Dance of the Cave Bear: Honouring the Scientific Legacy of Björn Kurtén

Rewinding developmental trajectories shows how bears break a developmental rule

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Received 28 Feb. 2024, final version received 27 June 2024, accepted 27 June 2024

Stenberg, O. E., Moustakas-Verho, J. E. & Jernvall, J. 2024: Rewinding developmental trajectories shows how bears break a developmental rule. — *Ann. Zool. Fennici* 61: 377–386.

Mammals have evolved a broad variety of dental morphologies. Nevertheless, the development of the mammalian dentition is considered highly conserved. Molar size proportions exemplify this as a system where small changes in shared developmental mechanisms yield a defined range of morphological outcomes. The Inhibitory Cascade (IC) model states that as molars develop in a sequence, the first developing anterior molars inhibit the development of subsequent posterior ones. The IC model thus predicts a trend of linear tooth size change along the molar row, as has been observed in a wide range of mammalian taxa with otherwise differing dental morphologies. Perhaps the starkest exceptions to the IC rule are bears, in which the second molar is the largest and the third one is disproportionally small. Here we sought to calculate when and how during development, the bear dentition moves away from the IC prediction. We examined molar proportions in eight bear species, and estimated tooth sizes during development. The results indicate that development of bear molars already deviates from IC expectation during patterning. However, during the earlier cap stage, size proportions of bear molars still seem to adhere to the IC model predictions. Overall, these analyses suggest that irrespective of the final outcome, the process of initial splitting of the molar-forming region into individual teeth is conserved and follows the IC rule.

Introduction

Bear dentitions have received considerable research interest, undoubtedly due to factors such as bears being typically considered as apex predators. Different bear taxa also show remarkable diversity in their ecology making, them highly suitable for comparative studies from multiple

Edited by Oscar E. Wilson, University of Helsinki

perspectives. Historically, Björn Kurtén pioneered quantitative approaches that linked intra- and interspecies variation in bears, starting with cave bear teeth (Kurtén 1953). Some of his studies also incorporated perspectives relevant to developmental biology. Links to development are already evident in Kurtén's thesis work in which he examined the minimum size when teeth can develop,



Fig. 1. The IC model predicting linear tooth size change along the molar row. -A: Visualised in a morphospace of molar size ratios, the largest bear molar being m2 is a departure from the expectations of the IC model. -B: Molars developing in a sequence, during which the previous tooth regulates initiation of the next one in an inhibitory cascade, producing a linear trend in tooth size along the molar row.

and also visualised integration of dentitions using correlation fields of tooth sizes (Kurtén 1953, *see* also Gomez-Robles & Polly 2012).

During the recent 30 years, developmental biology studies have advanced to incorporate molecular evidence that has provided mechanistic insights into the processes regulating dental variation. One of these new insights is the interplay of signals regulating dental development along the anteroposterior sequence. This signalling logic has been experimentally studied in molar teeth of the mouse, in which the presence of the first molar (m1) has been observed to inhibit the development of subsequent distal molars (Kavanagh et al. 2007). Cultivating mouse lower molars ex vivo, Kavanagh et al. (2007) microdissected explants, separating the developing m1 from the posterior tail that gives rise to the second (m2) and third (m3) molars. Comparing explants to intact molars, Kavanagh et al. (2007) noted that the absence of m1 significantly accelerated the initiation and growth of m2 and m3, elementally altering the morphology and size proportions of the resulting tooth row. This work implied that as molars are developing in succession, they are subject to cumulative effects of prior developmental events. In short, the state of the first tooth increasingly affects the

development of subsequent ones. The authors introduced this proposed developmental ratchet as an inhibitory cascade (IC) model (Fig. 1A).

Formally, the IC model states that the proportions of molars are determined by the ratio of mesenchymal activation and intermolar inhibition during development, with a balance between these signals yielding a row of molars equal in size (m1 = m2 = m3). Characterised as a cascade, the model by Kavanagh et al. (2007) predicts that any increase in inhibition has a cumulative effect on the size of distal molars, producing a pattern of decreasing size along tooth row (m1 > m2 > m3) that even extends to the complete loss of distal molars — as can be seen in felids. Accordingly, decreased inhibition should inversely affect development, increasing the size of distal molars (m1 < m2 < m3). These predictions by the IC model entail covariance between teeth; the known sizes of two molars can be used to predict the size and presence of the third one. Instead of calculating correlation matrices among fully formed teeth, here the starting point was the reasoning derived from experiments on developing mouse molars.

