

Conserved evolution of skull shape in Caribbean head-first burrowing worm lizards (Squamata: Amphisbaenia)

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In contrast to the extraordinary adaptive radiation of Caribbean *Anolis* lizards, head-first burrowing worm lizards (Amphisbaenia) of the Greater Antilles show a high degree of evolutionary conservatism in both taxonomic and phenotypic diversity. While Caribbean anoles reach over 160 endemic species and six ecomorphs, amphisbaenians peak at one to seven species per island and are characterized by two distinct head shapes, each associated with a specific burrowing behaviour. Using three-dimensional landmark-based geometric morphometrics, we found that Caribbean amphisbaenians also occupy a relatively confined area of skull morphospace, with considerable overlap between species from different islands and strong morphological integration between crania and mandibles. The only exceptions were the bizarre keel-headed cadeids (*Cadea*) from Cuba, which appear to be unlike other round-headed Caribbean forms and closer to Mediterranean blanids (*Blanus*), their putative sister group. The only significant differences in skull shape were found between insular amphisbaenians and their mainland relatives, indicating that fossorial vertebrates may respond differently to ecological opportunity than other terrestrial fauna. Given their highly specialized subterranean niche, we suggest that worm lizards are under strong stabilizing selection to maintain cranial proportions for head-first digging, thus limiting their ability to exploit novel resources (e.g. microhabitat, prey) encountered in insular environments.

ADDITIONAL KEYWORDS: *Amphisbaena* – amphisbaenians – biogeography – *Blanus* – burrowing – *Cadea* – fossoriality – geometric morphometrics – Greater Antilles – skull.

INTRODUCTION

Due to their discrete nature, islands are considered model regions for the study of biogeography, describing the distribution of species in space and time and its relation to the physical environment (Wallace, 1876, 1880; MacArthur & Wilson, 1967; Ricklefs & Bermingham, 2008). Such locations provide ideal conditions for adaptive radiation, in which single lineages rapidly diversify to inhabit a variety of environments that differ in the traits required to exploit them (Schluter, 2000). Particularly for terrestrial organisms, dispersal to oceanic islands is often haphazard but unidirectional in movement (MacArthur & Wilson, 1967; Houle, 1998; Bellemain & Ricklefs, 2008; Shaw & Gillespie, 2016), leading to *in situ* diversification and a high degree of local endemism.

Indeed, faunal assemblages on island archipelagos or ‘island-like’ habitats have best exemplified the principles of adaptive radiation: Darwin’s finches on the Galápagos Islands (Grant, 1986; Grant & Grant, 2002), Hawaiian honeycreepers (Lovette *et al.*, 2002) and cichlid fishes in East African lakes (Meyer, 1993; Salzburger *et al.*, 2005) are all classic, well-studied systems that demonstrate the interplay between colonization, adaptation and speciation underlying diverse endemic biotas.

The widely distributed and speciose *Anolis* lizards of the Caribbean provide a textbook example of adaptive radiation based on the exploitation of different structural niches within and between islands (Williams, 1983; Losos, 2007, 2009). Variation in body size, shape, diet, physiology and behaviour are strongly linked to habitat partitioning among perch types, leading to similar sets of ecomorphs on each island despite their independent evolution (Losos *et al.*, 1998; Losos, 2009). These associations provide strong evidence for the role

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of ecological opportunity in generating biological diversity, by which species colonizing new environments (e.g. islands) experience a reduction in competition for shared resources, allowing them to diversify and adapt to an array of unoccupied niches (Yoder *et al.*, 2010). While Caribbean anoles have provided major insights into the relationship between habitat specialization and evolutionary diversification (Pinto *et al.*, 2008; Losos, 2009; Mahler *et al.*, 2010; Yoder *et al.*, 2010), such clear associations between environment and phenotype have yet to be identified in other lizard groups within the rich Caribbean herpetofauna, whose species far outnumber all other terrestrial (non-avian) Caribbean vertebrates combined (Crother & Guyer, 1996; Stroud & Losos, 2016; Hedges, 2018).

Fossorial amphisbaenians, or ‘worm lizards’, provide a compelling counterexample to the well-sampled Caribbean *Anolis* system, in that they are present on many of the same islands, co-occur in sympatric habitats, and descend from multiple invasions of mainland ancestors in a similar time frame, yet they fail to exhibit the extraordinary taxonomic and phenotypic diversity as seen in ground- and tree-dwelling anoles. Amphisbaenians are a bizarre clade of predominantly limbless, head-first burrowing squamates that live buried under loose and sandy soils in tropical and subtropical regions of the world (Kearney, 2003a; Gans, 2005). Their derived morphology is thought to reflect adaptations to the stresses associated with a subterranean lifestyle, including an elongate body and robust skull with distinct snout shapes (shovel, spade, keel and round) corresponding to specific burrowing behaviours, a high degree of interdigitization among dermal roofing bones, and reduction or loss of the eyes and ears (Kearney, 2003a; Gans & Montero, 2008; Müller *et al.*, 2016). These features indicate strong selective pressures related to microhabitat use, suggesting that variation in soil type or other ecological variables may drive observed differences in skull shape across species. At the same time, stabilizing selection to maintain adequate digging performance throughout ontogeny is thought to constrain amphisbaenian allometry (Hipsley *et al.*, 2016), potentially limiting their ability to respond to novel environments and to diversify into new forms.

Among the Greater Antilles, 17 amphisbaenian species occur on four island groups – Cuba, Hispaniola, Puerto Rico and the Virgin Islands (Fig. 1A; Hedges, 2018). Of these, 15 belong to the most speciose family Amphisbaenidae (known as amphisbaenids), while two of the five species on Cuba are sole members of the monogeneric family Cadeidae (Vidal *et al.*, 2008). Within Amphisbaenidae, two separate radiations, both originating in South America, have been identified through molecular phylogenetics: the first dated

to the middle Eocene 43–40 Mya, while the second appears more recent (Oligocene, 27–23 Mya; Vidal *et al.*, 2008; Zheng & Wiens, 2016). The species comprising the older and younger radiations, henceforth referred to as CA1 and CA2 respectively (Fig. 1B), are also differently distributed. The CA1 radiation encompasses most of the amphisbaenids of the Caribbean and its taxa occupy all four of the island groups, whereas the CA2 radiation contains three species, all of which are restricted to south-western Hispaniola.

Although little is known of amphisbaenian ecology, detailed analyses of morphological variation among taxa have the potential to reveal the selective forces influencing their biogeographical patterns. Here, we use three-dimensional landmark-based geometric morphometrics (GM) to compare skull shape across 17 amphisbaenian species, and to assess morphological variation in relation to geographical distributions and evolutionary history across the Caribbean radiations. We also quantify the degree of shape covariation between the two main components of the skull, the crania and mandibles, which form functionally linked yet distinct developmental regions. We predict that because the snout is directly involved in burrowing, cranial shape will be conserved across closely related taxa occupying similar soil types, while variation in mandibles may reflect extrinsic factors related to diet. This is the first time that GM has been applied across amphisbaenian species, providing new insights into the processes of biogeographical diversification in this enigmatic clade.

