

“Septal compass” and “septal formula”: a new method for phylogenetic investigations of the middle ear region in the squirrel-related clade (Rodentia: Mammalia)

Cathrin Pfaff¹ · Thomas Martin² · Irina Ruf³

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Abstract Here, we introduce the “septal compass” and the “septal formula” as a new method for phylogenetic investigations of the middle ear region in squirrel-related clade. The middle ear cavity is characterized by bony septa that divide the dorsally lying epitympanic recess and the ventrally lying tympanic cavity into several segments or diverticula. The distribution patterns of these septa are conservative among the squirrel-related clade and are restricted to the species, genus, and family level. In the studied outgroups represented by †*Ischyromys typus* and lagomorphs, no septa are found in the epitympanic recess and tympanic cavity. Therefore, the “septal compass” and the “septal formula” provide a new approach for phylogenetic interpretations of the middle ear region. It is user-optimized and can be modified for other rodent families and mammalian taxa and will facilitate phylogenetic assumptions in future investigations. Additionally, this method will enable the allocation of isolated tympanic bullae to the respective genus level.

Keywords Rodentia · Sciuromorpha · Middle ear · Epitympanic recess · Tympanic cavity · Bony septa

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✉ Cathrin Pfaff
cathrin.pfaff@univie.ac.at

¹ Department of Palaeontology, University of Vienna, Vienna, Austria

² Steinmann-Institut für Geologie, Mineralogie und Paläontologie, Rheinische-Friedrich-Wilhelms-Universität Bonn, Bonn, Germany

³ Senckenberg Forschungsinstitut und Naturmuseum Frankfurt, Abteilung Paläoanthropologie und Messelforschung, Frankfurt am Main, Germany

Introduction

Resolving systematic issues of extant and extinct rodents is mainly based on anatomical investigations of teeth (e.g., Thenius 1989; Martin 1992). However, rodents with similar diets display convergent dental morphology (Samuels 2009). Since the nineteenth century, the study of the ear region has become an important aspect of comparative anatomical analyses (Hyrtl 1845; Doran 1879; van Kampen 1905; Bondy 1908; Fleischer 1978). A principal feature of the ear region is the pneumatization of the middle ear region and the auditory bulla. Diverticula are lying in the epitympanic recess and the tympanic cavity, which are dividing the middle ear cavity into differently sized chambers (Hyrtl 1845; van der Klaauw 1931; Fleischer 1978; Lavocat and Parent 1985; Meng 1990; Potapova 2001; Wible 2011). This anatomy often is associated with bone pneumatization of the occipital, squamosal, and basicranium (van Kampen 1905; Hooper 1968; Lay 1972; MacPhee 1981; Potapova 2001; Wible 2009). First attempts for comparative investigations of the mammalian ear regions were made by introducing a simplified nomenclature of the middle ear cavities. These nomenclatures focus on the space between the septa (Saban 1956; MacPhee 1981; Wible 2011) and the bulla pneumatization or pockets (Potapova 2001). However, these approaches were not consistent and consequently hampered phylogenetic investigations within and between taxa.

The squirrel-related clade is positioned at the base of the extant rodents (Huchon et al. 2002; Mercer and Roth 2003; Blanga-Kanfi et al. 2009; Wu et al. 2012) and normally is used to reconstruct the basal rodent morphotype in anatomical studies (Wöhrmann-Repenning 1982; Ade 1999; Frahnert 1999; Mess 1999). The squirrel-related clade comprises three families: the Sciuridae (squirrels), Gliridae (dormice), and Aplodontidae (mountain beavers) (Adkins et al. 2003; Blanga-Kanfi et al. 2009; Fabre et al. 2012). Phylogenetic

analyses are based on morphological (Lavocat and Parent 1985; Vianey-Liaud 1985) and molecular studies (Adkins et al. 2003; Blanga-Kanfi et al. 2009), revealing contradicting results. A sister-group relationship between sciurids and apodontids is supported by immunological studies (Sarich 1985) and anatomical investigations of the middle ear (Lavocat and Parent 1985; Vianey-Liaud 1985), which also is supported by molecular investigations (Adkins et al. 2003; Blanga-Kanfi et al. 2009). This contradicts the anatomical similarities of the cephalic artery pattern in more basal glirids (*Eliomys* and *Graphiurus*) and the genus *Sciurus* (Bugge 1974) and implies a closer relationship. We consider, therefore, the comparative anatomy of the middle ear region as an important step towards resolving contradictory phylogenetic hypotheses of molecular and morphological studies in the squirrel-related clade.