Because the experiments on mouse teeth produced large, interspecies-level changes in tooth proportions, Kavanagh *et al.* (2007) investigated molar sizes in murine rodents and reported molar size patterns that mostly adhered to the predictions of the IC model. Since then, a wide range of taxa have been used to evaluate the IC model on a macroevolutionary scale (e.g. Polly 2007, Asahara 2013, Bernal et al. 2013, Halliday & Goswami 2013, Schroer & Wood 2015, Evans et al. 2016, Carter & Worthington 2016, Selig et al. 2021). Overall, the IC model appears to explain much of the variation in several lineages, suggesting that evolutionary change in molar proportions has largely obeyed shared developmental logic. This logic is not, however, the driving force of evolutionary change. Rather, it is ecology. Herbivory in mammals is linked to larger and more complex molars, and faunivory to smaller simpler ones (Evans et al. 2007, Selig et al. 2021). The inhibitory cascade can be hypothesised to have been the developmentally simplest solution to modifying tooth size along the jaw (Kavanagh et al. 2007). This mode of development intrinsically carries the side effect that the more distal, later developing molars show the largest changes.

Whereas most mammalian taxa fit the predictions of the IC model to a high degree, a number of groups are known to deviate from the expectations of this model. One such group, already noted by Polly (2007), is bears. He observed that the dentitions of three bear species fell well outside the area of morphospace predicted by the IC model, exhibiting a pattern in which the largest molar is the second one (m1 < m2 > m3). This type of deviation has been found to be quite common (e.g. Bernal *et al.* 2013, Halliday & Goswami 2013), raising the question how these departures from the IC predictions occur during development.

To study the deviations from the IC predictions, first it is important to consider what is truly being measured to test the IC model. The predominant measures used are twodimensional (2D) areas of the teeth, whether as simple as length and width estimates of an area or more accurate direct measurements of 2D area. Although three-dimensional measures of size can also be used, they provide comparable results to the 2D measures (Evans *et al.* 2016). What these different measures of size share is the fact that they are measuring the very end point of the developmental process generating each tooth (Fig. 1B). This process includes the initiation, patterning, growth, matrix secretion, and mineralization of the dental hard tissues (Fig. 1B) — any of which may affect final tooth size. In addition to the interactions producing the inhibitory cascade dynamics, other genetic and hormonal factors affecting tooth development can affect the final outcome. Consequently, decomposing the inhibitory cascade from all the other factors regulating tooth size is undoubtedly more difficult at the lower taxonomic and population levels where the range of phenotypic variation is smaller (e.g., Roseman & Delezene 2019, Boughner et al. 2021, Bermúdez de Castro et al. 2021). Nevertheless, a recent analysis of a large dataset of primate dental variation suggests that IC aligns microevolution with macroevolution (Machado et al. 2023). Yet, exceptions to the IC such as bear molars are of special interest as they can be used to examine when and how these deviations occur during development. To state this in more general terms, understanding exceptions to developmental models and rules should help us explain how these rules function in the first place.

We examined how the lower molars of different bear taxa obtain the m1 < m2 > m3 size pattern, and therefore how these dentitions fall below the line predicted by the IC model (Fig. 1A). Following the logic of sequential development of molars (Fig. 1B), we assumed that most of the deviation is due to the last tooth of the cascade, the third molar, being too small, and focus on explaining deviation from the expected m1 < m2 < m3 pattern. We specifically asked when during development this deviation occurs. Fully formed bear molars are almost uniquely suitable for this particular question because their crowns are highly complex, having arrangements of several cusps. In general, cusp features responsible for the surface complexity are principally formed during patterning stage of development, a process from which fully formed bear molars preserve a fine-grained proxy. Moreover, by numerically rewinding developmental trajectories, we estimated tooth sizes at the end of patterning stage and at the earlier cap stage when the patterning process is only just beginning (Fig. 1B). Taken together, these analytical approaches allowed us to peer into earlier stages of development without direct experimentation, and ask whether the bear molars fall off the expected developmental trajectory before or after the patterning stage.