MATERIAL AND METHODS

DATA COLLECTION

We sampled specimens from 11 of the 17 Caribbean amphisbaenian species, in addition to two South American amphisbaenids and four Mediterranean species from the family Blanidae ($n = 41$, mean = 2.4 specimens per species; Table 1). The South American and Mediterranean taxa are the closest mainland relatives of the two Caribbean families (Amphisbaenidae and Cadeidae, respectively; Vidal *et al.*, 2008; Zheng & Wiens, 2016), and were included for outgroup comparisons.

Ethanol-preserved specimens were scanned using high-resolution X-ray computed tomography (CT) at the Museum für Naturkunde Berlin, Germany, and School of Earth Sciences, University of Melbourne, Australia. Both locations were equipped with a Phoenix|x-ray nanotom (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany) using a 180-kV nanofocus tube and a tungsten target. Specimens

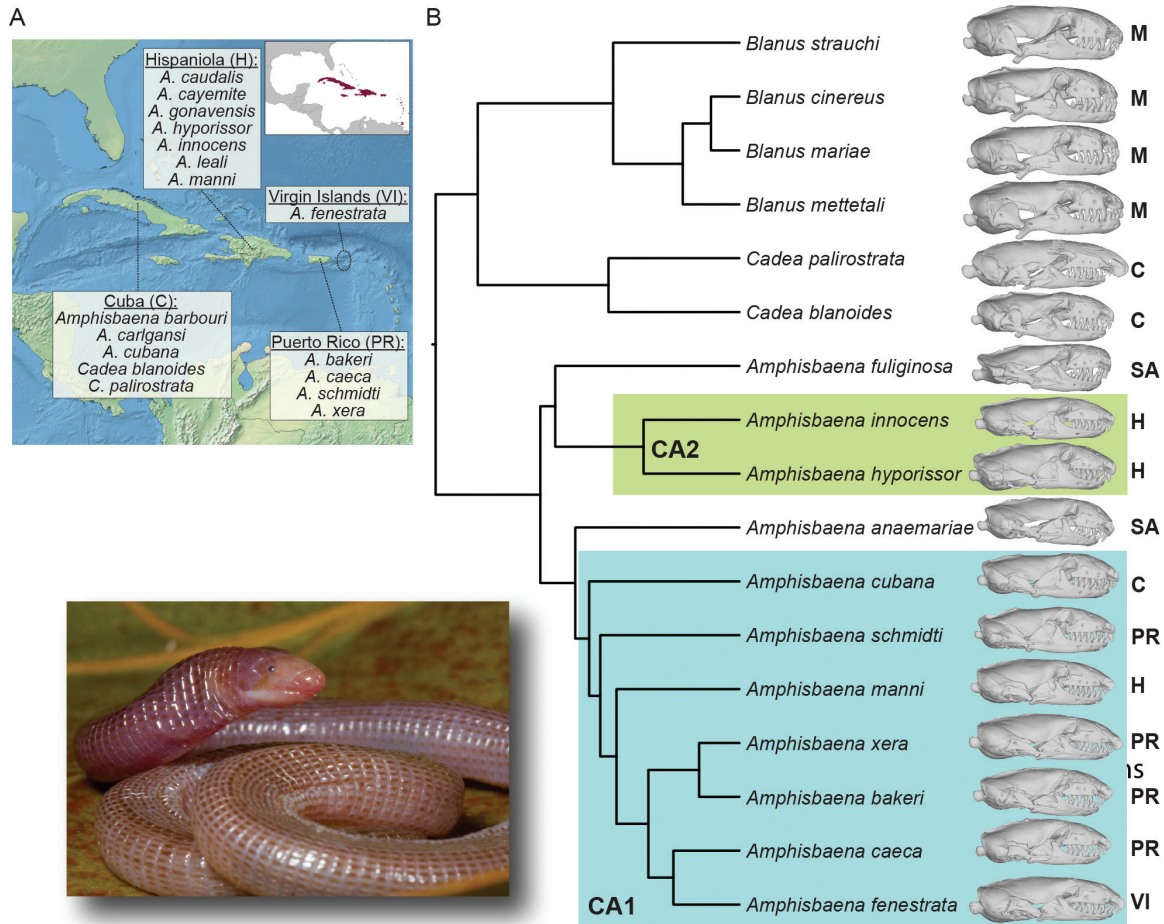


Figure 1. A, distribution of amphisbaenians on the Greater Antilles, highlighted in red in the Caribbean inset map. B, molecular phylogeny of amphisbaenian species in the present study, modified from Zheng & Wiens (2016). Caribbean radiations CA1 and CA2 are indicated in blue and green boxes, respectively. Examples of 3D skull models for each species are shown in right lateral view, with locations of occurrence given as abbreviations (C, Cuba; H, Hispaniola; M, Mediterranean; PR, Puerto Rico; SA, South America; VI, Virgin Islands). Photo: *Amphisbaena xera*, courtesy of Father Alejandro Sánchez-Muñoz.

were CT scanned in plastic tubes at 80–85 kV and 150–220 μ A for 500–1000 ms over 800 projections, resulting in a final voxel size of approximately 10 μ m. Volumetric reconstructions were made in datos|x-reconstruction software (GE Sensing & Inspection Technologies GmbH phoenix|x-ray), and mandibles and crania were separated from the body in VGStudio Max 2.1 (Volume Graphics, Heidelberg, Germany).

To adequately capture amphisbaenian skull shape, we used a combination of point and sliding landmarks. Point landmarks correspond to a single location, whereas sliding landmarks create a set of pseudolandmarks (here, consisting of ten points each) placed at equidistant locations along a designated curve. Fifty-three point landmarks and three sliding landmarks were digitally placed on crania, and 24 point landmarks and two sliding landmarks were

placed on mandibles in the program Landmark Editor v3.6 (Institute of Data Analysis and Visualisation, UC Davis, USA) (Fig. 2). Because individual specimens were preserved with their mouths opened in different positions, skull components were landmarked as separate structures and rearticulated in geometric space using the R tool ShapeRotator to remove the effects of random translation and rotation (Vidal-García *et al.*, 2018). The final landmark dataset was exported as *x*, *y* and *z* coordinates and subjected to a generalized Procrustes fit to remove variation in scaling, location and orientation among landmark configurations (Klingenberg *et al.*, 2002; Table S1). This generated a set of Procrustes coordinates which were averaged by species and used as shape variables in all analyses. The effect of size on individual skull shape was small (multivariate regression of Procrustes coordinates on