Sciurids represent the major group of this study. Extant sciurids comprise 273 species and 50 genera in the two sub-families, Sciurinae and Pteromyinae, respectively (Thorington et al. 2002; Thorington and Hoffmann 2005). The locomotory mode (Nowak 1991) as well as dental (Major 1873) and cranial characters (Miller and Gidley 1918; Moore 1959, 1961) support the phylogenetic distinction between ground, tree, and gliding squirrels (Simpson 1945; Moore 1959). The cranial morphology (Roth and Thorington 1982) and molecular studies (Oshida et al. 1996) furthermore indicate closer relationships between tree squirrels (Sciurinae) and gliding squirrels (Pteromyinae). Ground squirrels (Xerinae) are considered to be paraphyletic (Mercer and Roth 2003).

In this study, we developed the “septal compass” and the “septal formula”, to solve phylogenetic controversies between the families of the squirrel-related clade. This new approach enables a better comparability of morphological differences in the intrabullar anatomy of the investigated taxa. Furthermore, our approach can easily be modified and used for further investigations of other groups of mammals, and additionally allows the assignment of isolated and fossilized bullae tympanicae of rodents to the respective species.

Materials

In a total, the middle ear region of 38 extant species representing 28 genera of sciurids, five genera of glirids, and the last living representative of apodontids, *Aplodontia rufa*, were investigated (Table S1). The generic arrangement of the extant squirrel-related clade taxa is based on molecular studies (Mercer and Roth 2003; Herron et al. 2005). Additionally, we included fossil specimens of sciurids, which were assigned according to Vianey-Liaud (1974) and McKenna and Bell (1997): †*Palaeosciurus feignouxi* from the Miocene (MN2, Aquitanian) of France, representing the earliest known sciurid with a sciuriform masseter muscle topography (Vianey-Liaud

1974), †*Heteroxerus costatus* from the Oligocene (MP28, Chattian) of southern Germany, and †*Urocitellus undulatus* from the Upper Pleistocene of Siberia. For elucidating the ancestral character state for the squirrel-related clade, †*Ischyromys typus* from the Oligocene (MP21, Orellan) of North America and 12 species of Lagomorpha representing both living families (Leporidae, Ochotonidae) were included as outgroups. In order to record the intraspecific morphological variability of bony septum distribution, we examined the middle ear region of 18 specimens of *Sciurus vulgaris* (Table S1).

Methods

The right middle ear region was studied non-destructively by using high-resolution computed tomography (μ CT). Specimens of the squirrel-related clade were scanned with the μ CT device v|tome|x s (GE phoenix|x-ray) at the Steinmann-Institut für Geologie, Mineralogie und Paläontologie, Universität Bonn, Germany. The specimen of †*I. typus* was scanned with a SkyScan 1173 (Bruker) at the Department of Palaeontology, University of Vienna, Austria. *Nesolagus netscheri* was scanned with a SkyScan 1172 (Bruker) at the Netherlands Centre for Biodiversity Naturalis, Leiden, the Netherlands. The middle ear region was virtually opened by using several different orientated “clipping planes” of the reconstruction software Avizo 6.1.1[®] (Visualization Sciences Group) or, alternatively, by “oblique slice” of the reconstruction software Amira 5.4.1[®] (Visualization Sciences Group).

We subsequently mapped the distribution patterns of the septa on previous phylogenies (Vianey-Liaud 1974; Harrison et al. 2003; Huchon et al. 2002; Mercer and Roth 2003; Marivaux et al. 2004; Herron et al. 2005; Blanga-Kanfi et al. 2009). Ancestral character states of the septal distribution between the families and tribes were reconstructed employing parsimony analysis implemented in Mesquite 3.02 (Maddison and Maddison 2015). A phylogenetic analysis of sciurids based on ear features, however, is beyond the scope of this paper due to the small number of characters compared to 45 taxa (Wiley et al. 1991), which ultimately would result in a high amount of polytomies (Schwarz 2012).

Septal compass

The “septal compass” displays the graphical representation of the anatomy of the middle ear region. In the center of the septal compass, the virtually opened cochlea with the respective cochlea turns is represented (Fig. 1). If a bony septum attaches medially in the tympanic cavity, it is drawn with a dashed line. If the septum attaches laterally on the inner surface of the auditory bulla, it is drawn with a solid line. The bony canal of the stapedia artery (if present) is illustrated by a circled cross.