Material and methods

Sample

We sampled the lower molar dentition of eight bear species, including the extinct cave bear (Ursus spelaeus) and all extant ursids but the spectacled bear (Tremarctos ornatus). The specimens (Table 1) originated from the collections of the Finnish Museum of Natural History (FMNH), Zoological Museum of the University of Copenhagen (ZMUC), the National Museum of Nature and Science of Tokyo (NSMT), and the Smithsonian National Museum of Natural History (USNM). Although the cave bear specimens do not necessarily represent a single species, here we treated them as such for illustrative purposes. For the analyses of dental complexity, tooth rows were selected with the criteria of being complete and as unworn as available. Size measurements were taken also from worn teeth as long as they preserved the maximum dimensions.

Data acquisition and mesh preparation

Lower molar rows were captured as surface scans using a Planmeca PlanScan intraoral dentistry scanner (PlanMeca, Helsinki, Finland). Scanned tooth rows were original specimens except for USNM 259400 that was an epoxy cast. The surfaces were prepared for analysis in MeshLab ver. 2020.07 (Cignoni et al. 2008) as described in Christensen et al. (2023) with the following modifications: no smoothing was deemed necessary for surface scans and the target face count for mesh simplification was set to 20 000. Because our focus was on calculating ratios within each individual, our analyses should be relatively robust against the choice of mesh simplification strategy (see methods in Christensen et al. 2023). After this, molars were separated (Fig. 2). As the

bear m3 is generally tilted more lingually than the other molars, each m3 was manually realigned to have the occlusal surface aligned horizontally. Additional specimens were photographed for two-dimensional size estimations. To ensure parity of area measurements, any lingually tilted m3 was photographed separately from its respective occlusal direction.

Measurements and estimation of tooth size during development

Molar complexity was quantified from surface scans using OPCR (orientation patch count rotated), and OPC maps (Fig. 2) were produced following Christensen et al. (2023). In addition, the two-dimensional projection area (mm²) of each tooth scanned was measured using MorphoTester ver. 1.1.2 (Winchester 2016) RFI functionality. For photographed specimens, Fiji (Schindelin et al. 2012) was used to measure molar area. Maximum width (mm) was measured for each molar. Feature density was obtained as a ratio of complexity to area (OPCR/ mm²) for each tooth, and area, complexity, and feature density proportions were calculated (m2/ m1, m3/m1). For species with several sampled specimens, medians of these ratios were calculated and used in the figures. Distances from the IC line (y = 2x - 1) of ratios were calculated for individual specimens.

For each species, we estimated tooth area at two points of development. Employing equations from Christensen *et al.* (2023), we used final tooth width (mm) and area (mm²) to estimate developing molar size (mm²) at the end of patterning (A_{pat} , Eq. 1) and at the onset of patterning (A_{cap} , Eq. 2). These stages correspond to the late bell and cap stages of crown formation, respectively (Fig. 1B). The equations we used are:

$$\begin{split} A_{\text{pat}} &= [\exp 10(\log_{10}L_{\text{width}} \times 0.640 \\ &+ 0.885)/L_{\text{width}}]^2 \times A_{\text{final}} \end{split} \tag{1} \\ A_{\text{cap}} &= [\exp 10(\log_{10}L_{\text{width}} \times 0.043) \end{split}$$

$$+ 2.095)/L_{\rm width}]^2 \times A_{\rm final} \tag{2}$$

where L_{width} is the maximum width and A_{final} is the area of the fully formed tooth, respectively. For calculations, measurements were converted to

micrometres. Note that the original equations in Christensen *et al.* (2023) are for the widths of the developing teeth at the cap and late bell stage. We assumed the width/length proportions of the

crowns to be roughly constant from the cap stage onwards. This is supported by the observation that length differences between developing teeth appear first (Christensen *et al.* 2023). Thus, the

Table 1. The measurements taken from the sampled bear specimens. Specimen data with and without complexity values are from 3D scans and photographs, respectively. In the figures, median values were used in case of multiple specimens.