Table 1. Amphisbaenian species included in the present study, with location of occurrence, family and number of specimens landmarked

Location	Species	Family	Clade	Number of specimens landmarked
Caribbean Islands				
Cuba	<i>Amphisbaena cubana</i>	Amphisbaenidae	CA1	2
	<i>Cadea blanoides</i>	Cadeidae	–	4
	<i>C. palirostrata</i>	Cadeidae	–	2
Hispaniola	<i>A. hyporissor</i>	Amphisbaenidae	CA2	3
	<i>A. innocens</i>	Amphisbaenidae	CA2	3
	<i>A. manni</i>	Amphisbaenidae	CA1	2
Puerto Rico/Virgin Islands	<i>A. bakeri</i>	Amphisbaenidae	CA1	1
	<i>A. caeca</i>	Amphisbaenidae	CA1	1
	<i>A. fenestrata</i>	Amphisbaenidae	CA1	1
	<i>A. schmidtii</i>	Amphisbaenidae	CA1	2
	<i>A. xera</i>	Amphisbaenidae	CA1	3
South America	<i>A. anaemariae</i>	Amphisbaenidae	SA	1
	<i>A. fuliginosa</i>	Amphisbaenidae	SA	4
Mediterranean	<i>Blanus cinereus</i>	Blanidae	–	4
	<i>B. mariae</i>	Blanidae	–	2
	<i>B. mettetalii</i>	Blanidae	–	2
	<i>B. strauchi</i>	Blanidae	–	4

For the family Amphisbaenidae, the Caribbean clade in [Figure 1B](#) is also noted, with South American amphisbaenids marked as SA. See [Appendix 1](#) for specimen voucher numbers.

log-transformed centroid size for crania: $R^2 = 0.067$, $P = 0.017$; and mandibles: $R^2 = 0.072$, $P = 0.049$, and was therefore not considered here.

For all comparative tests we incorporated the molecular phylogeny of [Zheng & Wiens \(2016\)](#), pruned to match our taxon sampling ([Fig. 1B](#)). That tree, based on a maximum likelihood analysis of 52 genes from two datasets ([Wiens et al., 2012](#); [Pyron et al., 2013](#)), received strong support for a sister group relationship between Cadeidae and Blanidae consistent with previous studies (e.g. [Vidal et al., 2008](#); [Vidal & Hedges, 2009](#)). *Blanus mariae* was not included in the original study, so it was manually added to the tree as the sister taxon to *B. cinereus* based on the molecular phylogeny of [Tonini et al. \(2016\)](#), and its elevation to species status from being considered a separate population of the latter ([Albert & Fernández, 2009](#); but see [Ceríaco & Bauer, 2018](#) for taxonomic discussion). *Cadea palirostrata*, which was also absent from the original tree, was added as sister taxon to its congener *C. blanoides* ([Dickerson, 1916](#)).

GEOMETRIC MORPHOMETRIC ANALYSES

Several approaches were used to describe morphological variation in amphisbaenian skulls, and to test the

effects of location and phylogeny on skull shape. For all analyses, crania and mandibles were considered separately to explore the potential influence of head-first digging on snout shape versus the lower jaws. Principal component analysis (PCA) was performed on each skull component, and the molecular phylogeny in [Figure 1B](#) was projected into morphospace to visualize the evolutionary history of phenotypic diversification using squared-change parsimony to reconstruct internal nodes ([Klingenberg, 2011](#)).

To determine the degree of evolutionary association between skull partitions, we tested for integration between cranial and mandibular shape in a phylogenetic context ([Adams & Felice, 2014](#)). Integration is defined as the strength of covariation between sets of traits, or blocks, arising from developmental or functional interactions ([Klingenberg, 2008](#)). A two-block partial least squares (PLS) analysis was used to quantify integration, resulting in a PLS coefficient (r_{PLS}) with values ranging from 0 to 1, higher values indicating a greater degree of evolutionary covariation between the two sets of variables across tips of the phylogeny.

The effect of location of occurrence (i.e. islands/continents of habitation) on skull shape was tested using a phylogenetic ANOVA on the shape variables with location and family as factors for the dataset containing all

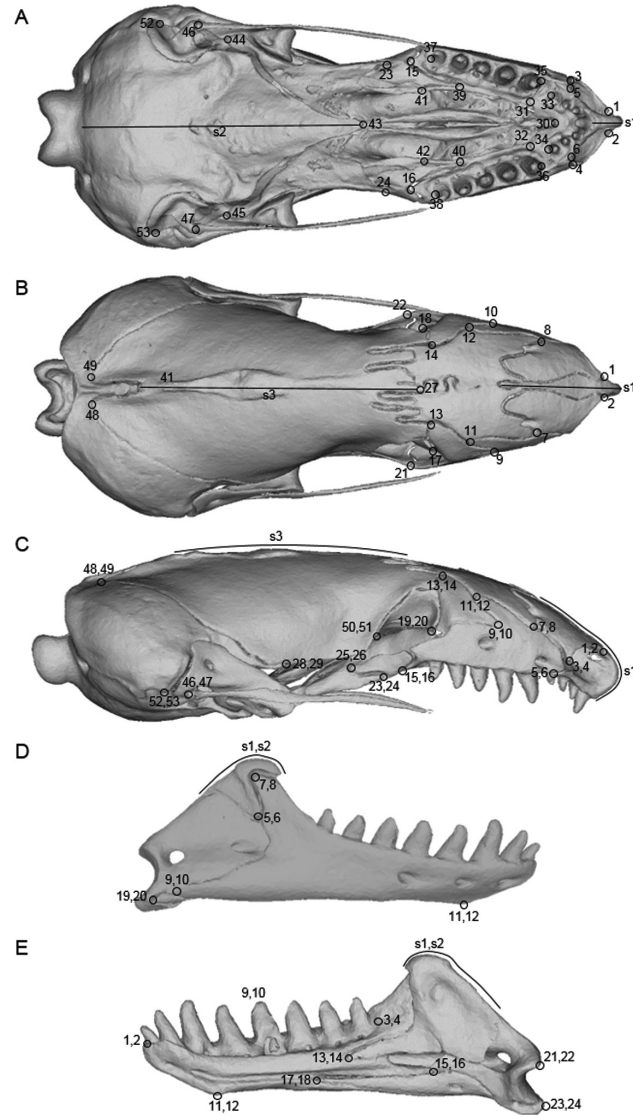


Figure 2. Locations of landmarks on the amphisbaenian skull: A, ventral; B, dorsal; and C, right lateral cranium; D, labial right and E, lingual right mandible. ‘s’ denotes sliding landmarks. See Appendix 2 for landmark descriptions.

species, and location and clade (CA1, CA2, SA; Fig. 1B) for amphisbaenids only. The datasets were divided to first compare shape variation between insular taxa and their mainland relatives, while the second was restricted to Amphisbaenidae to explore morphological evolution within a single lineage across islands. This method uses a phylogenetic generalized least squares regression of the Procrustes coordinates to compare observed results with a prediction based on Brownian motion (BM; Adams, 2014a).