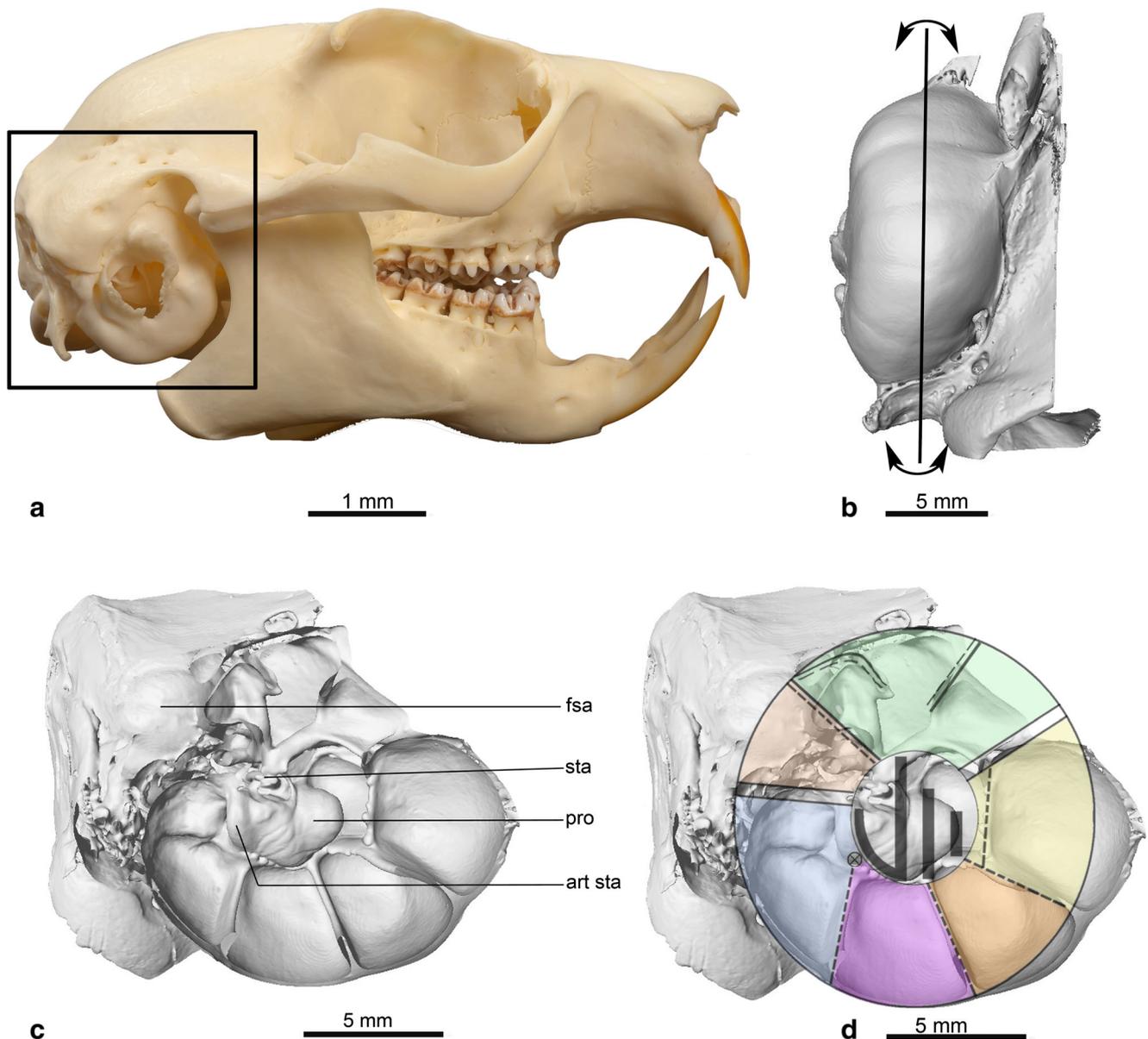


Fig. 1 Application of the septal compass. **a** Skull and right middle ear region in lateral view of *Petaurista petaurista*. **b** The right ear region of *Euxerus erythropus* in ventral view. The relative position of all septa is gathered by using several different orientated clipping planes. **c** Lateral

wall of the ear region of *Euxerus erythropus* is removed. **d** “Septal compass”. *art sta* arteria stapediales (=stapedial artery), *fsa* fossa subarcuata (=subarcuate fossa), *sta* stapes, *pro* promontorium, circled cross represents the position of stapedial artery

