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Use of scalation landmarks in geometric morphometrics of squamate reptiles: a comment on homology

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Geometric morphometrics (GM) is a powerful analytical approach for evaluating phenotypic variation relevant to taxonomy and systematics, and as with any statistical methodology, requires adherence to fundamental assumptions for inferences to be strictly valid. An important consideration for GM is how landmark configurations, which represent sets of anatomical loci for evaluating shape variation through Cartesian coordinates, relate to underlying homology (Zelditch *et al.* 1995; Polly 2008). Perhaps more so than with traditional morphometrics, anatomical homology is a crucial assumption for GM because of the mathematical and biological interpretations associated with shape change depicted by deformation grids, such as the thin plate spline (Klingenberg 2008; Zelditch *et al.* 2012). GM approaches are often used to analyze shapes or outlines of structures, which are not necessarily related to common ancestry, and in this respect GM approaches that use linear semi-landmarks and related methods are particularly amenable to evaluating primary homology, or raw similarity between structures (De Pinna 1991; Palci & Lee 2019). This relaxed interpretation of homology that focuses more on recognizable and repeatable landmarks is defensible so long as authors are clear regarding the purpose of the analyses and in defining their landmark configurations (Palci & Lee 2019). Secondary homology, or similarity due to common ancestry, can also be represented with GM methods and is often assumed to be reflected in fixed Type 1 (juxtaposition of tissues) or Type 2 (self-evident geometry) landmarks (Bookstein 1991).

The arrangement (i.e., developmental macro-patterning) of epidermal scales has long been paramount to the taxonomy of squamate reptiles (see Tomovic *et al* 2008). For traditional morphological analyses, standardized counts of scales (or scale rows) are defined based on their position relative to other anatomical landmarks, and collectively these traits are referred to as "scalation" or "scutellation." In general, scalation traits are perceived as meristic (although some are defined from a binary or multimodal perspective), and more emphasis is placed on standardization and repeatability rather than an explicit consideration of secondary homology. As a natural outgrowth of the popularity of GM methods, scalation traits have increasingly been used in taxonomic studies where the junctions of scale sutures often serve as fixed Type 1 landmarks (e.g., Kaliontzopoulou 2011; Sindaco *et al* 2014; Tamagnini *et al.* 2018). GM approaches are particularly appropriate for scalation landmarks placed on scale sutures that are present and invariant in number within the study group, such as the large scutate scales on the prefrontal region of the heads of colubrid snakes (Ruane 2015) and lacertid lizards (Edwards *et al.* 2016). Meaningful shape and size variation can be extracted from these types of landmark configurations, as the secondary homology of individual scutes and correspondence of landmarks is usually unambiguous. However, fixed landmarks placed on sutures of scales from rows or skin surfaces that vary in scale count or arrangement within the study group are usually intractable in terms of homology and have the potential to bias inferences from GM studies.

The gold standard for selecting landmarks based on the homology criterion in GM is that individual landmarks are repeatable and recognizable across all specimens of the study, and that they do not alter topological positions relative to other landmarks (Bookstein 1991; Zelditch *et al.* 2012). Selecting fixed loci from a variable meristic series of epidermal scales often violates both of these assumptions. We illustrate this point using the example of supralabial scales (i.e., the scales that line the periphery of the upper jaw) from the western rattlesnake (*Crotalus viridis*, Rafinesque 1818) species complex. *Crotalus viridis* was the subject of a recent taxonomic work using GM methods (Davis *et al.* 2016), and the study incorporated several landmarks defined by the authors as fixed and homologous from areas of the head with vari-