Amphisbaenian skull morphology is marked by homoplasy across phylogenetically divergent taxa (Kearney, 2003a; Mott & Vieites, 2009; Müller *et al.*, 2016). To test the relationship between lineage

divergence and morphological disparity, we quantified the strength of phylogenetic signal in skull shape using a multivariate version of the K -statistic, K_{mult} (Adams, 2014b). A $K_{\text{mult}} < 1$ indicates lower phylogenetic signal (i.e. taxa appear less similar) than expected under a BM model of evolution, while $K_{\text{mult}} > 1$ indicates that close relatives resemble one another more than expected under a neutral model of trait evolution.

The PCA and averaging of landmarks were conducted in MorphoJ (Klingenberg, 2011). All other analyses were made in the R v3.3.3 package geomorph (Adams *et al.*, 2017) using a randomized residual permutation procedure of 10 000 iterations to test for statistical significance.

RESULTS

CRANIAL AND MANDIBULAR SHAPE VARIATION

The first two PC axes captured approximately three-quarters of the total shape variation (63–70%) in each skull partition, with the remaining axes accounting for less than 10% each. The phylomorphospace defined by PC1 and PC2 revealed clusters according to family for crania (with the exception of Cadeidae, see below), but less tightly so for mandibles (Fig. 3). For crania, movement along PC1 describes the degree of lateral compression, with positive values reflecting a broad, shallow cranium and rounded snout, while negative values indicate a laterally compressed cranium and a tall, elongated narrow snout. This pattern is clearly demonstrated by *Cadea palirostrata*, the most extreme keel-headed species in our study, having the lowest value along this axis (Fig. 3A). PC2 captured more subtle differences in the relative length and width of

the postorbital region, with positive values describing a wider and longer occipital than negative ones.

Patterns of mandibular shape variation among species differed from the cranium, in that blanids and cadeids grouped together on the positive side of PC1, reflecting a vertically shortened coronoid and lengthened compound bone (the fusion of several bones posterior to the tooth-bearing dentary; Kearney, 2003a), while amphisbaenids fell mainly on the negative side, corresponding to a taller but more anteroposteriorly shortened jaw. Variation along PC2 separated blanids and cadeids, along which positive values were associated with a narrow angle between the right and left mandibles (and thus a laterally compressed skull; e.g. *C. palirostrata*), while negative values reflected a wider jaw and thus broader skull (e.g. *Blanus strauchi*) (Fig. 3B).

For both skull components, the two South American species *Amphisbaena fuliginosa* and *A. anaemariae* appeared closer to each other in morphospace than

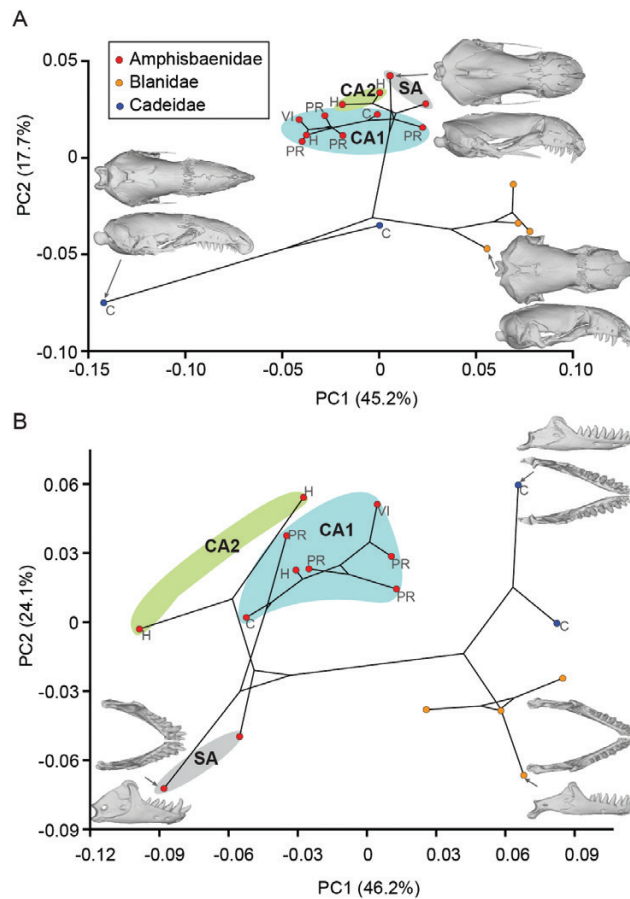


Figure 3. Phylomorphospace of shape variation in (A) crania and (B) mandibles of Caribbean amphisbaenians and their closest relatives, coloured by family. Shaded areas correspond to the Caribbean clades CA1 and CA2, and their South American relatives (SA) as shown in Figure 1B. Example skulls of species at the ends of each axis illustrate the extremes of morphological variation in our sample: *Amphisbaena anamariae*, *Blanus strauchi* and *Cadea palirostrata*. Island of occurrence for each Caribbean species is shown as abbreviations (C, Cuba; H, Hispaniola; PR, Puerto Rico; VI, Virgin Islands).

they did to either of their nearest relatives in the Caribbean clades (CA1 and CA2, respectively).

SKULL INTEGRATION AND PHYLOGENETIC SIGNAL

We found a high degree of phylogenetic morphological integration in amphisbaenian skulls ($r_{\text{PLS}} = 0.99$, $P < 0.001$), indicating tight covariation between cranial and mandibular shape among species. Covariation between partitions revealed a gradient from the round-headed South American taxon *A. fuliginosa* on the far right side of the PLS plot to its congener, *A. xera* from Puerto Rico, on the far left (Fig. 4). This shift described variation from species with tall blunt snouts and robust compact jaws (negative PLS scores) to those with thinner skulls possessing more elongate pointed snouts coupled with narrow, vertically shortened jaws (positive PLS scores). In contrast to the PCA plots, no obvious phylogenetic structure was observed in patterns of skull integration, with the two South American species falling on opposite sides of the PLS axes and members of both Caribbean clades being widely scattered. Similar phylogenetic signal was also observed in each of the skull components ($K_{\text{mult}} \text{ crania} = 0.54$, $P = 0.003$; $K_{\text{mult}} \text{ mandibles} = 0.58$, $P < 0.0001$), although both values were lower than expected under a null (BM) model of evolution.

