High morphological variation of vestibular system accompanies slow and infrequent locomotion in three-toed sloths

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The semicircular canals (SCs), part of the vestibular apparatus of the inner ear, are directly involved in the detection of angular motion of the head for maintaining balance, and exhibit adaptive patterns for locomotor behaviour. Consequently, they are generally believed to show low levels of intraspecific morphological variation, but few studies have investigated this assumption. On the basis of high-resolution computed tomography, we present here, to our knowledge, the first comprehensive study of the pattern of variation of the inner ear with a focus on Xenarthra. Our study demonstrates that extant three-toed sloths show a high level of morphological variation of the bony labyrinth of the inner ear. Especially, the variation in shape, relative size and angles of their SCs greatly differ from those of other, faster-moving taxa within Xenarthra and Placentalia in general. The unique pattern of variation in three-toed sloths suggests that a release of selection and/or constraints on their organ of balance is associated with the observed wide range of phenotypes. This release is coincident with their slow and infrequent locomotion and may be related, among other possible factors, to a reduced functional demand for a precise sensitivity to movement.

Keywords: inner ear; shape; variability; constraint; Xenarthra; semicircular canals

1. INTRODUCTION

The semicircular canal (SC) system constitutes a major part of the organ of balance in the inner ear of vertebrates and is primarily specialized for sensitivity to rotational acceleration/deceleration of the head [1–3]. Accordingly, its bony morphology presents diverse morphological patterns correlated to the modes of locomotion and agility [1,2,4–6]. A common assumption is that, notably owing to adaptive constraints, the morphology of the mammalian inner ear, especially the SC, shows low levels of intraspecific variation [7–11]. However, possible fluctuations of these levels of intraspecific variation have never been widely investigated or compared with locomotor behaviours.

Extant sloths in the genera Bradypus (three-toed) and Choloepus (two-toed) offer an opportunity to address this question. They are unique among extant placentals in exhibiting an activity pattern with very infrequent and slow motion. Their daily trips rarely exceed 100 m [12], and average speed does not exceed 0.5–0.6 km h⁻¹ [13–15]. A few studies have previously investigated a possible relationship between these species’ vestibular system morphology and their slow behaviour. As long ago as 1907, Gray [16] noted an irregular shape and small size of the SC in one specimen attributed to Bradypus tridactylus and hypothesized that this ‘may be in some way related to the sloth’s clumsy and slow movements’. More recent studies have focused on the size of the SC in sloths [5,17], but their previously reported irregular shape has not been closely investigated to date.

Our study explores SC morphology in sloths and asks whether their irregular shape is part of a larger pattern of variability. Indeed, Darwin ([18], p. 455) hypothesized that ‘an organ, when rendered useless, may well be variable, for its variations cannot be checked by natural selection’. Links between reduced function, relaxed natural selection and greater variation has been acknowledged by more recent studies [19] and demonstrated to characterize certain vestigial traits of insects [20]. More distantly related but still relevant examples of reduced selection and increased variation include certain birds and fishes, which show greater sperm morphological variation coincident with a release of sexual selection [21,22]. In addition, reduced competition in Garter snakes may have increased variation heritability of colour patterns [23]. Accordingly, one would expect to find among sloths a substantial degree of morphological variation in their SC, because significant travelling distances, speed and agility are not part of their locomotor repertoire. In this context, sloths constitute an ideal case to address Darwin’s hypothesis.

Using a large dataset of inner ear bony labyrinths, we compare the pattern of variation exhibited by the SC in sloths (especially three-toed sloths) to that of faster-moving species in order to test the possibility that lethargy in sloths...
corresponds to their patterns of SC variation. Our results demonstrate that three-toed sloths depart from the low levels of morphological variation of the SC system observed in other xenarthrans and placentals. Echoing Darwin's hypothesis, we discuss the probability of a relaxed selective pressure on the balance organ as a partial cause explaining the persistence of these varying SC morphologies, and tentatively relate it to three-toed sloths' slow behaviour. This hypothesis, amenable to further testing, suggests that even highly conservative organs such as the inner ear may exhibit substantial morphological variation when functional demand is decreased.

