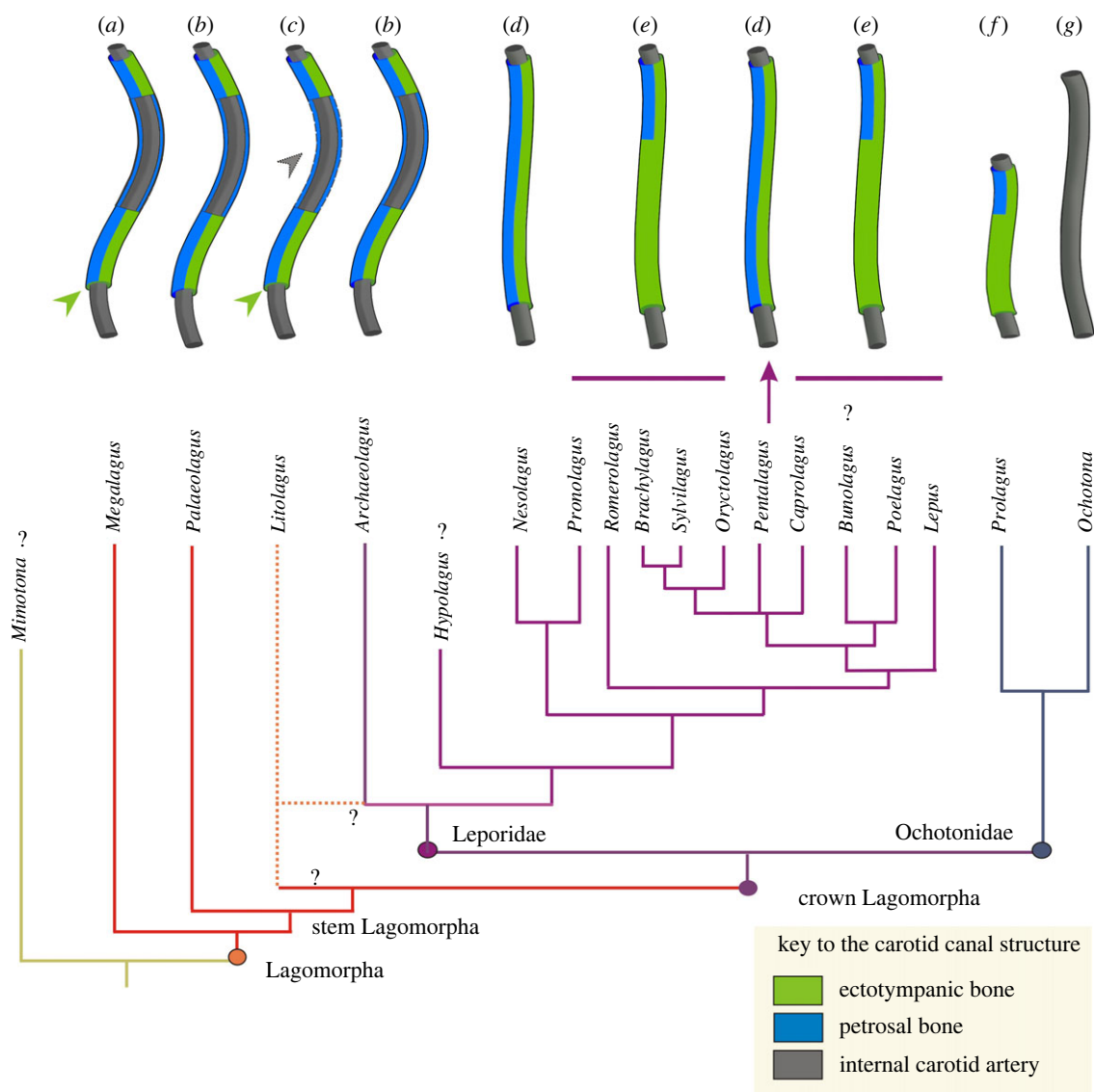


Figure 7. The carotid canal structure in Lagomorpha. Schematic drawings in ventral view, anterior to the top. (a) Posterior and anterior carotid canals present, the posterior carotid foramen formed exclusively by ectotympanic (green arrow), complete sulcus on the promontorium; (b) posterior and anterior carotid canals present, the posterior carotid foramen formed by ectotympanic and petrosal, complete sulcus on the promontorium; (c) posterior and anterior carotid canals present, the posterior carotid foramen formed mostly by ectotympanic (green arrow), incomplete sulcus on the promontorium (grey arrow); (d) complete carotid canal with the posterior carotid foramen formed by ectotympanic and petrosal; (e) complete carotid canal formed mostly of the ectotympanic; (f) short version of (e); (g) extra-bullar carotid canal. Lagomorph phylogeny based on Matthee *et al.* [41], Fostowicz-Frelik [22], Fostowicz-Frelik & Meng [42] and Cano-Sánchez *et al.* [43], modified. The dotted lines denote alternative phylogenetic position.