EFFECT OF BIOGEOGRAPHY AND EVOLUTIONARY HISTORY ON SKULL SHAPE

Significant variation in skull morphology was detected at the family level for both crania and mandibles, whereas location of occurrence had no effect on skull shape divergence among species (Table 2). No significant shape differences were found within the family Amphisbaenidae, either among localities

(Caribbean Islands, South America) or between subclades, despite their deep (43–23 Myr; Vidal *et al.*, 2008) evolutionary divergences (Table 2). These results were consistent with the PC plots in Figure 3, in which members of Amphisbaenidae from different islands exhibited substantial overlap in cranial and mandibular morphospace. A post-hoc pairwise comparison of least squares means between families revealed that blandids differed from both amphisbaenids and cadeids in cranial and mandibular shape, while the latter two were not significantly different from one other. Pairwise results are given on maps of the Caribbean and mainland regions with soil textures describing the relative proportions of silt, sand and clay in topsoil (0–30 cm depth; Fig. 5) as listed in the Harmonized World Soil Database v1.2 (FAO/IIASA/ISRIC/ISS-CAS/JRC, 2009).

DISCUSSION

The ecological opportunity theory predicts that populations freed from competitive pressure, such as through invasion of novel habitats or the evolution of key innovations, will experience an ecological release characterized by rapid speciation and phenotypic diversification (Schluter, 2000; Yoder *et al.*, 2010). Our results, based on the first GM analysis of skull shape across amphisbaenian species, indicate that worm lizards have failed to undergo significant diversification in skull morphology within the Greater Antilles, despite multiple colonization events, phylogenetic lineages and ecological (burrowing) behaviours. The only significant differences in skull shape were found between mainland Mediterranean and insular taxa, suggesting that fossorial vertebrates may be restricted in their ability to respond to novel resources (e.g. soil

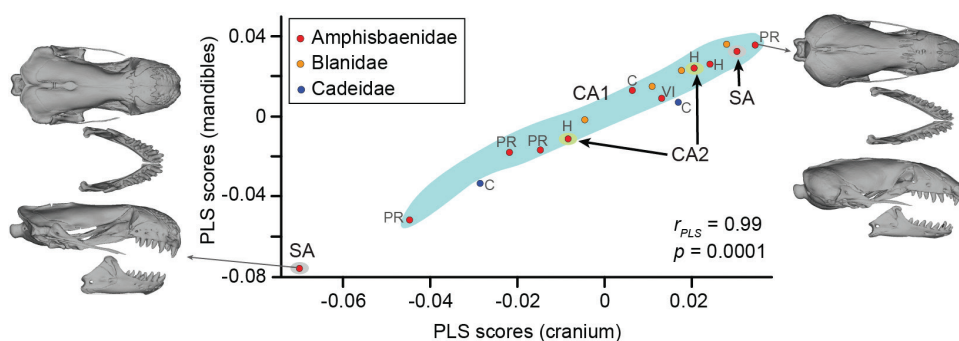


Figure 4. Plot of species scores along the first partial least squares (PLS) axes for cranial vs. mandibular shape in Caribbean amphisbaenians and their relatives, coloured by family. Shaded areas correspond to the Caribbean clades CA1 and CA2, and their South American relatives (SA) as shown in Figure 1B. Examples of species skulls, separated into crania and mandibles in dorsal and right lateral view, are shown for *Amphisbaena fuliginosa* (negative PLS scores) and *A. xera* (positive PLS scores). Island occurrence of each Caribbean species is shown as abbreviations (C, Cuba; H, Hispaniola; PR, Puerto Rico; VI, Virgin Islands).

types, prey) encountered in island habitats. This observation is in direct contrast to diversification patterns of *Anolis* lizards occupying the same islands, which experienced rapid early accumulation of lineages and bursts of phenotypic evolution following colonization from mainland South America in a similar time frame (Mahler *et al.*, 2010).

Table 2. Results of phylogenetic ANOVA of cranial and mandibular shape for (A) all species and (B) amphisbaenids only

	d.f.	SS	MS	R ²	F	Z	P
(A) All species							
Crania:							
location	4	0.013	0.003	0.262	1.067	1.274	0.167
family	2	0.008	0.004	0.176	1.496	4.065	< 0.0001
Mandibles:							
location	4	0.01	0.003	0.289	1.222	1.425	0.108
family	2	0.007	0.003	0.195	1.697	4.426	< 0.001
(B) Amphisbaenidae							
Crania:							
location	3	0.006	0.002	0.253	0.79	0.708	0.592
clade	2	0.005	0.002	0.191	0.943	1.181	0.215
Mandibles:							
location	3	0.005	0.002	0.223	0.671	0.551	0.676
clade	2	0.004	0.002	0.169	0.812	0.934	0.34

Factors are location of occurrence and family for the dataset including all species, and location of occurrence and clade (CA1, CA2, South American; Fig. 1B) for Amphisbaenidae.

Within the Greater Antilles, only Cuban cadeids appeared dramatically distinct, in having strongly laterally compressed snouts unlike the other round-headed forms. The two cadeid species also differed from one other in patterns of skull shape, falling distantly apart in cranial and mandibular morphospace (Fig. 3). Although these species are united by general confirmation of head shields and number of teeth (Dickerson, 1916), *C. blanoides* possesses an obviously wider and more cylindrical head than *C. palirostrata*, which together with extensive variation in other traits (e.g. numbers of dorsal annuli, anal segments and preanal pores) has led some authors to believe that they may be unrelated (Barbour & Ramsden, 1919; Zug & Schwartz, 1958). At the same time, *C. blanoides* appeared no closer in morphospace to other Caribbean (or Cuban) taxa, and was most similar to Mediterranean blanids in overall skull shape, further corroborating their sister group relationship (e.g. Vidal *et al.*, 2008; Zheng & Wiens, 2016). Determining whether *C. palirostrata* is also a member of Cadeidae or if its divergent morphology represents an entirely distinct genus or even family must therefore await further, ideally molecular-based, investigations.