2. MATERIAL AND METHODS

We investigated the shape of the bony labyrinth of the inner ear by high-resolution computed tomography in a total of 65 adult specimens: 33 extant xenarthran skulls representing nine genera and 14 species, and 32 other skulls for two well-sampled species outside Xenarthra (the red squirrel *Sciurus vulgaris* and European mole *Talpa europaea*) for outgroup comparison (see some selected specimens in figure 1; and see the electronic supplementary material). Species and subspecies identification is based on collection data, geographical origin and cranial anatomy [24–26]. The bony labyrinths were imaged using high-resolution computed microtomography ($\mu$CT). Three-dimensional reconstruction and visualization of the bony labyrinth were performed using stacks of digital $\mu$CT images with Avizo v. 6.1.1 software (Visualization Sciences Group). We then assessed the morphological variation from values and ratios of linear and angular measurements taken directly from virtual endocasts of bony labyrinths, which reflect the geometrical attributes of the membranous semicircular ducts [2,27].

Measurements were taken on the SC as well as on other parts of the bony labyrinth, such as the cochlea, in order to look for distinctive variation levels between different inner ear subsets and especially to address the assumption that high variation would be more concentrated on the SC in parts of the bony labyrinth, such as the cochlea, in order to look for distinctive variation levels between different inner ear subsets and especially to address the assumption that high variation would be more concentrated on the SC in sloths. The following linear and angular measurements were taken with the software POLYWORKS v. 10.0.5 (Innov-Metric Inc.) on different parts of bony labyrinth endocasts (see the electronic supplementary material, figure S1):

- height (Coc H) of cochlea in profile, 
- width (Coc W) of cochlea in profile, 
- number of cochlear canal turns (TC), 
- length of cochlea (Coc L), 
- cube root of entire inner ear volume (R3Vol), 
- mean thickness of SC ducts (SC D), 
- length and width diameters of SC arc (ASC L, ASC W, PSC L, PSC W, LSC L, LSC W), 
- SC radii (ASC R, PSC R, LSC R; R = half the average of the arc length and width diameters), 
- inner perimeter of area enclosed by SC (ASC P, PSC P, LSC P), 
- vestibule width (VW) = maximal distance in dorsal view between anterior and posterior ampullae, 
- vestibule height (VH) = shortest distance from the line joining anterior and posterior ampullae (VW) to the posteroverentralmost point of the fenestra vestibuli, and 
- angles ASC/PSC, ASC/LSC, PSC/LSC, cochlea/LSC; with ASC, PSC and LSC being the respective abbreviations for anterior, posterior and lateral SCs.

Figure 1. Bony labyrinth morphology and variation pattern in selected xenarthrans and *Sciurus*. (a) Ventral view (anterior to the top) of the skull of *Bradypus variegatus* ZFMK 60157 (see the electronic supplementary material). (b) Ventral view (anterior to the top) of virtually reconstructed transparent basicranium and bony labyrinths of same specimen. (c) Ventrolateral view (anterior to the right) of isolated left bony labyrinth of same specimen. (d)–(w) Views of bony labyrinths showing profile of anterior and lateral SCs and illustrating greater SC variation in *B. variegatus* (d)–(h), than in *Tamandua tetradactyla* (i)–(m), *Dasypus novemcinctus* (n)–(r) and *Sciurus vulgaris* (s)–(w). In *D. novemcinctus*, (n)–(p) correspond to subspecies *mexicanus* and (q) and (r) to subspecies *novemcinctus*; in *S. vulgaris*, (s) corresponds to subspecies *infuscatus*, (t) and (u) to *fuscater* and (v) and (w) to *vulgaris* (see the electronic supplementary material). (d)–(w) not to scale. ASC, anterior semicircular canal; PSC, posterior semicircular canal; LSC, lateral semicircular canal;
Several ratios were calculated from these measurements in order to standardize the data and compare proportions of different inner ear parts (see the electronic supplementary material, dataset S1). Ratios are:

- **R1.** ASC L/ASC W (R1a), PSC L/ PSC W (R1b), LSC L/LSC W (R1c);
- **R2.** ASC P/ PSC P (R2a), ASC P/LSC P (R2b), PSC P/ LSC P (R2c);
- **R3.** SC D / mean SC R;
- **R4.** Coc H/Coc W;
- **R5.** Coc L/R3Vol; and
- **R6.** VW/VH.