with the primatomorph *Plesiadapis* ([35]; see [36] for phylogenetic position) and above-mentioned earliest rodents (ctenodactyloids, ischyromyids and sciuravids). The exact spatial relationships between the promontorium and ICA course within the tympanic cavity are, however, not the same in stem Lagomorpha as in primatomorphs and other Euarchontoglires, which have the transpromontorial ICA course. Furthermore, the ICA (especially its promontorial part) in stem lagomorphs is positioned differently from the reconstructed ICA morphology in early eutherians (see [7,13,35]). In lagomorphs, the sulcus for the ICA on the promontorium is more medial (especially in *Palaeolagus* and *Litolagus*) than in other Euarchontoglires displaying this character state.

The transpromontorial course of the ICA is observed in some Euarchontoglires groups, such as treeshrews (*Ptilocercus* and *Tupaia*idae; see [37]), Strepsirhini, Paromomyidae and Plagiomenidae (see [12]), but in all these groups the course of the ICA runs mostly in the middle of the promontorium.

In Dermoptera the ICA is lost, although the presence of the internal carotid nerves indicates a previous transpromontorial course in a similar position (see fig. 6 in [35]). Therefore, the ICA course in stem Lagomorpha cannot be interpreted strictly as ancestral compared with the totality of Euarchontoglires. Its position is already derived, although, being still transpromontorial, it should be regarded as a modified character state in relation to the primitive course known for other Euarchontoglires. A somewhat similar situation occurs in *Tupaia*, in which a bony canal encloses the ICA, but still within the tympanic cavity, and thus it is a derived state compared with an open transpromontorial sulcus [8,35].

Nevertheless, the transpromontorial course of the ICA and the presence of a sulcus in the Oligocene stem lagomorphs and especially early Miocene *Archaeolagus* are yet further confirmation of the notable morphological conservatism observed in Lagomorpha (see [18,23,30,38]).

The only other derived feature in stem Lagomorpha seems to be a lack of the stapedia artery originally existing

Table 1. The structure of the posterior carotid foramen (PCF) and the carotid canal composition in fossil and extant Lagomorpha.

taxon	ICA course	PCF composition	proximal carotid canal composition	distal carotid canal composition	sulcus on the promontorium between proximal and distal carotid canal
<i>Megalagus turgidus</i>	transpromontorial	ectotympanic	ectotympanic + petrosal	ectotympanic + petrosal	complete
<i>Palaeolagus haydeni</i>	transpromontorial	ectotympanic + petrosal	ectotympanic + petrosal	ectotympanic + petrosal	complete
<i>Palaeolagus burkei</i>	transpromontorial	ectotympanic + petrosal	ectotympanic + petrosal	ectotympanic + petrosal	complete
<i>Litolagus molidens</i>	transpromontorial	ectotympanic + petrosal)	ectotympanic + petrosal	ectotympanic + petrosal	incomplete
<i>Archaeolagus ennisianus</i>	transpromontorial	ectotympanic + petrosal	ectotympanic + petrosal	ectotympanic + petrosal	complete
<i>Prolagus sardus</i>	perbullar	ectotympanic	ectotympanic	ectotympanic + petrosal	—
extant Leporidae	perbullar	ectotympanic ^a	ectotympanic ^b	ectotympanic + petrosal	—
extant Ochotonidae (<i>Ochotona</i>)	extrabullar	—	—	—	—

^aIn *Nesolagus timminsi*, the posterior carotid foramen is formed by both the ectotympanic and petrosal. This is also the case in *Pentalagus furnessi* (right side).

^bIn *Nesolagus* spp. and *P. furnessi*, a short carotid canal is completely formed by the ectotympanic and petrosal.

in their placental ancestors [8]. However, the stapedial foramen, normally passed by the stapedial artery, is retained as a plesiomorphic mammalian character in Lagomorpha (see [39]). The uniform pattern of the ICA route in stem lagomorphs stands in opposition to the rapid evolutionary changes observed in rodents and even advanced eurymylid Glires, such as *Rhombomylus*, which has already lost the PCF and a whole ICA, whereas in the closely related *Matutinia* the ICA presumably showed an extratympanic bifurcation of the ICA into a branch representing the anterior continuation of this vessel and the stapedial artery, with separate entrances [8,40].