Proportional changes were not uniformly expressed by different functional units of the skull, despite their strong phylogenetic signal and morphological integration. The shape of the lower jaws generally mirrored that of the facial (snout) region, in being either wide and robust or narrow and gracile (Fig. 4). This relationship varied, however, in the extent of

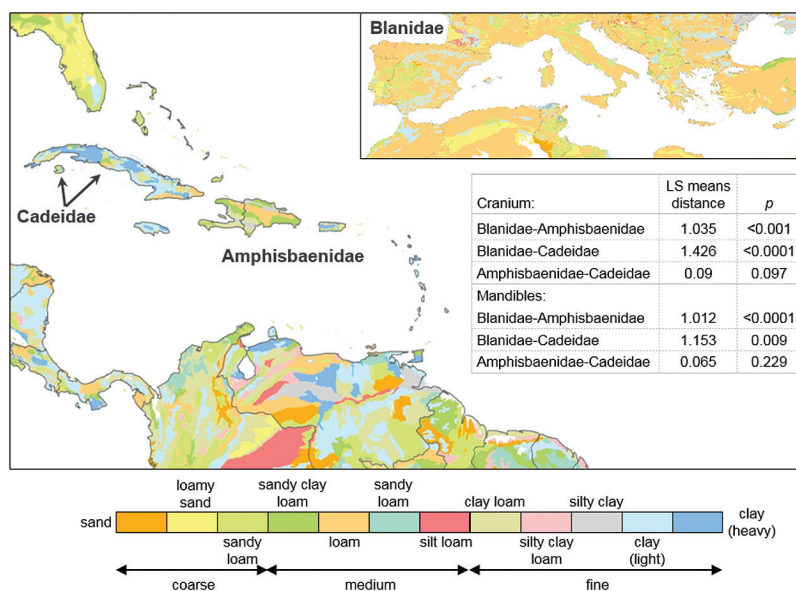


Figure 5. Map of the Caribbean and Mediterranean (inset) regions with soil texture class colour-coded in 30 arc-second (~1 km²) resolution. Pairwise Procrustes distances and associated P-values are given in the table as least squares (LS) means between families for the cranium and mandibles.

the coronoid process and compound bones, which for members of Amphisbaenidae tended to be taller than for other groups (Fig. 3B). Among amphisbaenians, snout shape is highly correlated with specific excavatory movements, with round-headed forms using a forward-driving stroke to penetrate and compress the soil, while keel-headed forms swing their snouts laterally side to side (Gans, 1968). The mandibles, in contrast, are directly involved in biting and mastication, which for most worm lizards occurs inside the burrow where they use specialized sensory systems and interlocking dentition to find and crush arthropod prey (Gans, 1968, 1978). Individuals with narrower heads have been shown to be more efficient at digging (the energetic costs of which increase exponentially with body diameter; Navas *et al.*, 2004), but are also associated with a weaker bite force, potentially restricting the dietary spectrum to softer prey (Baeckens *et al.*, 2017). These conflicting selective pressures are thought to limit the evolution of skull shape in head-first burrowers, by imposing a trade-off between diet and locomotory performance (Barros *et al.*, 2011; Vanhooydonck *et al.*, 2011).

Although bite capacity has only been recorded in a single amphisbaenian species (the unusually molluscivorous *Trogonophis wiegmanni*), they produced a remarkably strong bite force when compared to other lizards of similar size, even after accounting for phylogenetic relationships (Baeckens *et al.*, 2017). These results suggest that some fossorial taxa have evolved alternative muscle architecture or physiology to facilitate a crushing bite while maintaining adequately narrow skulls for digging. Limbless caecilians (Gymnophiona) provide a primary example of fossorial animals overcoming this dilemma, by developing a unique accessory jaw-closing muscle to compensate for the limited range of motion imposed on the jaw joint (Nussbaum, 1983; Kleinteich *et al.*, 2008). Likewise, certain amphisbaenians (e.g. amphisbaenids) may have evolved adaptations to feed on hard-bodied prey in fossorial environments, such as an increase in the surface area of the coronoid and compound bones that serve as attachment sites of the jaw adductor (chewing) muscles (Daza *et al.*, 2011). Posterior extension of the retroarticular process, observed in both the cadeids and blanids sampled in our study (Fig. 3B), may serve a similar purpose by providing greater area for insertion of the pterygoid muscles involved in protraction of the lower jaw (Daza *et al.*, 2011), as well as a longer outlever as seen in caecilians and other insectivorous and omnivorous lizards (e.g. McBrayer, 2004; Kleinteich *et al.*, 2008; Barros *et al.*, 2011; Fabre *et al.*, 2014). Dietary observations of Caribbean amphisbaenians are few, but suggest opportunist feeding on subterranean arthropods such as termites and beetle larvae (Cusumano & Powell, 1991; White *et al.*, 1992).

It would therefore be interesting to test if the hardness of encountered prey types across the Caribbean and mainland regions correlates with lower jaw morphology, thus explaining differences in mandibular shape.

Regardless of slight variation in cranial and mandibular shape, we found no support for adaptive diversification in skull morphology of Caribbean amphisbaenians. Only Mediterranean blanids differed from the other two families, with no significant differences in skull shape among Caribbean Islands or between independent Caribbean radiations (Table 2). The presence of the extreme keel-headed *C. palirostrata*, as well as the distant relationship between blanids and amphisbaenids, probably explains the first observation, although the absence of significant morphological variation within insular amphisbaenians is surprising, given that (1) they comprise at least three separate colonization events of different ages and regions (*Cadea* on Cuba, CA1 on all islands except Jamaica, and CA2 on Hispaniola; Fig. 1), (2) inhabited islands vary in soil texture and proportions of sand, silt and clay (Fig. 5), both features known to influence burrowing energetics and type and abundance of invertebrate prey (Martín *et al.*, 1991; Civantos *et al.*, 2003; Navas *et al.*, 2004; Barros *et al.*, 2011; Wu *et al.*, 2015), and (3) skull shape among amphisbaenians is notoriously homoplasious (Kearney, 2003a; Gauthier *et al.*, 2012; Müller *et al.*, 2016), indicating that worm lizards are capable of evolving new morphologies in various lineages and ecological contexts.

Our observations are based on relatively small sample sizes of individuals per species (1–4; Table 1) and also exclude several Caribbean taxa: *Amphisbaena barbouri* and *A. carlgansi* from Cuba, as well as four amphisbaenids from Hispaniola (*A. caudalis*, *A. cayemite*, *A. gonavensis*, *A. leali*). Although additional data must be collected, superficial descriptions of these species characterize them as round-headed forms with only subtle morphological differences (i.e. scalation, tail-to-body length ratio, coloration) distinguishing them from sympatric congeners (Thomas & Hedges, 1998, 2006). We also did not consider sexual dimorphism in skull shape, which is hypothesized to be limited in head-first burrowers by the burrow/bite trade-off discussed above (Teodecki *et al.*, 1998; Heideman *et al.*, 2008). Evidence for sexual dimorphism in amphisbaenians is mixed (see Hipsley *et al.*, 2016, and references therein), although Baeckens *et al.* (2017) found no intersexual differences in head dimensions or bite force in *T. wiegmanni*, nor have intersexual diet or microhabitat differences been reported for other amphisbaenian species (e.g. Martín *et al.*, 1991; Civantos *et al.*, 2003; Kearney, 2003b; Balestrin & Cappellari, 2011), which would be expected if the larger of the sexes also possessed

a greater bite capacity. Although amphisbaenians are typically limited in herpetological collections due to the difficulties in finding small fossorial animals, targeted collecting efforts and detailed inter- (and intra-) specific investigations may provide more information on the ecologies and habits of these secretive reptiles. Potential sampling issues notwithstanding, our results suggest strong evolutionary constraints on skull shape of Caribbean Island amphisbaenians.