Also TC noted as R7 in figures and datasets (see details of earlier-mentioned abbreviations).

Principal component analysis (PCA) and the calculation of variation indices allowed us to visualize and quantify the amount of variability in our sample. PCA (see the electronic supplementary material, dataset S2) was performed in PAST v. 2.06 on a set of 54 specimens (excluding Tal. europaea §2) in each species. Asterisks (*) denote the absence of data for Tal. europaea (see §2).

The calculated SAAD demonstrates that, in comparison with the nine-banded armadillo, southern tamandua (Tamandua tetradactyla), red squirrel and European mole, three-toed sloths always exhibit the highest measured intraspecific variation, i.e. highest SAAD values, for each investigated character of the bony labyrinth (figure 2). For the SC characters, the three-toed sloths show an amount of variability always twice or more than that of most other taxa. Other structures such as the vestibule (also related to balance with the detection of linear acceleration/deceleration) and the cochlea (related to hearing) are also variable in three-toed sloths. This variation mostly concerns the vestibule and cochlear canal coiling shape (R6, R4 and R7). By contrast, three-toed sloths show a less variable vestibule and cochlear canal coiling shape (R6, R4 and R7) for almost all measured bony labyrinth characters (Levene’s test, p < 0.05). Three-toed sloths also exhibit significantly higher variation than nine-banded armadillos (Dasypus novemcinctus) for eight out of 10 characters of the SC but not for the cochlea (Levene’s test, p < 0.05). Conversely, non-sloths do not show significantly different levels of variation for most of the SC and cochlear characters (Levene’s test, p > 0.05).

Some measurements could not be performed in Tal. europaea concerning the angle made between the ASC and other SCs, owing to the overly high planar deviation of the former, and concerning R3 and R5, which were not possible to estimate confidently owing to insufficient precision on the thickness of structures on the reconstructed endocasts due to low scan resolution.

### 3. RESULTS

Data from our best-sampled species indicate that three-toed sloths (Bradypus variegatus) present a significantly higher level of variation than red squirrels (Sciurus vulgaris) and European moles (Talpa europaea) for almost all measured bony labyrinth characters (Levene’s test, p < 0.05). Three-toed sloths also exhibit significantly higher variation than nine-banded armadillos (Dasypus novemcinctus) for almost all measured bony labyrinth characters (Levene’s test, p < 0.05) present a significantly higher level of variation than red squirrels (Sciurus vulgaris) and European moles (Talpa europaea) for almost all measured bony labyrinth characters (Levene’s test, p < 0.05).}

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significant morphological differentiation between the

ASC/LSC, R2a, R2b, R2c. MANOV AS indicate

angle LSC/PSC, R1a, R1b, R1c and R3; negatively with

second axis is positively correlated with angle ASC/PSC,

principal component is positively correlated with angle

thran taxa represented by fewer specimens. The first

xenarthrans. The area shaded in red delimits variation exhibited across individuals of B. variegatus (Brad_v); purple indicates D. novemcinctus (Das_n); green indicates Tam. tetradactyla (Tam_t); and yellow indicates S. vulgaris. Dashed and solid lines delineate xenarthran subordinal or familial groups (Folivora, Vermilingua and Dasypodidae) and the species S. vulgaris. Other taxonomic abbreviations are B. tridactyla (Brad_t), Choloepus didactylus (Cho_d), C. hoffmani (Cho_h), Cyclopes didactylus (Cyc_d), Myrmecophaga tridactyla (Mym_t), Dasyus hybridus (Das_h), Chaetophractus vellerosus (Cha_v), Euphractus sexcinctus (Eup_s), Chlamyphorus truncatus (Chl_t), Priodontes maximus (Pri_m), Tolypeutes matacus (Tol_m).