There is a disparity in the ICA course and the carotid canal structure among the stem and crown Lagomorpha (figure 7) as well as within the crown groups themselves (table 1 for details). In general, all studied stem fossil taxa and *Archaeolagus* allowed the reconstruction of a more or less medial transpromontorial course of the ICA. The extrabullar ICA course of the extant *Ochotona* is apparently highly derived, whereas the ICA course in extant Leporidae shows principally a similar pattern to that observed in stem lagomorphs (and *Archaeolagus*). However, both groups differ in the formation of the PCF, lack of the promontorial sulcus and total enclosure of the carotid canal, especially by the bullar wall.

Among extant leporids, *Nesolagus* and *Pentalagus* are exceptions having the posterior part of the carotid canal and PCF formed also by both the ectotympanic and petrosal (figure 7), in this respect slightly resembling the ICA structure in stem lagomorphs (disregarding the absence of a transpromontorial sulcus). It is hard to discern whether such similarity is a result of a preserved ancestral structure, or a secondary derived character state, and its adaptive meaning is also unclear. The other feature of the ear structure that both extant genera (*Nesolagus* and *Pentalagus*) have in

common with *Megalagus* is a relatively small auditory bulla. On the other hand, *Litolagus* and *Palaeolagus*, which also have the carotid canal built by both the ectotympanic and petrosal, display large auditory bullae. The size of the bullae in lagomorphs is regarded as an adaptive character related to the habitat type [22]; the species living in open landscape habitats or mixed forest/grassland environments have larger bullae than typical forest/jungle inhabitants (such as *Nesolagus* and *Pentalagus*). In rodents, bullar hypertrophy is frequently associated with increased aridity and xeric habitats (see [44]).

As there is no direct functional relation between the carotid canal structure and the size of the auditory bullae, we can assume that the ICA-related structures such as the PCF and presence/absence of the canal, and its composition, can be perceived as features of phylogenetic meaning in Lagomorpha. Although our study does not cover many Archaeolinae and early Leporinae, it captures the ICA route and structure in most crucial points of the lagomorph evolution: stem taxa, early crown members and all two/three crown groups (depending on whether or not *Prolagus* is placed within Ochotonidae).

The character polarization is clear, and it follows the character states observed by Wible [12] for Placentalia: the transpromontorial course of the ICA with a well-displayed sulcus is an ancestral state for Lagomorpha, whereas the perbullar and extrabullar states should be considered as derived.

Furthermore, the PCF formed in parts by the ectotympanic and petrosal represents another ancestral character state for Lagomorpha, whereas the PCF formed solely by the ectotympanic can be considered derived. We can hypothesize that the carotid canal formed equally by the ectotympanic and petrosal (as in *Nesolagus* and *Pentalagus*) is less advanced than that formed prevalently by the ectotympanic (the rest of

the studied leporids). Following this hypothesis, it can be assumed that *Nesolagus* and *Pentalagus* are relatively 'basal' leporid taxa, which at least partly agrees with the recent molecular-based phylogenies of extant Lagomorpha (see e.g. [43]). Nevertheless, we cannot rule out that this is a secondary character because evolutionary parallelisms are frequent in lagomorph evolution [38,42]. The question remains unanswered until a more exhaustive study of the Neogene leporids is conducted. However, the persistence of the 'stem lagomorph' pattern of the ICA-related structures in *Archaeolagus* shows the evolutionary stability of this structure. The developmental aspect supports our conclusions as the pattern observed in the stem lagomorphs resembles the one described in prenatal ontogeny of *Oryctolagus cuniculus* [45]. In the European rabbit, the proximal part of the ICA becomes enclosed by the growing ectotympanic, and the distal part runs in a sulcus on the anterior promontorium but becomes enclosed by the ectotympanic and petrosal later.