Classification of bony septa

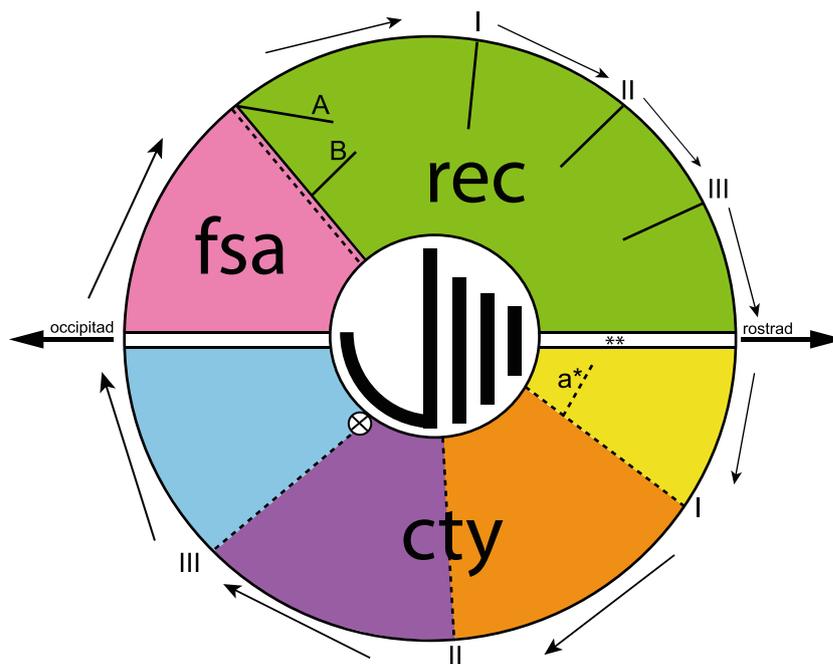
Bony septa are classified according to their orientation, position, and attachment line in the epitympanic recess and in the tympanic cavity. However, the size and position of the septa are schematized in the septal compass to provide a user-optimized simplified topography of the middle ear cavity (Figs. 1 and 2).

“Primary septa” proceed radially along the dorsal margin of the epitympanic recess and the ventral wall of the tympanic cavity towards the promontorium and are consecutively numbered by Roman numerals. The reading direction starts at the

subarcuate fossa and proceeds clockwise. If the subarcuate fossa is not seen from lateral view, the reading direction starts at the most occipital position (Figs. 1 and 2).

“Secondary septa” never proceed towards the promontorium, as it is postulated for “primary septa”. Letters number “secondary septa”: upper case letters are used in the epitympanic recess and lower case letters in the tympanic cavity. The “secondary septum A” lies between the dorsal margin of the epitympanic recess and the margin of the subarcuate fossa. The “secondary septum B” of the epitympanic recess aligns with the septum A towards the promontorium (Fig. 2).

Fig. 2 Septal compass: general graphical illustration of the anatomy of the right middle ear region with the relative position of the primary septa (I–III) and the secondary septa (A, B, a). One asterisk (a*) indicates the “lateral secondary septum” of MacPhee (1981), two asterisks (**) indicate the “anterior septum” of Wible (2009). *cty* cavum tympani (=tympanic cavity), *fsa* fossa subarcuata (=subarcuate fossa), *rec* recessus epitympanicus (=epitympanic recess)



Septal formula

The “septal formula” corresponds to a numeric description of the topography of the bony septa in the epitympanic recess and the tympanic cavity in addition to the septal compass. The numerator or top of the fraction describes the dorsal septa inside the epitympanic recess, whereas the denominator or bottom of the fraction describes the ventral septa of the tympanic cavity. The expansion of the epitympanic recess is depicted with the symbols for “greater than” (>) and “smaller than” (<). “Greater than” represents the expansion of the epitympanic recess to the occipital bone (e.g., >Re I,II,III/a,I,II,III), whereas “smaller than” represents the possibility of a lateral cutting plane to open the subarcuate fossa (e.g., <Re A,B,I,II,III/a,I,II,III).

Results

Illustrations of the septal compasses of representative species and the septal formula of all investigated taxa are found in the supplementary material (Fig. S1–S4). The ancestral character state reconstructions of the septal compass of the tribes are illustrated in the phylogenetic tree (Fig. 3) and, additionally, listed in the supplementary material (Table S3).

Sciuridae

The middle ear region of the investigated sciurids shows similarities and differences in the occurrence of the bony septa in the epitympanic recess and the tympanic cavity at the genus level. Based on the ancestral character analysis, the ancestral

character state of the intrabullar septa for Sciuridae is a primary septum I and a secondary septum A in the epitympanic recess and in the tympanic cavity the secondary septum a and the primary septa I and II (>Re A,I,II/a,I,II; Fig. 3).

Marmotini All investigated species of Marmotini possess a secondary septum a and the primary septa I and II in the tympanic cavity (Fig. 3; Table S2). However, the distribution of septa in the epitympanic recess is not uniform. *Notocitellus annulatus*, *Callospermophilus lateralis*, *Spermophilus citellus*, and *Spermophilus erythrogegens* are characterized by the primary septum I and the secondary septum A in the epitympanic recess (>Re A,I/a,I,II). Additionally, *S. citellus* displays a primary septum III in the tympanic cavity (>Re A,I,II/a,I,II,III). Both extant and extinct *U. undulatus* and *Spermophilus suslicus* possess the secondary septum B in the epitympanic recess (>Re B,I/a,I,II). The epitympanic recesses of the investigated *Cynomys* species are characterized by an additional septum representing the primary septum II. These two species can be differentiated by the occurrence of the dorsal secondary septum A, which occurs in the middle ear of *Cynomys leucurus* (>Re A,B,I,II/a,I,II), but not in the middle ear of *Cynomys ludovicianus* (>Re B,I,II/a,I,II).