Figure 3. Shape differentiation of the bony labyrinth of xenarthrans on the first two axes (60% of the among group variance) of the PCA performed on a set of 10 variables on the SCs and 54 specimens (axes 3 and 4 available in the electronic supplementary material, figure S3). Data points are labelled as follows: squares, sloths; crosses, armadillos; triangles, anteaters; circles, red squirrels. The area shaded in red delimits variation exhibited across individuals of B. variegatus (Brad_v); purple indicates D. novemcinctus (Das_n); green indicates Tam. tetradactyla (Tam_t); and yellow indicates S. vulgaris. Dashed and solid lines delineate xenarthran subordinal or familial groups (Folivora, Vermilingua and Dasypodidae) and the species S. vulgaris. Other taxonomic abbreviations are B. tridactyla (Brad_t), Choloepus didactylus (Cho_d), C. hoffmani (Cho_h), Cyclopes didactylus (Cyc_d), Myrmecophaga tridactyla (Mym_t), Dasyus hybridus (Das_h), Chaetophractus vellerosus (Cha_v), Euphractus sexcinctus (Eup_s), Chlamyphorus truncatus (Chl_t), Priodontes maximus (Pri_m), Tolypeutes matacus (Tol_m).

characters is much higher than the mean SAAD of cochlear characters (see the electronic supplementary material, dataset S1). Such a difference in variation between the SC and cochlea is not apparent for other taxa except for red squirrels, which show, in contrast to Bradypus, very low levels of variation for both regions. The analysis of the SRV mostly parallels the SAAD results but also demonstrates that the less well sampled two-toed sloth Choloepus hoffmani also presents substantial variation for some SC characters. Although we had access to only three specimens for this species, it nevertheless showed a large SRV of the anterior and lateral SC shape, much higher than for nonsloth species (see the electronic supplementary material, figure S2).

The PCA allowed us to examine the morphological space defined by the overall SC morphological variation and enabled us to include in our sample additional xenarthran taxa represented by fewer specimens. The first principal component is positively correlated with angle ASC/LSC, angle LSC/PSC, R1b, R1c and R2a; negatively with the angle ASC/PSC, R1a, R2b, R2c and R3. The second axis is positively correlated with angle ASC/PSC, angle LSC/PSC, R1a, R1b, R1c and R3; negatively with the angle ASC/LSC, R2a, R2b, R2c. MANOVAS indicate a significant morphological differentiation between the different suborders (Wilk’s λ-test: value = 0.003185, F = 24.57, p < 0.001). This analysis shows a large, random and scattered distribution of the specimens of three-toed sloths in the morphological space representing the SC shape (figure 3). There is no clear cluster among the seven specimens of B. variegatus (on PCA graphs with PC1–PC2 and PC3–PC4; figure 3 and see the electronic supplementary material) that would support recognition of taxonomic subsets of this species in our sample. The species B. variegatus occupies a morphological space of the SC shape larger than that of any other species sampled, and even larger than that of all specimens of armadillos or anteaters combined.

Therefore, our results demonstrate that the variation of the bony labyrinth in B. variegatus is very high and that it is especially apparent in their SC. Most of this variation concerns the overall, irregular shape of the SC, and the relative size, thickness and angles delimiting, each SC (figure 2). Such variation is clear from a visual inspection of the endocasts (figures 1 and 4). Interestingly, the high level of intraspecific variation among three-toed sloths is not apparent intra-individually. That is, our data do not indicate appreciable left-right asymmetry (see the electronic supplementary material, figure S3).