In terms of the general ICA pattern found in Leporidae, it can be treated as an advanced variant of the basic stem lagomorph structure, especially when the fully extrabullar ICA course of modern ochotonids is considered (figure 7). In this respect, the pattern displayed by *Prolagus sardus* seems halfway between the typical leporid and ochotonid structures, as it is extrabullar in its posterior part but then becomes perbullar and the canal structure is similar to that of leporids (figure 7). Furthermore, the PCF is shifted far anteriorly and thus resembles a transitional stage. The *Prolagus* lineage is old, originating around the end of the Oligocene [46]. Whether *Prolagus* indeed shares a direct common ancestor with *Ochotona* is another matter, but the ICA structure and canal pattern show some similarity to the leporid structure, which suggests an independent evolutionary history of the *Prolagus* lineage from the rest of

Ochotonidae. Until more fossil ochotonids are studied, it is hard to determine if the *Prolagus* pattern is ancestral for the entire ochotonid lineage and the fully extrabullar condition appeared later in the *Ochotona* lineage, or *Prolagus* is an independent group of the crown Lagomorpha.

Data accessibility. Virtual reconstructions of the auditory region of six fossil and two extant lagomorphs are available from the Dryad Digital Repository: doi:10.5061/dryad.prr4xgxr [31]. Detailed data for all specimens, including μ -CT scan parameters, are provided in electronic supplementary material, table S1.

Authors' contributions. I.R.: conceptualization, funding acquisition, investigation, methodology, resources, supervision, validation, visualization, writing—original draft, writing—review and editing; J.M.: conceptualization, investigation, methodology, resources, supervision, validation, writing—original draft, writing—review and editing; L.F.-F.: conceptualization, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, visualization, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed herein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. This research was funded by National Science Centre (Kraków, Poland) grant no. 2015/18/E/NZ8/00637 to L.F.-F. and by Deutsche Forschungsgemeinschaft (DFG) grant no. DFGRU 1496/4-1 to I.R. Funding for the Open Access fees was approved by University of Chicago Library based on an agreement between University of Chicago Library and The Royal Society.

Acknowledgements. We are grateful to Bill Simpson (Field Museum of Natural History, Chicago: FMNH) and to Ruth O'Leary (American Museum of Natural History, New York: AMNH) for access to the fossil specimens in their care. Thanks are also extended to Morgan Hill (AMNH) and James Thostenson (currently Duke University) for the scanning of fossil lagomorph specimens at the AMNH μ -CT scanning facility. We thank Katrin Krohmann for technical support with μ CT scans of *Pentalagus furnessi* and Juliane Eberhardt for visualizations of the carotid canal structure in figure 7 (both SGN). Finally, we thank J. Wible and an anonymous reviewer, whose comments helped to improve the manuscript considerably.

References

- Krause W. 1884 *Die Anatomie des Kaninchens in topographischer und operativer Rücksicht [The anatomy of the rabbit regarding topography and surgery]*. Leipzig, Germany: Verlag von Wilhelm Engelmann. [In German]