EVOLUTIONARY CONSERVATION OF SKULL SHAPE IN INSULAR HEAD-FIRST BURROWERS

Several factors could account for the lack of morphological variation observed in insular amphisbaenians. First, adaptive radiation requires both the proliferation of species and diversification in resource use to fill divergent ecological niches (Stroud & Losos, 2016). Compared to other lizard groups within the Greater Antilles, amphisbaenians account for a minor proportion (< 4%) of the total squamate diversity, despite having a substantial temporal window during which to undergo *in situ* diversification (Vidal *et al.*, 2008; Zheng & Wiens, 2016). They are also conspicuously absent from Jamaica, an island similar in size to Puerto Rico but with fewer endemic lizards (Hedges, 2018). Why these disparities in biogeographical patterns occur among Greater Antillean lizards is unclear, although it seems that not all clades will radiate in the presence of ecological opportunity (Stroud & Losos, 2016). For example, many animal groups almost never speciate on islands smaller than a particular size (Coyne & Price, 2000; Pyron & Burbrink, 2014), including *Anolis* lizards on the Lesser Antilles (Losos & Schluter, 2000). It is also unknown whether rates of speciation among insular amphisbaenians following colonization exceeded those of their mainland relatives (a benchmark of adaptive radiation; Schluter, 2000), particularly as the Caribbean radiation appears non-monophyletic and probably arose from multiple overseas dispersal events (Vidal *et al.*, 2008; Longrich *et al.*, 2015).

Another explanation for the lack of morphological variation is that ecological opportunity is not actually present (Stroud & Losos, 2016). In the case of fossorial reptiles, other earlier-colonizing vertebrates may have already exploited available resources such as excavatable soils or invertebrate prey, thus hindering the diversification of later arrivals. Within the Greater Antilles, several other insectivorous groups with fossorial or semi-fossorial ecologies exist, including solendons and the recently extinct *Nesophontes* (shrew-like mammals), the Cuban night lizard *Cricosaura typica* (Xantusiidae), blind snakes (Typhlopidae) and thread snakes (Leptotyphlopidae). Molecular divergence estimates for thread snakes indicate that leptotyphlopids arrived in the West Indies after amphisbaenians (~34 Mya),

with the Hispaniolan ancestor diversifying *in situ* starting 16–10 Mya (Adalsteinsson *et al.*, 2009). At least among carnivorous mammals, low productivity of the subterranean ecotope is associated with strong resource competition for vertebrate prey, leading to the evolution of cooperative social systems and group-living when compared to ecologically similar non-fossorial species (Noonan *et al.*, 2015). In contrast, burrowing insectivores such as worm lizards encounter a range of diggable soil types and arthropod prey in their environments (López *et al.*, 1991; Martín *et al.*, 1991, 2013; Civantos *et al.*, 2003; Kearney, 2003b; Baeckens *et al.*, 2017), making it unlikely that the fossorial niche is limiting in terms of resource partitioning.

Alternatively, ecological opportunity itself may differ across taxa, meaning that the spectrum of exploitable resources is not the same for all species. While most worm lizards are thought to be generalist insectivores (Gomes *et al.*, 2009; Balestrin & Cappellari, 2011), some species exhibit a narrow dietary niche indicating selective foraging of prey types or sizes. For example, in the African amphisbaenid *Trogonophis wiegmanni*, Baeckens *et al.* (2017) found that individuals with different head sizes have access to different gastropod prey, with larger worm lizards able to crush larger (and thus harder) snail shells while smaller ones enter the shell via the aperture to feed from inside. *Blanus cinereus*, one of the Mediterranean species included here, is also known to be a selective forager, favouring large insect larvae and avoiding certain types of ants (López *et al.*, 1991). Although dietary preferences are still unknown for most amphisbaenians, the diversity of resources available on the Greater Antilles may have provided ecological opportunities for some colonizers but not others, potentially contributing to differences in diversification patterns among worm lizards and other endemic squamate groups (e.g. *Anolis*, *Sphaerodactylus* geckos).

Finally, Caribbean amphisbaenians may have failed to radiate morphologically because they lack the ability to readily evolve into new forms. Variation in evolvability, the capability of populations to rapidly adapt to novel environments, is strongly linked to modularity, which describes the organization of biological entities as functional, loosely connected subunits (Clune *et al.*, 2013). For morphological traits, modularity can occur at different levels (i.e. genetic, developmental, evolutionary) and is considered the counterpart to morphological integration (Klingenberg, 2008). The extreme degree of integration detected in our dataset suggests that worm lizards may be less able to alter their skull morphology than species in which the crania and mandibles evolve more independently. As previously demonstrated in the African amphisbaenid *Cynisca leucura*, the snout is probably under strong stabilizing selection throughout ontogeny to maintain

adequate proportions for head-first digging, while the back of the skull grows longer and thinner to maximize burrowing efficiency (Hipsley *et al.*, 2016). While that study did not include the mandibles, similar patterns of morphological conservatism were observed in our dataset, with closely related species generally resembling one another in snout shape (Fig. 3A). Because evolutionary changes in morphological characters require changes in the developmental processes that produce them (Klingenberg, 1998), it is plausible that a conserved ontogenetic trajectory constrains size–shape relationships at higher taxonomic (intra- and inter-specific) levels, thus restricting organisms’ abilities to modify proportions among traits. At the same time, conserved allometries may themselves be the result of natural selection, such that the highly derived skull morphology of amphisbaenians represents an adaptive peak constrained by functional trade-offs that limit their ability to move to new such peaks (Crisp & Cook, 2012). For species in sandy soils where digging is easier, relaxation of selection on the skull may allow the evolution of alternative digging strategies and head shapes, potentially explaining the variety of forms found throughout the clade. For example, Hispaniolan soils are coarser in texture and contain less clay than those of the Caribbean Islands, while the Mediterranean region (home to *Blanus* and probable colonization source of caecilians) is generally sandier than the Caribbean and northern South America (Fig. 5).