Tamiini The investigated species of Tamiini, *Neotamias townsendii* and *Eutamias sibiricus*, display a secondary septum A and a primary septum I in the epitympanic recess (Fig. 3; Fig. S1 A, B; Table S2). Additionally, *N. townsendii* possess a dorsally laying primary septum II. In the tympanic cavity, both species possess the secondary septum a and the primary septa I and II (>Re A,I/a,I,II).

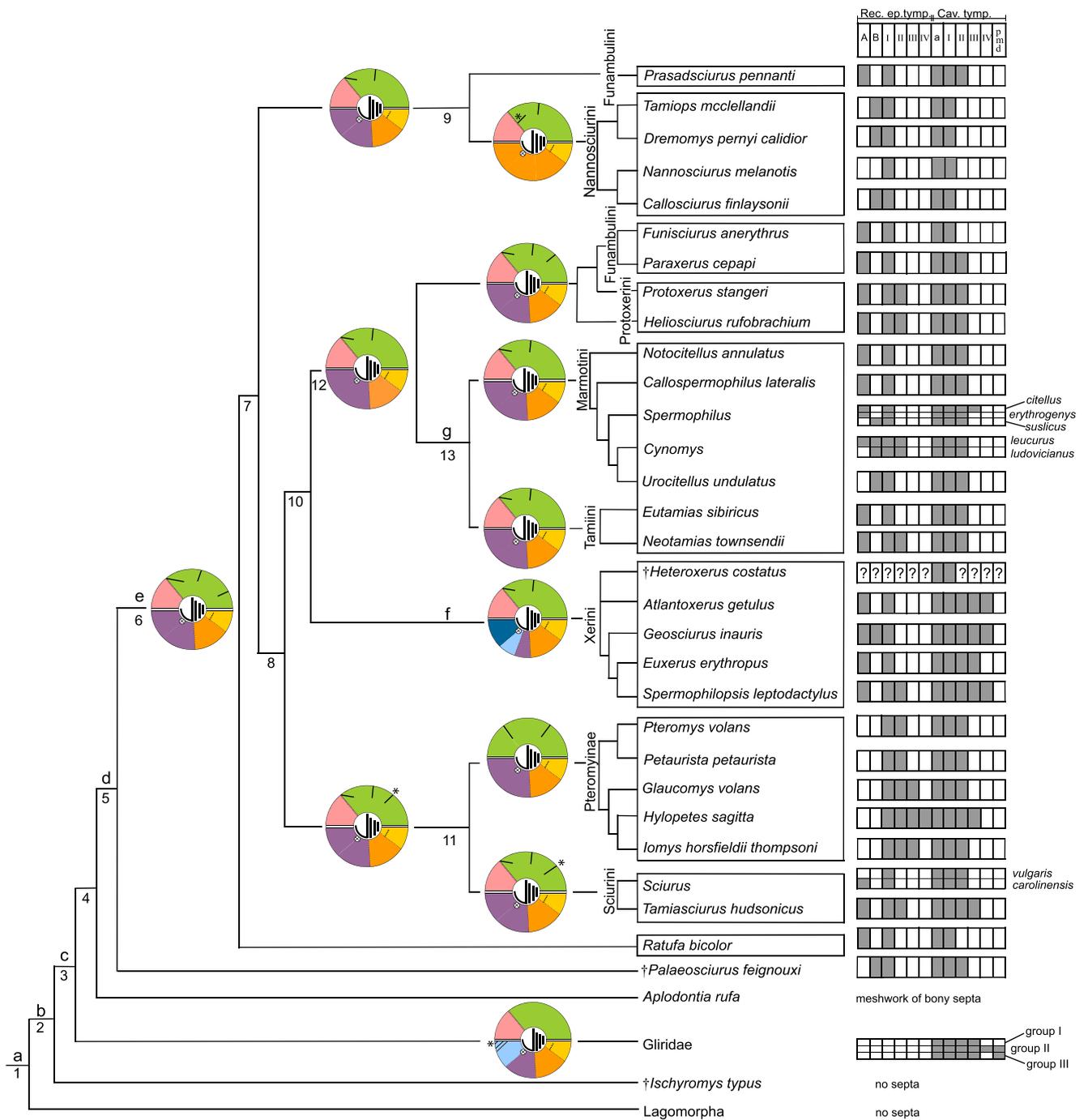


Fig. 3 Patterns of “primary septa” and “secondary septa” in the epitympanic recess and the tympanic cavity in the squirrel-related clade, †*Palaeosciurus feignouxii*, †*Ischyromys typus*, and investigated outgroups: *pmd* posterior medial diverticulum, *filled square* character present, *white square* character absent, *question mark* character not preserved. Species of investigated glirids: group I=*Glis glis*; group II=*Dryomys nitedula intermedius*, *Eliomys quercinus*, and *Graphiurus parvus*; group III=*Muscardinus avellanarius*. Schemas of septal compass