- Davis DD, Story HE. 1943 The carotid circulation in the domestic cat. *Fieldiana Zool. Ser.* **28**, 1–47.
- Bugge J. 1968 The arterial supply of the rabbit nose and oral cavity. *Acta Anat. (Basel)* **70**, 168–183. (doi:10.1159/000143125)
- Evans HE, de Lahunta A. 2013 *Miller's anatomy of the dog*. St Louis, MO: Elsevier-Saunders.
- Anderson RH, Bamforth SD. 2022 Morphogenesis of the mammalian aortic arch arteries. *Front. Cell Dev. Biol.* **10**, 892900. (doi:10.3389/fcell.2022.892900)
- Novacek MJ. 1985 Cranial evidence for rodent affinities. In *Evolutionary relationships among rodents—a multidisciplinary analysis* (eds WP Luckett, J-L Hartenberger), pp. 59–81. New York, NY: Plenum.
- Wible JR. 1986 Transformations in the extracranial course of the internal carotid artery in mammalian phylogeny. *J. Vertebr. Paleontol.* **6**, 313–325. (doi:10.1080/02724634.1986.10011628)
- Meng J, Hu Y, Li C-K. 2003 The osteology of *Rhombomylus* (Mammalia, Glires): implications for phylogeny and evolution of Glires. *Bull. Am. Mus. Nat. Hist.* **275**, 1–247. (doi:10.1206/0003-0090(2003)275<0001:TOORMG>2.0.CO;2)
- Ekdale EG, Archibald JD, Averianov AO. 2004 Petrosal bones of placental mammals from the Late Cretaceous of Uzbekistan. *Acta Palaeontol. Pol.* **49**, 161–176.
- Wible JR, Novacek MJ, Rougier GW. 2004 New data on the skull and dentition in the Mongolian Late Cretaceous eutherian mammal *Zalambdalestes*. *Bull. Am. Mus. Nat. Hist.* **281**, 1–144. (doi:10.1206/0003-0090(2004)281<0001:NDOTSA>2.0.CO;2)
- Wible JR, Rougier GW, Novacek MJ, Asher RJ. 2009 The eutherian mammal *Maelestes gobiensis* from the Late Cretaceous of Mongolia and the phylogeny of Cretaceous Eutheria. *Bull. Am. Mus. Nat. Hist.* **327**, 1–123. (doi:10.1206/623.1)
- Wible JR. 1983 The internal carotid artery in early eutherians. *Acta Palaeontol. Pol.* **28**, 281–293.
- MacPhee RDE. 1981 Auditory regions of primates and eutherian insectivores: morphology, ontogeny, and character analysis. *Contrib. Primatol.* **18**, 1–282.
- Wible JR, Rougier GW, Novacek MJ, McKenna MC. 2001 Earliest eutherian ear region: a petrosal referred to *Prokennalestes* from the Early Cretaceous of Mongolia. *Am. Mus. Novit.* **3322**, 1–44. (doi:10.1206/0003-0082(2001)322<0001:EEERAP>2.0.CO;2)
- Matthew WD. 1909 The Carnivora and Insectivora of the Bridger Basin, Middle Eocene. *Mem. Am. Mus. Nat. Hist.* **9**, 289–567.
- Presley R. 1979 The primitive course of the internal carotid artery in mammals. *Acta Anat.* **103**, 238–244. (doi:10.1159/000145015)
- Bugge J. 1967 The arterial supply of the middle ear of the rabbit with special reference to the contribution of the stapedia artery to the development of the superior tympanic artery and the petrosal branch. *Acta Anat. (Basel)* **67**, 208–220. (doi:10.1159/000142980)
- Bugge J. 1974 The cephalic arterial system in insectivores, primates, rodents and lagomorphs, with special reference to the systematic classification. *Acta Anat. (Basel)* **87**(Suppl. 62), 1–159. (doi:10.1159/000144205)
- Wood AE. 1940 The mammalian fauna of the White River Oligocene. Part III. Lagomorpha. *Trans. Am. Phil. Soc.* **28**, 271–362. (doi:10.2307/1005524)
- Dawson MR. 1958 Later Tertiary Leporidae of North America. *Univ. Kansas Paleontol. Contrib. Vertebrata* **6**, 1–75.