Species/specimen	Side	Width (mm)			2D area (mm ²)			Complexity (OPCR)		
		m1	m2	m3	m1	m2	m3	m1	m2	m3
Ailuropoda melanoleuca										
USNM 259400	right	17.1	19.9	19.3	416.9	432.5	261.4	87.6	128.5	114.5
NSMT M30000	right	15.9	17.7	18.9	385.9	376.8	286.5			
NSMT M31458	right	18.1	19.6	20.3	471.5	466.8	272.4			
NSMT M32901	right	15.6	18.5	18.5	426.3	422.7	288.6			
Helarctos malayanus	•									
FMNH 40_1976	left	8.3	9.7	11.1	108.7	130.7	113.1	69.1	104.6	113.1
FMNH 1973	left	9.5	10.3	10.5	130.1	143.9	94.6			
FMNH 1084	right	8.2	9.9	8.5	119.9	132.1	60.3			
FMNH 3288	right	7.3	8.7	8.7	89.2	112.2	79.8			
Melursus ursinus	•									
ZMUC 4290	left	9.4	10.4	8.8	125.5	135.5	75.1	50.6	80.6	67.1
Ursus americanus										
FMNH 545 1960	left	7.9	10.8	10.4	107.4	167.2	111.9	69.3	111.4	103.4
FMNH 991	left	7.9	11.1	12.4	108.7	168.1	125.4	71.6	115.9	143.1
FMNH 996 1952	left	8.7	11.9	13.1	136.1	206.3	173.0	60.4	112.1	109.0
FMNH 3292	left	7.6	10.5	10.8	108.4	164.2	112.5			
FMNH 3293	left	8.2	10.7	11.5	120.6	172.5	147.0			
FMNH 3165	right	8.2	11.8	11.9	124.5	205.0	140.7			
Ursus arctos	-									
FMNH 1362	left	10.4	12.9	14.6	187.9	251.0	203.5	80.0	128.5	94.5
FMNH 1369	left	9.9	12.9	13.0	171.5	245.3	192.3	71.0	125.8	111.8
FMNH 22.679	left	9.5	12.2	13.5	164.5	223.3	200.7	80.5	124.1	151.8
FMNH 39733	left	10.0	13.5	13.7	176.4	261.4	204.7	77.8	118.0	111.1
FMNH 6457	left	10.5	13.9	13.1	186.1	286.5	215.7			
FMNH 3614	right	9.6	14.0	14.1	169.0	278.3	177.6			
Ursus maritimus	•									
ZMUC 5365	left	8.5	9.8	10.8	136.5	154.5	135.0	60.5	71.5	96.6
ZMUC 5378	left	9.1	11.2	12.9	163.5	209.4	160.9	63.6	93.0	112.6
ZMUC 5620	left	8.2	10.8	9.8	132.8	171.0	93.4	60.4	99.3	86.6
FMNH 2342 201	left	8.9	11.8	12.5	168.5	222.3	161.1	65.0	106.9	131.6
ZMUC 5472	right	8.7	11.4	12.4	157.8	194.6	163.2	67.3	85.3	114.4
Ursus spelaeus	•									
FMNH P10477 2290	right	15.2	19.9	21.2	395.5	540.7	472.4	92.5	202.0	181.4
FMNH P941	left	12.4	16.8	19.3	264.4	414.8	421.7			
FMNH P947	left	16.4	20.6	21.4	435.7	587.4	529.3			
FMNH P725	right	14.6	18.3	21.0	367.4	521.6	552.8			
FMNH P948	right	13.9	18.1	19.6	307.8	500.9	388.9			
FMNH P949	right	15.0	19.0	20.8	365.1	521.3	506.1			
FMNH P950	right	14.3	19.7	19.7	326.8	494.3	448.7			
Ursus thibetanus	2									
FMNH 1.962	left	8.5	11.7	13.0	152.9	210.5	190.3	66.6	99.3	100.0
ZMUC 603	right	8.3	11.1	10.6	150.5	171.8	113.5	73.9	98.4	66.4
FMNH 1796	left	8.0	10.8	10.9	143.9	196.3	152.5			



Fig. 2. Three-dimensional surfaces and OPC maps illustrating differences in dental complexity across the eight bear species sampled. For species with several specimens, a representative tooth row is shown. Left-hand-side molar rows were mirrored for consistency. Scale bars = 5 mm.

cap stage when the forming crown base is established by lateral expansion of the cervical loops, is the earliest stage when the crown proportions can be observed. In addition, although the original equations were derived from data on individual teeth from different species, here we used them also for the teeth in the same tooth row.