represent ancestral character states; *asterisk* character is stated as present and absent in the ancestral character state analysis (Mesquite 3.02). Source of splits: *a* Huchon et al. (2002), *b* Marivaux et al. (2004), *c* Blanga-Kanfi et al. (2009), *d* Vianey-Liaud (1974), *e* Mercer and Roth (2003), *f* Herron et al. (2005), *g* Harrison et al. (2003). Number of nodes correspond to septal ancestral character states (see supplementary material Table S3)

Funambulini All investigated taxa are characterized by the dorsally positioned secondary septum A and primary septum I (Fig. 3; Table S2). The ventrally located

primary septum II is found in all funambulins (>Re A, I/a,I,II; Fig. S1 C, D), except in *Funisciurus anerythrus* (>Re A,I/a,I).

Protoxerini All of the investigated taxa of Protoxerini possess the same number of septa in the middle ear region (Fig. 3; Table S2). Species can be characterized by the secondary septum A and the primary septa I and II in the epitympanic recess and the secondary septum a and the primary septa I and II in the tympanic cavity (>Re A,I,II/a,I,II).

Xerini The investigated taxa of Xerini show the highest number of ventrally positioned primary septa in the middle ear cavity of all examined sciurids (Fig. 3; Table S2). In the dorsal epitympanic recess, all members of this tribe are characterized by a secondary septum A. Additionally, *Geosciurus inauris* possesses a secondary septum B (>Re A,B,I/a,I,II,III,IV) and *Spermophilopsis leptodactylus* a primary septum II (>Re A,I,II/a,I,II,III,IV). Except for *Euxerus erythropus* (>Re A,I/a,I,II,III), Xerini have four primary septa in the tympanic cavity. The epitympanic recess of the investigated specimen of †*H. costatus* is partly destroyed; a secondary septum a and a primary septum I can be identified ventrally in the tympanic cavity (?/a,I,?).

Pteromyini The epitympanic recess is enlarged in latero-occipital direction in all investigated taxa of Pteromyini. Based on this anatomy and the corresponding definition of the septal compass, no dorsal secondary septa can be found in gliding sciurids (Fig. 3; Table S2; Fig. S1 E, F). The spatial arrangement of the dorsal and ventral primary septa discriminates the investigated taxa. The epitympanic recess and the tympanic cavity of *Pteromys volans* and *Petaurista petaurista* are characterized by the primary septa I and II (<Re I,II/a,I,II), whereas the middle ear regions of *Glaucomys volans* and *Iomys horsfieldii thompsoni* display the dorsal primary septa I to III and ventral primary septa I and II (<Re I,II,III/a,I,II). *Hylopetes sagitta* displays the highest number of septa of all investigated pteromyines comprising four primary septa in the epitympanic recess and three primary septa in the tympanic cavity (<Re I,II,III,IV/a,I,II,III; Fig. S1 E, F).

Sciurini In this tribe, the investigated taxa of the genus *Sciurus* show the secondary septum a and the primary septa I and II in the tympanic cavity (Fig. 3; Table S2). The investigated specimen of *Tamiasciurus hudsonicus* possesses a secondary septum A and the primary septa I and II in the epitympanic recess. Additionally, a secondary septum a and the primary septa I to III are found in the tympanic cavity (>Re A,I,II/a,I,II,III).

Nannosciurini The investigated taxa of Nannosciurini have the lowest number of septa of all investigated species in this study (>Re I/a,I; Fig. 3; Table S2). Except for *Nannosciurus melanotis*, all studied species of this tribe show dorsally the secondary septum B and the primary septum I. A secondary septum a and the primary septum I are located within the tympanic cavity (>Re B,I/a,I).

Ratufini The investigated species of this tribe, *Ratufa bicolor*, possesses a secondary septum A and the primary septum I in the epitympanic recess (Fig. 3; Table S2). In the tympanic cavity, the secondary septum a and the primary septum I are present (>Re A,I/a,I; Fig. S2 I, J).

†*Palaeosciurus feignouxi* The auditory region of the investigated specimen of †*P. feignouxi* is characterized by the secondary septum B and the primary septum I in the epitympanic recess (Fig. 3; Table S2). In the tympanic cavity, we observe the primary septa I and II and, additionally, the secondary septum a (>Re B, I/a,I,II).

