

Additional discussion: 1) landmark selection; 2) morphometric regions and *Hox* expression domains**1) Landmark selection**

Our analysis quantifies vertebral morphology as a series of shape gradients along the anterior-posterior axis (Extended Data Fig. 1). Morphology varies as both a continuum of change in the relative size and shape of homologous structures and in the appearance or loss of discrete structures along the axis, and the landmarking scheme we use must capture both aspects of this variation (Extended Data Fig. 3). The method performs best when all structures that vary gradationally are included in the landmarking scheme, especially those that are unique to a particular region. Both the gradation within regions and differences in the gradations between regions are used by our SLR analysis of regionalization.

Because there are extensive taxonomic and intracolumnar differences in vertebral morphology between Squamata, *Alligator*, and *Mus* in our analysis, no single set of landmarks can adequately describe morphological variation in all three taxa¹. For example, squamate vertebrae can be characterized by variation in synapophyseal shape, but this structure is not present in mammals or crown archosaurs. We therefore chose the most parsimonious set of landmarks that best described the vertebral column of the taxon in question.

Twelve structurally homologous landmarks were used for squamates (Fig. 1A, Methods). For *Alligator* and *Mus* we used an overlapping but different set of 12 and 13 landmarks respectively (Extended Data Fig. 3, Extended Data Table 2). Landmarks for structures that are not present in all vertebrae were collapsed to their nearest homologous structure where they were absent as recommended by Ref. 2.

In *Alligator*, The transition from rib-bearing thoracic “dorsal” vertebrae with dia- and parapophyses located at the distal margins of transverse processes to lumbar vertebrae with ribless processes was described by translating the landmarks that define the thoracic apophyses to the distal edges of the lumbar process (Extended Data Fig. 3b). The structural and/or topographic homology of these two features is not precisely known, but the presence of rib projections on the first two lumbar vertebrae² in embryonic specimens suggests thoracic and lumbar transverse processes may be homologous.

2) Morphometric regions and *Hox* expression domains*Morphometric regional boundaries and Hox expression in Mus and Alligator*

In *Mus*, the four region model recovered to within one position the boundary between the cervical and thoracic regions and anterior expression boundary of *HoxC6*; the boundary in the anterior thoracic series corresponding to *HoxC8*; and exactly recovered the boundary between the thoracic and lumbar series corresponding to *Hox10* (Fig. 3). In *Alligator*, the four-region model recovered morphometric boundaries adjacent to the expression domain of *HoxA5* (ref. 3) which corresponds to the transition within the cervical series from ventrolateral to laterally oriented rib attachments. The model exactly recovered the transition of the vertebral parapophysis from the lateral margin of the centrum and neural arch to the transverse process between thoracic vertebrae two and three, which corresponds to anterior expression of *HoxA7* (ref. 3). The model additionally recovered the thoracic-lumbar transition, although *Hox* domains have not yet been mapped for this region in *Alligator* (Fig. 3).

Cervical-thoracic boundaries in squamates.

Complete *Hox* codes have been mapped for a only few of the taxa included in our analyses^{4,5}, and *Pantherophis* is the only snake taxon to have been comprehensively mapped for expression boundaries⁵. However, several additional taxa have partial *Hox* maps that allow for comparison of morphometric regions and expression domains for subregions of the primaxial skeleton. The first morphometric regional boundary in *Pogona vitticeps* occurs between vertebrae five and six (The first three vertebrae [Extended Data Fig. 4] plus the atlas-axis complex). This boundary corresponds to the anterior expression of *HoxC5* and *HoxB8* between the fifth and sixth prevertebra and expression of *HoxC6* between the sixth and seventh prevertebrae⁵ in developing embryos, all of which are associated with the primaxial cervical-thoracic transition⁶. The position of this boundary in *Pogona* is consistent with its location in the snake *Pantherophis*, the elongate, limb reduced *Eumeces*, and the limbed *Tupinambis* and *Physignathus*, which suggests that the location of the *Hox* expression boundary is conserved across these phenotypically divergent taxa.

Hox10 expression boundaries and morphometric regions in Pantherophis

The ~15 vertebral segment offset between the posterior morphometric boundary and *Hox10* expression in *Pantherophis* may be explained by vertebral count variation between our sample and *Hox*-mapped specimens⁵. Snakes demonstrate variation in precloacal vertebral counts associated with sexual dimorphism⁷. The formation of somites in the precaudal paraxial mesoderm and expression of *Hox* domains occurs antero-posteriorly both in timing and direction^{4,8,9} and changes in the relative numbers of precaudal vs. caudal vertebrae are sexually dimorphic⁷. As a result, if *Hox10* expression patterns for posterior precloacal morphology in snakes, then dimorphic shifts in the relative position of the cloacal along the axial skeleton may explain the offset between morphometric and *Hox* expression boundaries. The relative positions of both the *Hox10* anterior expression boundary and morphometric regional boundaries are nearly identical: the morphometric boundary is 81% (181/224) along the length of the precloacal vertebral column, the *Hox10* anterior boundary is 81% (195/242) along the length of the column in mapped specimens (Fig. 4 in Ref. 10), suggesting the differences in boundary position result from changes in total number of precloacal vertebrae. Because sex cannot be determined for domain-mapped embryos, relative changes in the position of *Hox10* expression based on sex cannot be directly examined. Additionally, at least one *Hox10* mapped late-stage embryonic specimen of *Pantherophis* showed anterior expression of *HoxA10* at prevertebral position 180 (Ref. 10, Fig. 4d), statistically corresponding to the regional boundary recovered in our analysis (Extended Data Fig. 5).

Data acquisition methods

Morphometric data was obtained by digitizing images of vertebral morphology in anterior view using Ref. 11.

Data sources for figures

Figure 2: References for consensus phylogeny

The consensus phylogeny in Figure 2 is based on molecular phylogenetic analyses of Squamata higher order clades¹², and multiple snake clades¹³⁻¹⁷.

Figure 4: Primaxial skeletal representations

Primaxial skeletal reconstructions from Figure 4 are based on specimen examination (Extended Data Table 1) and literature references¹⁸⁻²⁴. Reconstruction based on Ref. 21 is used by permission of the Society of Sedimentary Geology (SEPM).

Figure 4: Generalized ancestral *Hox* code

The generalized sequential pattern of *Hox* genes along the A-P axis in total clade amniotes is based on literature references^{3-5,25}.

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