21. Dawson MR. 1969 Osteology of *Prolagus sardus*, a Quaternary ochotonid (Mammalia, Lagomorpha). *Palaeovertebrata* **2**, 157–190. (doi:10.18563/PV.2.4.157-190)
22. Fostowicz-Frelik Ł. 2013 Reassessment of *Chadrolagus* and *Litolagus* (Mammalia: Lagomorpha) and a new genus of North American Eocene lagomorph from Wyoming. *Am. Mus. Novit.* **3773**, 1–76. (doi:10.1206/3773.2)
23. Wolniewicz AS, Fostowicz-Frelik Ł. 2021 CT-informed skull osteology of *Palaeolagus haydeni* (Mammalia: Lagomorpha) and its bearing on the reconstruction of the early lagomorph body plan. *Front. Ecol. Evol.* **9**, 634757. (doi:10.3389/fevo.2021.634757)
24. Wu S-Y. 2003 The cranial morphology and phylogenetic relationship of *Alloptox gobiensis* (Lagomorpha, Ochotonidae). *Vert. Palasiat.* **41**, 115–130.
25. Meng J, Wu W-Y, Ye J, Bi S-D. 2001 Two petrosal of gliiform mammals from Late Oligocene of Tiersihabahe, Xinjiang Uygur Autonomous Region, China. *Vert. Palasiat.* **39**, 43–53.
26. Maier W, Tröscher A, Ruf I. 2018 The anterior process of the malleus in extant Lagomorpha (Mammalia). *J. Morphol.* **279**, 132–146. (doi:10.1002/jmor.20759)
27. Ruf I, Meng J, Fostowicz-Frelik Ł. 2021 Anatomy of the nasal and auditory regions of the fossil lagomorph *Palaeolagus haydeni*: systematic and evolutionary implications. *Front. Ecol. Evol.* **9**, 636110. (doi:10.3389/fevo.2021.636110)
28. López-Torres S, Bhagat R, Bertrand OC, Silcox MT, Fostowicz-Frelik Ł. 2023 Locomotor behavior and hearing sensitivity in an early lagomorph reconstructed from the bony labyrinth. *Ecol. Evol.* **13**, e9890. (doi:10.1002/ece3.9890)
29. Kraatz BP *et al.* 2021 Lagomorpha as a model morphological system. *Front. Ecol. Evol.* **9**, 636402. (doi:10.3389/fevo.2021.636402)
30. López-Torres S, Bertrand OC, Lang MM, Silcox MT, Fostowicz-Frelik Ł. 2020 Cranial endocast of the stem lagomorph *Megalagus* and brain structure of basal Euarchothoglires. *Proc. R. Soc. B* **287**, 20200665. (doi:10.1098/rspb.2020.0665)
31. Fostowicz-Frelik Ł, Ruf I, Meng J. 2023 Data from: Auditory region circulation in Lagomorpha: the internal carotid artery pattern revisited. Dryad Digital Repository. (doi:10.5061/dryad.prr4xgxr)
32. Wible JR. 2007 On the cranial osteology of the Lagomorpha. *Bull. Carnegie Mus. Nat. Hist.* **39**, 213–234. (doi:10.2992/0145-9058(2007)39[213:OTCOOT]2.0.CO;2)
33. Bugge J. 1985 Systematic value of the carotid arterial pattern in rodents. In *Evolutionary relationships among rodents* (eds WP Luckett, J-L Hartenberger), pp. 355–379. Boston, MA: Springer.
34. Meng J. 1990 The auditory region of *Reithroparamys delicatissimus* (Rodentia, Mammalia) and its systematic implications. *Am. Mus. Novit.* **2972**, 1–35.
35. Wible JR. 1993 Cranial circulation and relationships of the colugo *Cynocephalus* (Dermoptera, Mammalia). *Am. Mus. Novit.* **3072**, 1–27.
36. Seiffert ER, Tejedor MF, Fleagle JG, Novo NM, Cornejo FM, Bond M, De Vries D, Campbell Jr KE. 2020 A parapithecoid stem anthropoid of African origin in the Paleogene of South America. *Science* **368**, 194–197. (doi:10.1126/science.aba1135)
37. Wible JR. 2011 On the treeshrew skull (Mammalia, Placentalia, Scandentia). *Ann. Carnegie Mus.* **79**, 149–230. (doi:10.2992/007.079.0301)
38. Fostowicz-Frelik Ł. 2017 Convergent and parallel evolution in early Glires (Mammalia). In *Evolutionary biology: self/nonself evolution, species and complex traits evolution, methods and concepts* (ed. P Pontarotti), pp. 199–216. Cham, Switzerland: Springer.
39. Schultz JA, Ruf I, Martin T. 2018 Oldest known multituberculate stapes suggests an asymmetric bicrural pattern as ancestral for Multituberculata. *Proc. R. Soc. B* **285**, 20172779. (doi:10.1098/rspb.2017.2779)
40. Ting S-Y, Meng J, McKenna MC, Li C-K. 2002 The osteology of *Matutinia* (Simplidontata, Mammalia) and its relationship to *Rhombomylus*. *Am. Mus. Novit.* **3371**, 1–33. (doi:10.1206/0003-0082(2002)371<0001:TOOMSM>2.0.CO;2)
41. Matthee CA, Vuuren van BJ, Bell D, Robinson TJ. 2004 A molecular supermatrix of the rabbits and hares (Leporidae) allows for the identification of five intercontinental exchanges during the Miocene. *Syst. Biol.* **53**, 433–447. (doi:10.1080/10635150490445715)
42. Fostowicz-Frelik Ł, Meng J. 2013 Comparative morphology of premolar foramen in lagomorphs (Mammalia: Glires) and its functional and phylogenetic implications. *PLoS ONE* **8**, e79794. (doi:10.1371/journal.pone.0079794)
43. Cano-Sánchez E, Rodríguez-Gómez F, Ruedas LA, Oyama K, León-Paniagua L, Mastretta-Yanes A, Velázquez A. 2022 Using ultraconserved elements to unravel lagomorph phylogenetic relationships. *J. Mammal. Evol.* **29**, 395–411. (doi:10.1007/s10914-021-09595-0)
44. Scarpitti EA, Caledo JJM. 2022 Ecological correlates of the morphology of the auditory bulla in rodents: application to the fossil record. *J. Anat.* **240**, 647–668. (doi:10.1111/joa.13579)
45. Wible JR. 1984 The ontogeny and phylogeny of the mammalian cranial arterial pattern. PhD thesis, Duke University, Durham, NC, USA.
46. López-Martínez N. 2001 Paleobiogeographical history of *Prolagus* an European ochotonid (Lagomorpha). *Lynx (Praha)* (New Series) **32**, 215–231.