Aplodontidae

A spongy meshwork of bony trabeculae characterizes the entire middle ear region in all investigated specimens of *A. rufa* (Fig. S2 A). The septal compass and the septal formula cannot be applied.

Gliridae

None of the investigated taxa of glirids possesses septa in the epitympanic recess (Fig. 3; Table S2). The ancestral character state of the septa distribution for glirids are the primary ventrally lying septa I to III and the secondary septum a (>Re -/a,I,II,III^{pmd}). The appearance of the posterior medial diverticulum (pmd) is coded as two character states (present, absent). The number of bony septa and the occurrence of a posterior medial diverticulum distinguish three groups of middle ear cavities within this group:

Group I: *Glis glis* is characterized by three ventrally positioned primary septa (>Re -/a,I,II,III; Fig. S2 B, C).

Group II: The investigated specimens of *Eliomys quercinus*, *Dryomys nitedula intermedius*, and *Graphiurus parvus* (Table S2, Fig. S2 D, E) show the same pattern as *G. glis*. Additionally, these species possess a posterior diverticulum, similar to the condition found in *Muscardinus avellanarius*.

Group III: The middle ear cavity of *M. avellanarius* houses four primary septa in the tympanic cavity and has an additional posterior diverticulum (>Re -/a,I,II,III,IV^{pmd}; Fig. S3 A, B).

Outgroups

Investigated specimens of †*Ischyromys typus* and lagomorphs entirely lack septa in the middle ear region (Figs. S3 E, S4 A, B, Table S3). The anatomy of the auditory bulla in lagomorphs is distinct (Fig. S4). In Ochotonidae, the auditory bulla shows

a highly spongy wall (Fig. S4 A), which also is present in †*I. typus* (Fig. S3 E), but is absent in *Oryctolagus cuniculus* (Fig. S4 B) and other leporids.

Morphological variability

We observed no variation in number and spatial arrangement of dorsal and ventral primary septa and the secondary septum a of the tympanic cavity in 20 specimens of *S. vulgaris* (Table S2). We were not able to clearly determine the occurrence of the dorsally situated secondary septa A and B in all of the studied individuals of *S. vulgaris*.

Discussion

It was possible to distinguish the intrabullar septal patterns of the middle ear region in all investigated species of the squirrel-related clade (Sciuridae, Gliridae, *A. rufa*) as well as in the outgroup taxa (†*I. typus*, Lagomorpha) by our new approach of the “septal compass” and the “septal formula.” Septum combinations are highly conservative and specific to species, genus, and family levels (Fig. 3, Table S2). Consequently, we were able to distinguish higher taxonomic levels and elucidate phylogenetic relationships easily with this simplified method.

The septal distribution pattern of the primary septa and the secondary septum a among sciurids and glirids are morphologically different from the bony meshwork of *A. rufa* as it is indicated in the ancestral character analyses using Mesquite (Fig. 3, Table S3). A secondary septum a and the primary septa I and II of the tympanic cavity are representing the ancestral character states for sciurids and glirids and support their sister group relationships (>Re A,I,II/a,I,II). This also is supported by comparative studies of the molars (Stehlin and Schaub 1951), the cranial artery pattern (Bugge 1974), the shape of the auditory ossicles (Farr and Mason 2008), and, as demonstrated here, in the septal pattern of the middle ear region. However, this hypothesis contradicts results from molecular analyses (DeBry and Sagel 2001; Huchon et al. 2002; Adkins et al. 2003; Blanga-Kanfi et al. 2009), immunological information (Sarich 1985), as well as the artery pattern in the middle ear region (Lavocat and Parent 1985; Vianey-Liaud 1985). According to these studies, aplodontids are closely related to sciurids forming the clade of Sciuroidea, which is not supported by our analyses.

However, a functional relevance of a subterranean lifestyle for *A. rufa* cannot be entirely excluded, as a general functional correlation between a higher number of septa and enhanced lower frequency perception was proposed by several previous studies (e.g., Lay 1972; Webster and Webster 1975; Parent 1980; Lange et al. 2007; Begall and Burda 2006). For instance, the investigated fossorial Xerini possess more primary

and secondary septa in the tympanic cavity (>Re A,I/a,I,II,III, IV) than arboreal species of Sciurini (>Re A,I,(II)/a,I), Tamiini (>Re A,I, (II)/a,I,II), Protoxerini, Funambulini, Nannosciurini (>Re (B),I/a,I), and Ratufini (>Re A,I/a,I), a pattern that supports the abovementioned correlation. However, it remains unclear whether this anatomy of a bony meshwork of septa, as observed in *A. rufa*, represents adaptations to hearing low frequencies in taxa with a subterranean lifestyle. The other possibility is that it reflects a phylogenetic heritage that cannot be linked to a specific habitat, as already suggested by Fleischer (1978). This needs to be investigated in future studies with additional subterranean taxa (e.g., bathyergids, spalacids).