4. DISCUSSION

(a) Inner ear variation pattern compared with other placentals

Our analyses demonstrate that three-toed sloths (B. variegatus) present a much higher level of intraspecific
variation in the structure of their SC compared with other xenarthrans (D. novemcinctus, Tam. tetradactyla) and non-xenarthran placentals (S. vulgaris, Tal. europaea). Additionally, data for other well-documented mammals [8,27,30–33] support the fact that SC variation in three-toed sloths is unusually high. Neither large variation in SC shape nor differences in the relative sizes of each SC, as found in three-toed sloths (figures 1 and 4), have, to our knowledge, previously been documented (although sample sizes in previous studies rarely permit good characterization of intraspecific diversity). Another striking example concerns the 44° range of variation of the angle between the anterior and lateral SCs observed in B. variegatus (figure 4d). Our sample (n = 7) spans a range of variation for this angle comparable to that observed among multiple genera from various placentals [7], and much higher than the variation exhibited by D. novemcinctus, Tam. tetradactyla, S. vulgaris (see the electronic supplementary material, figure S2; data on this angle could not be collected for Talpa—see §2), Homo sapiens, Monodelphis domestica, Mus musculus and Cavia porcellus [27,31,34,35]. Hence, we regard high variation in the SC of three-toed sloths to be a remarkable exception to the conservatism exhibited by other mammals.

The varying morphologies of other inner ear regions in three-toed sloths might also depart from phenotype observed in other mammals (see §3 and data on cochlear coiling variation in [8]), but to a lesser extent than the SC, as shown in our sample.

(b) Constraints and selection on semicircular canal morphological variation
Considering the observed high morphological variation of their inner ear, and most specifically of their SC, we would expect three-toed sloths to exhibit a marked release of the processes normally limiting vestibular variation. Specifically, processes limiting the extent and directions of morphological variation in living organisms can be of several kinds [36–39]. Constraints that bias the production of variant phenotypes should be distinguished from selection that limits the persistence of the produced variants [39]. The pattern of selection of a morphological trait is theoretically influenced by its functional architecture, i.e. the complex relationships uniting a morphological trait with its function(s) [40]. Morphological variations in the SC system can dramatically change the animal’s temporal response dynamic (i.e. the behaviour of the cupulae with regard to time during a rotation) and sensitivity to motion [1,2], and this is probably the case in three-toed sloths. In particular, the high variability of SC relative proportions in B. variegatus (figures 2 and 4) should lead to great differences in relative sensitivity of canals among specimens. Moreover, the high variation in angles between the anatomical planes of the SC (figure 4) suggests varying directions of maximal sensitivity [2,41]. However, these morphological and functional variants of the inner ear in B. variegatus do not lead to apparent perception impairment nor are they pruned by natural selection. The persistence of such varying sensitivity patterns of the SC in three-toed sloths thus suggests that their functional architecture is not associated with a high level of selection on their shape. On the other hand, absence of left-right asymmetry in our sample of sloths, as usual in other mammals [7], indicates that intra-individual variation of the inner ear is limited, possibly in order to avoid vertigo pathophysiology [27], or as a result of a constraint at the level of individual development, which remains to be determined.

Therefore, we hypothesize that relaxed selective pressure on the SC has permitted the persistence of the observed variants in three-toed sloths. The level of genetic and/or developmental constraints on the production of these variants might also depart from that of other placentals.

(c) Phenotypic variation in sloths
The relative roles of selection and constraint in determining the degree of inner ear variation are not yet established. Whether a release of these limiting processes is restricted to certain organs such as the SC or applies to

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Figure 4. Bony labyrinth reconstructions showing the extreme cases in three-toed sloth B. variegatus of the (a), ratio of PSC P/LSC P (R2c); (b), ratio of SC P/SC P (R2a); (c), ASC aspect (R1a); (d), angle ASC/LSC; (e), angle ASC/PSC; (f), LSC aspect (R1e). Values in grey and black represent, respectively, the lower and higher extremes of the respective character. ASC, anterior semicircular canal; PSC, posterior semicircular canal; LSC, lateral semicircular canal; P, inner perimeter; L, length diameter; W, width diameter (see §2).

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most of the phenotype of three-toed sloths constitutes a critical issue. Unfortunately, the overall genetic and phenotypic variability of sloths, especially of Bradypus, is not well-known [24,42], but some data are available.

Concerning B. variegatus, the great outlier of our study, Anderson & Handley [42], previously noted variability in skull shape as a function of geographical provenance for this species. While authors usually point out the overall high variability of this three-toed sloth species, they also emphasize the lack of clearly differentiated forms, which has until now impeded the recognition of different subspecies [42,43]. Similarly, our results do not show clearly recognizable subsets in our sample for this species. It is otherwise noteworthy that the recognition of different subspecies, or even species, in B. variegatus would not be a sufficient explanation for their high SC variation, because this variation exceeds that observed in entire families of armadillos or suborders of anteaters (encompassing six different genera of Dasypodidae, and three of Vermilingua in our sample; figure 3).