The classic scenario of island radiation posits that most evolutionary differences between related taxa are accumulated in allopatry (via genetic drift or adaptations to locally divergent conditions), followed by secondary contact and competition for resources (Schluter, 2000; Stroud & Losos, 2016). Ecological opportunities encountered in novel environments spur nascent populations to further diverge in resource use, permitting coexistence and phenotypic diversification. For Caribbean Island anoles, partitioning of the environment into perch types has enabled sympatry of multiple reproductively isolated populations, probably through reduced predation pressure rather than adaptive divergence (Losos, 2009; Yoder *et al.*, 2010). Among fossorial lizards, dispersal limitations associated with limb reduction may also facilitate genetic differentiation of populations through reduced gene flow, promoting speciation and partitioning of habitats (Lee *et al.*, 2013). Although amphisbaenians are assumed to have low dispersal ability (so much so that their presence on either side of the Atlantic was contributed to continental drift; e.g. Kearney, 2003a; Hembree, 2006), molecular divergence estimates post-dating Gondwanan fragmentation demonstrate that transoceanic dispersal has played an important role in shaping their

biogeographical patterns (Vidal *et al.*, 2008; Longrich *et al.*, 2015). Colonization of the Caribbean as well as other offshore islands (e.g. Fernando de Noronha, Chafarinas Islands, Socotra; Gans, 2005) indicates an ability to cross marine barriers while buried in floating vegetation, and to establish viable populations once they reach there. At the same time, their poor species richness and constrained morphological variation within the Caribbean Islands suggest low evolvability and propensity to speciate when compared to other terrestrial insular fauna.

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APPENDICES

Appendix 1. Voucher numbers of landmarked specimens. FMNH, Field Museum of Natural History; MCZ, Museum of Comparative Zoology Harvard University; MZUSP, Museum of Zoology of the University of São Paulo; NMV, Museums Victoria; USNM, National Museum of Natural History; ZMB, Berlin Zoological Museum; ZSM, Zoologische Staatssammlung München.

Voucher number	Species
MZUSP 97171	<i>Amphisbaena anaemariae</i>
USNM 327157-172208	<i>A. bakeri</i>
ZMB 8949	<i>A. caeca</i>
ZMB 6904	<i>A. cubana</i>
ZMB 9383	<i>A. cubana</i>
ZMB 4346	<i>A. fenestrata</i>
NMV D6329	<i>A. fuliginosa</i>
ZMB 1369	<i>A. fuliginosa</i>
ZMB 1372	<i>A. fuliginosa</i>
ZMB 31950	<i>A. fuliginosa</i>
FMNH 264821-5345	<i>A. hyporissor</i>
FMNH 264828	<i>A. hyporissor</i>
FMNH 264829	<i>A. hyporissor</i>
MCZ 07864-66316	<i>A. innocens</i>
MCZ Y-18664 R-121829	<i>A. innocens</i>
MCZ Y-18734 R-121833	<i>A. innocens</i>
FMNH 264851	<i>A. manni</i>
FMNH 264852-3811	<i>A. manni</i>
USNM 327159-161376	<i>A. schmidtii</i>
USNM 327160-172209	<i>A. schmidtii</i>
FMNH 265021-3378	<i>A. xera</i>
USNM 212327-043708	<i>A. xera</i>
USNM 327161-101727	<i>A. xera</i>
ZMB 29178	<i>Blanus cinereus</i>
ZMB 9626b	<i>B. cinereus</i>

Appendix 1. Continued

Voucher number	Species
ZSM 204–1975	<i>B. cinereus</i>
ZSM 227–1975	<i>B. cinereus</i>
ZSM 27-1998-1	<i>B. mariae</i>
ZSM 27-1998-2	<i>B. mariae</i>
FMNH 109456	<i>B. mettetalii</i>
FMNH 109457	<i>B. mettetalii</i>
ZMB 14116	<i>B. strauchi</i>
ZSM 37-1993-1	<i>B. strauchi</i>
ZSM 37-1993-2	<i>B. strauchi</i>
ZSM 37-1993-3	<i>B. strauchi</i>
NMV D6274	<i>Cadea blanooides</i>
ZMB 10496	<i>C. blanooides</i>
ZMB 4082	<i>C. blanooides</i>
ZMB 9381	<i>C. blanooides</i>
MCZ R-13512	<i>C. palirostrata</i>
ZMB 30768	<i>C. palirostrata</i>

Appendix 2. Description of landmark locations. Paired landmarks are indicated by right and left (R, L). Landmark numbers preceded by 's' are sliding landmarks, consisting of ten points each.

Landmark	Description
Crania	
1, 2	anterior process of nasal (R, L)
3, 4	anteroventral process of nasal (R, L)
5, 6	posterolateral process of premaxilla (on outside of snout) (R, L)
7, 8	anteriolateral process of frontal (R, L)
9, 10	anterior process of prefrontal (R, L)
11, 12	dorsal process of maxilla (R, L)
13, 14	dorsal process of prefrontal (R, L)
15, 16	posteroventral process of maxilla (R, L)
17, 18	ventral process of prefrontal (inside orbit) (R, L)
19, 20	anterodorsal point of palatine (R, L)
21, 22	anterodorsal point of ectopterygoid (R, L)
23, 24	anteroventral process of pterygoid (R, L)
25, 26	anterodorsal process of pterygoid (R, L)
27	posterior junction of frontals
28, 29	posteroventral point of parietal (R, L)
30	posteroventral point of premaxilla (on midline)
31, 32	posteroventral process of premaxilla (R, L)
33, 34	posteriormost point of premaxillary tooth row (L, R)
35, 36	anteriormost point of maxillary tooth row (R, L)
37, 38	posteriormost point of maxillary tooth row (R, L)
39, 40	anteroventral process of ectopterygoid (R, L)
41, 42	anteroventral process of palatine/premaxilla (R, L)
43	anteroventral process occipital (on midline)
44, 45	posteriormost point of pterygoid (R, L)
46, 47	stapedial process (R, L)
48, 49	posterodorsal point of parietal (R, L)
50, 51	posteroventralmost point of frontal (along outside of orbit) (R, L)
52, 53	ventralmost point of occipital articular surface (R, L)

Appendix 2. Continued

Landmark	Description
s1: 54–63	anteriormost point of premaxillary tooth, along midline of dorsal premaxilla to posteroventral point of premaxilla
s2: 64–73	start landmark 43, along midline of ventral occipital to ventral base of occipital condyle
s3: 74–83	start landmark 27, along midline of sagittal crest to anterodorsal process of occipital
Mandibles	
1, 2	anteriormost point of tooth row (R, L)
3, 4	posteriormost point of tooth row (R, L)
5, 6	labial anteroventral process of coronoid (R, L)
7, 8	apex of coronoid process of dentary (R, L)
9, 10	labial posterior process of dentary (R, L)
11, 12	ventralmost point of dentary symphysis (R, L)
13, 14	lingual anterior process of coronoid (R, L)
15, 16	lingual anterior process of compound (R, L)
17, 18	lingual anterior process of angular (R, L)
19, 20	posterior process of angular (R, L)
21, 22	posterodorsal process of articular (R, L)
23, 24	posteroventral process of articular (R, L)
s1: 25–34, s2: 35–44	anterodorsal process of coronoid, along dorsal coronoid arch to posterodorsal process of coronoid (R, L)