Although, we can resolve higher taxonomic hypotheses of the squirrel-related clade and between investigated taxa, the phylogenetic relationship and evolutionary history between the tribes of Sciuridae cannot be resolved without additional anatomical characters. The studied Funambulini, *Prasadscurus pennanti* and *Paraxerus cepapi*, are both characterized by an identical septal pattern (>Re A,I/a,I,II; Fig. 3). This suggests closer relationships between these two genera, which contradicts molecular studies (Mercer and Roth 2003; Steppan et al. 2004). The middle ear region of the investigated members of Xerini shows the highest number of ventrally situated primary septa in all examined sciurids with an ancestral septal formula of >Re A,I/a,I,II,III,IV (Fig. 3). This supports the monophyly of Xerini, which is controversially to molecular results (Mercer and Roth 2003). In Pteromyini, the epitympanic recess is enlarged in the latero-occipital direction, which has already been described by van Kampen (1905). Therefore, the middle ear region of gliding sciurids possesses primary septa in the epitympanic recess, whereas no secondary septa are present (Fig. 3). This character state identifies pteromyines as a monophyletic group with the septal formula of <Re I,II/a,I,II. This also is supported by morphological investigations of the postcranium (Thorington et al. 1997; Thorington and Darrow 2000) and molecular analyses (Mercer and Roth 2003; Steppan et al. 2004; Blanga-Kanfi et al. 2009). In the studied Nannosciurini, we observed the same number of bony septa in the middle ear region of *Tamiopsis mccllellandii*, *Dremomys pernyi calidior*, and *Callosciurus finlaysonii* displaying the ancestral septal formula of >Re B, I/a,I. *N. melanotis* has the lowest number of septa of all investigated sciurids, which may represent a unique autapomorphic pattern and implies a secondary loss of bony septa within sciurids (>Re I/a,I). The septal pattern of the auditory region of †*P. feignouxi* also is seen in the investigated specimens of *U. undulatus*, but also in *S. suslicus*. This might indicate that †*P. feignouxi* belongs to the tribe Marmotini, which already was hypothesized by McKenna and Bell (1997).

Potapova (2001) also studied the middle ear region of glirids and distinguished different types of pneumatization in the mastoid region of glirids compared to other rodents with

enlarged bulla, e.g., gerbillids, dipodids, and heteromyids. She postulated closer relationships between *Dryomys* and *Eliomys*, which also is supported by our observations of the septal pattern in the middle ear cavity and the common septal formula consisting of $>Re -/a, I, II, III^{pmd}$ (Fig. 3). The middle ear of *G. parvus* is characterized by an arrangement of septa and diverticula (Potapova 2001), which also is present in the other investigated glirid species. The genus *Graphiurus* clearly is a representative of glirids, which already is indicated by the pseudohystricomorph topology of the masseter muscle (Maier et al. 2002), but is in contrast to the distinct anatomy of the teeth (Wahlert et al. 1993).

The middle ear regions of all investigated lagomorphs and †*I. typus* lack any intrabullar septa, a pattern also described for Capromyinae, Hystricidae, Erethizontidae, Lagostomidae, Dasyproctidae, Dinomyidae, as well as Caviidae (Winge 1888 cited by van Kampen 1905), Muridae except for Microtinae (Tullberg 1899), *Dipus jaculus*, and *Pedetes caffer* (van Kampen 1905). The lack of bony septa may represent a plesiomorphic character state of Rodentia, which has to be verified in further investigations.

The presence of septa A und B of the epitympanic recess in *S. vulgaris* is highly variable and contradicts the conservative anatomy of the intrabullar septal pattern of the tympanic cavity. However, it remains ambiguous whether this represents ontogenetic variability of the epitympanic recess in which the surrounding bones and mesenchyme become absorbed (Van Kampen 1905), or is caused by the position of the head of the malleus and the short crus of the incus inside this cavity (Fleischer 1973). The latter may influence the surrounding space and, as a consequence, the variability and presence of secondary septa A and B. Nevertheless, the variability of septa A and B and the homology of the intrabullar septa need to be investigated in further studies focussing on auditory bulla morphogenesis.

In conclusion, the occurrence of bony septa in the middle ear cavity of the squirrel-related clade bears highly informative phylogenetic signals. Primary septa can be distinguished from secondary septa in their position and spatial direction in the epitympanic recess and the tympanic cavity, and certain patterns are restricted to specific taxon levels. Both types of septa can be mapped by using the “septal compass” and “septal formula,” which were developed in this study and may also be applied to other extant and extinct rodent taxa as well as to other mammalian orders such as Carnivora.

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