In addition, substantial variation in the axial skeleton of extant sloths has also been demonstrated [44–46]. However, in our dataset, xenarthran taxa that do share a high level of vertebral thoracolumbar variability with sloths [44], such as D. novemcinctus, show a very low variation in the configuration of their SC, suggesting no association between high variation in these anatomical regions.

Interestingly, new data on the timing pattern of cranial suture closure in Xenarthra indentify both extant sloths genera as outliers, exhibiting very high variation [47]. This phenomenon might parallel that of the inner ear in sloths and, together with previous evidence, suggests that sloths could show high variation for many parts of their phenotype. The nesting of sloths within Xenarthra and Atlantogenata might already endow them with a relaxed level of constraint in patterning several characters as notably illustrated by the high variation of axial skeleton and dental eruption pattern reported in these clades [44,48,49]. However, as mentioned earlier, sloths (especially three-toed sloths) clearly constitute outliers within this clade for showing high level of intraspecific phenotypic variability of their SC.

**d. Sloth semicircular canal variation and slow locomotion**

We proposed earlier the hypothesis of a release of selective pressure on the SC in three-toed sloths to explain at least partially their morphological variation. The most plausible reason for such a released selective pressure on their SC morphology lies in their reduced activity pattern. Such high variation of the SC has not been detected in faster-moving xenarthrans, nor has it been documented in other fast-moving mammals. Furthermore, while slow locomotion and suspensory behaviour are likely to have evolved convergently in both extant genera of sloths [50–53], both display a rather highly variable configuration of the SC, even if this is much more distinctive in Bradypus than in Choloepus in our sample (figure 3 and the electronic supplementary material, figure S2). This latter fact coincides with the observation that three-toed sloths move less and more slowly than two-toed sloths [12,15]. According to our interpretation, the infrequent and slow motion of three-toed sloths could make the demand for precise detection of head angular movements relatively less important than in faster-moving animals. It is also possible that the input of the motion signal is so weak for them that a difference in the morphology of the SC does not make any significant difference for the output message sent to the brain.

Contrasting with these ideas however, Jones & Spells [54] suggested that the sensitivity required to detect slow head movements can be very demanding and increased for this purpose. On the one hand, fairly thick SC ducts, like those of most three-toed sloths, might correspond to some increase in sensitivity [54,55]. On the other hand, the general small size of the SC (radius) relative to body mass in sloths [5] and the small area they enclose do not apparently represent an increase in sensitivity [2,54,55]. Although these different possibilities are not yet fully elucidated, tolerance of varying locomotor sensitivity (e.g. directionality and relative sensitivity between canals) in three-toed sloths can still be associated with their peculiar behaviour. According to this hypothesis, slow and infrequent motion would render the wide range of morphological variation of the SC architecture more accessible to three-toed sloths. For two-toed sloths, further investigation on their pattern of SC variation is required to determine whether the same hypothesis could apply to them.

Numerous implications and prospects result from our discovery. It highlights the need for further research on the implications of SC variability on the functions of balance, which would help determine the effect of functional architecture on selection patterns for the inner ear. It also calls for further investigation detailing the pattern of variation of other anatomical structures in sloths in order to circumscribe more precisely the roles of selection and constraint. Moreover, this potential link between a decreased functional importance of the organ of balance and part of its phenotype is apparent at the species level, but not at that of the individual. In other words, the investigation of such variation requires quantification of size and shape across individuals, as does recognition of an apparent release of axial skeleton conservatism [44]. This is of primary importance as the investigation of variation patterns is a promising but poorly explored field in mammals.

Our study shows very high variation of a phenotype previously believed to be highly conservative, and suggests that this variation has been influenced by a reduced level of selection on the sensory modalities of balance and agility to which it is linked. This idea corroborates another of Darwin’s [18] predictions on the evolution of organs with decreased functional demand.

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