Results

Proportions of molar size and complexity in bears

A simple plot of molar sizes along the tooth row makes it immediately apparent that the second molar is largest in size while the third molar is smaller (m1 < m2 > m3) in all sampled bear dentitions (Table 1 and Fig. 3A). This pattern is inconsistent with the IC model of linear change from tooth to tooth as m3 is much smaller

than would be expected from the m2/m1 ratio (Fig. 3B). In the case of the *Ailuropoda* and *Melursus*, m3 is by far the smallest molar in the tooth row (Figs. 2 and 3B), so that these species fall further down in the IC morphospace (Fig. 3B; the mean distance from the IC line is 0.286 and the range is 0.094 to 0.556 for all the specimens with size measurements; *see* Table 1).

The pattern of m2 being the largest tooth holds for the OPCR values in most of the species (Fig. 3A). *U. maritimus* and *Helarctos* are exceptions in that their m3s have the largest OPCR values along the molar row, and they are also closest to the IC line (Fig. 3A and B). Overall, apart from *U. maritimus*, the species appear to still fall below the IC line (the mean distance from the IC line is 0.270 and the range is 0.069 to 0.629). Because the offset from the IC line remains roughly similar between the size and complexity, we also illustrated the feature density, calculated as the ratio of complexity to area. The



Fig. 3. Molar area, complexity, and feature density along the molar row (for the 18 specimens with complexity values, median values were plotted in case of multiple specimens; *see* Table 1). Molar area and complexity do not change linearly along the molar row, but feature density is highest in m3 as can be observed in (**A**) tooth sizes, and (**B**) tooth proportions plotted in the IC morphospace. Blue lines in **B** are the IC predictions, and coloured regions m1 > m2 < m3 above, and m1 < m2 > m3 below the IC line, respectively.

density of features increased along the tooth row (m1 < m2 < m3; Fig. 3A), and the sample straddled mostly along or above the IC line (Fig. 3B; the mean distance from the IC line is 0.148 and the range is 0.013 to 0.286). This result is indicative of both the patterning and final growth stages being roughly equally affected, pointing towards an early developmental divergence from the IC.

Rewinding developmental trajectories

Using two empirically derived equations of Christensen *et al.* (2023), we estimated the sizes of developing teeth at the late bell stage, which corresponds to the end of patterning stage, and at the earlier cap stage. Crown morphogenesis starts at the cap stage when the cervical loops begin to grow laterally to the primary enamel knot, and thus these two estimates, together with the final tooth size, bracket the whole crown morphogenesis (Fig. 1B). The resulting estimates show that late bell stage molars are closer to the IC line, but still fall below it (Fig. 4A and B; the mean distance from the IC line is 0.178 and the range is 0.069 to 0.318). Compared to the fully formed teeth, m2 is smaller and more equal in size to m1, or even smaller as in Ailuropoda. (Fig. 4A). The cap stage size estimates revealed a relatively linear reduction in sizes along the tooth row (m1 > m2 > m3; Fig. 4A). This in turn means that the cap stage sizes appear to agree with the IC prediction (Fig. 4B; the mean distance from the IC line is 0.045 and the range is 0.002 to 0.142). The change in tooth proportions in the rewind of the developmental trajectories is explained by differences in tooth lengths and widths. The size proportion estimates for the



Fig 4. (**A**) Tooth sizes and (**B**) their position in IC morphospace at three points of development (for all the 36 specimens, median values were plotted in case of multiple specimens; *see* Table 1). Estimates of size at the late bell and the cap stage are based on numerically rewinding development. Whereas the late bell stage estimates remain below the IC line, the cap stage estimates appear to follow the IC prediction of linear change is size between teeth. Blue lines in **B** are the IC predictions, and coloured regions m1 > m2 < m3 above, and m1 < m2 > m3 below the IC line, respectively.

three cap stage molars converge around 1.0, 0.8, and 0.6 for m1, m2, and m3, respectively. This point on the IC line equals activator/inhibitor ratio of 0.8 in the original inhibitory cascade formula (Kavanagh *et al.* 2007).

Discussion

The opportunity to study mammalian tooth development in action is limited to a handful of species. A common feature of these species that can be investigated experimentally is their small size. Despite its highly derived dentition, the mouse has remained the species that has contributed most to our understanding of tooth development. In this context, bears make poor model organisms. Yet, from a standpoint of evolutionary diversity, bears provide prime examples to test why generalisations made from mouse tooth development sometimes fall short.

The dental inhibitory cascade is a proposed, seemingly plesiomorphic system of development, which describes how a balance of inhibition and activation drives the sequential development of molars, resulting in linear patterns of tooth size along the molar row (Kavanagh *et al.* 2007). Though most mammals largely fit these predictions of molar proportions, bears are a noted exception. Moreover, the mode of departure from the IC prediction (m1 < m2 > m3) is commonly observed in other mammalian groups such as primates, and also at lower taxonomic levels (Bernal *et al.* 2013, Halliday & Goswami 2013). Explanations of how bears escape the IC may thus apply more broadly.

Here, we rewound developmental trajectories in bears by integrating recent advances in developmental theory with computational methods for quantifying tooth shape. Tooth shape is chiefly established by two partly concurrent developmental processes: patterning and growth (Fig. 1B). Measuring crown complexity along the molar row in the context of the IC model provided a proxy for the patterning process preceding the final growth in size. Selig et al. (2021) concluded that in primates and related groups, the complexity of tooth crowns generally increases from m1 to m3, and shows a better fit to the IC than the corresponding molar sizes. They found a size pattern more like the one in bears. The more complex bear dentitions paint a somewhat different picture: like the molar size ratios, the proportions of complexity in bears remain below the IC prediction (Fig. 3). This may imply that whereas in primates the development follows the IC prediction through the patterning, the bears are divergent already before patterning. This is supported when shape and size are considered together. A ratio of complexity to area — summarising how fine-grained the morphological detail of surface is - shows a closer match with the IC line (Fig. 3). Though m2 displayed higher feature density than m1, m3 far surpassed both in this aspect. A way to interpret this result is that both patterning and the growth after patterning are affected equally because the number of cusps and crown features in general are ultimately limited by the size of the growing tooth.

Our second approach to rewind tooth development took advantage of the empirical discovery that the size when the patterning is completed, and the size when the patterning begins, show strict scaling relationships with the final tooth size (Christensen et al. 2023). The mechanistic explanation for these relationships is the integration of patterning and growth processes by insulin-like growth factor (IGF) pathway. Whereas in the case of larger teeth, the patterning scales so that it happens at a larger size, the initiation of patterning is relatively size invariant (Christensen et al. 2023). We found that the estimated late bell size when the patterning is completed is closer to the IC line, but still below it (Fig. 4). This is consistent with the result on complexity and suggests that the relatively small size of bear m₃, for example, is not a result of a developmental arrest

late during development. Rather, the departure from the IC appears to involve most parts of the crown formation process.

The discovery that the estimated cap stage teeth are on the IC line is intriguing as it indicates that the initial splitting of the tooth-forming region follows the IC rule (Fig. 4). The cap stage is also closest in time to the actual inhibitory cascade tested experimentally by Kavanagh et al. (2007). That is, the microdissection separating the developing m1 from the posterior tail was done when m1 was at the cap stage. While our results on bear teeth should be considered preliminary, it is perhaps a plausible hypothesis that the IC mode of tooth formation is the conserved, or plesiomorphic (Halliday & Goswami 2013) feature in all mammals; and whereas bears are one group that has diverged greatly from the IC, at the onset of the crown formation they still follow the rule. These kinds of analyses may also help to classify and uncover different mechanisms that produce the divergent patterns. One important aspect related to the cascading mode of development is that the late bell and cap stage molars are not at the same developmental stage at the same time (Fig. 1B). This means that the plots placing the developing teeth into the IC morphospace manifest the process of development, not a static state of morphology (Fig. 4). In this context, it is also useful to keep in mind that molars in a fully formed tooth row, while typically analysed as a static representation of anatomy, actually reach their final sizes at different points in time (Fig. 1B).

In conclusion, analyses of morphology that utilise theories derived from developmental processes can be used to both test these theories, and to provide new insight into the kinds of developmental changes producing phenotypic diversity. In the case of mammalian teeth and the IC, it will be interesting to decipher the number of ways in which this rule can be broken, from the standpoints of both evolution and development.

Acknowledgments

We thank the members of Jernvall lab for comments and discussions on this work, and J. Granroth, Björn Kröger (Finnish Museum of Natural History, Helsinki), E. Lorenzen (Zoological Museum of the University of Copenhagen), and W. Morita (National Museum of Nature and Science, Tokyo) for access and help with museum collections. This study was supported by the Academy of Finland (J.J.), SYNTHESYS Grant DK-TAF-7064 (J.E.M.-V.), and the Finnish Doctoral Programme in Oral Sciences (O.E.S.).

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