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able scale count and arrangement. As part of their landmark configuration, Davis *et al.* (2016: pp 5, Fig. 3) anchored the supralabial scale series with fixed loci at the first and last supralabial, as well as selected one fixed locus at the center of the series. The exemplar specimen had 14 total supralabials, meaning that the center landmark was positioned at a suture spanning seven scales from either the first or the last supralabial as counted from each direction (Fig. 1A). However, not mentioned in the study is the fact that supralabial scales vary from 12–19 within the *C. viridis* complex. Placing the same landmark on another specimen with, for example, 16 supralabial scales (Fig. 1B), requires the subjective decision of whether to place the center landmark seven scale sutures from the first supralabial, seven scale sutures from the last supralabial, or at the new center suture (eight scales from first and last supralabials in each direction), yielding three different options for positioning the same anatomical locus, which in this example differ by only two scales in the series. Because a single locus on one specimen cannot correspond to three loci on another specimen, the options are to alter the topological placement of either the left- or right-of-center landmark of the supralabial series relative to either of the terminal supralabial landmarks, or to place the landmark on the new center suture, which changes the topological placement of both terminal supralabial landmarks relative to the specimen with 14 supralabials (Fig. 1B).

Each of these choices requires different assumptions regarding the anatomical homology of individual epidermal scales within a variable series, and the problem is exacerbated by the high variability of supralabial (and other) scale counts in the *C. viridis* complex. The option of selecting a consistent anchor landmark will require a change in topological position for any specimens added to the dataset that have a different number of scales in the row. The option of selecting the suture that demarcates the center scales would be problematic for a series that is quasi-normally distributed and includes specimens with odd or even numbers of scales in a row. Such a practice alternately transforms the nature of the landmark from one that reflects putative secondary homology of individual scales (Type 1) to one concerned simply with the distance between anchor points (Type 3). With this example we do not intend to dispute the inferences of Davis *et al.* (2016) but point out that intractable homology of several fixed landmarks would render the study non-replicable or comparable for future efforts using their published configuration. This problem is exacerbated because the authors did not provide any explanation as to whether fixed scalation landmarks were anchored consistently relative to other fixed landmarks. Moreover, these decisions related to landmarks can alter interpretations of results by artificially increasing the variation associated with some scale series relative to other components of the landmark configuration. Given these considerations, a more justifiable approach for the above example would have been to evaluate the shape and relative length of the upper jaw using a series of linear semi-landmarks.

The historical success of traditional morphometric approaches in squamate taxonomy might be attributed to the observation that traits are often defined as scale counts from rows or specific regions of skin that serendipitously capture aspects of the underlying developmental mechanism of epidermal scale formation. Scale morphogenesis in squamates follows a reaction-diffusion model where various conserved signaling molecules (i.e., morphogens), such as Sonic hedgehog (*Shh*) and ectodysplasin A (*Eda*), diffuse at different rates from initiation spots at the dermal-epidermal interface (Maderson & Alibardi 2000; Chang *et al.* 2009; Musser *et al.* 2015). As a result of this diffusion process, periodic fluctuations in morphogen concentrations develop spatially, and at specific thresholds of concentration these chemicals interact with each other and with morphogens from the dermis (e.g., bone morphogenetic proteins [*BMPs*]) to signal the differentiation of the placode precursors of individual scales. These scale tracts are homologous to placode tracts that form hair and feather follicles in mammals and birds, respectively, but were only recently confirmed in embryonic squamate skin because of their temporal and spatial transience (Di-Poi & Milinkovitch 2016). The reaction-diffusion model predicts that random perturbations will amplify noise in the process, which is consistent with the unique individual outcomes of vertebrate skin patterning (Turing 1952).

From a developmental perspective, the number, size, and arrangement of scales over a specific area of skin surface is determined by (i) number and location of initiation spots for scale tracts, (ii) relative diffusion rates of morphogens, (iii) the manner of interaction of these morphogens, and (iv) concentration thresholds for placode differentiation. Therefore, by adjusting few parameters, the exquisite and unique scalation patterns of individuals and species could be changed considerably. Without explicit consideration of the reaction-diffusion process of scale patterning, GM methods that use arbitrary scale sutures as fixed Type 1 landmarks along variable scale tracts will tend to alter the topological position of landmarks relative to a holistic view of scalation across individuals of the study group. Depending on the choice of landmark placement (even if landmarks are selected consistently relative to one anchor point), and the amount of within group meristic variation that is compressed between fixed anchor points, both the amount of variation and the trajectories of shape change depicted by deformation grids have the potential to be biased at these landmarks.

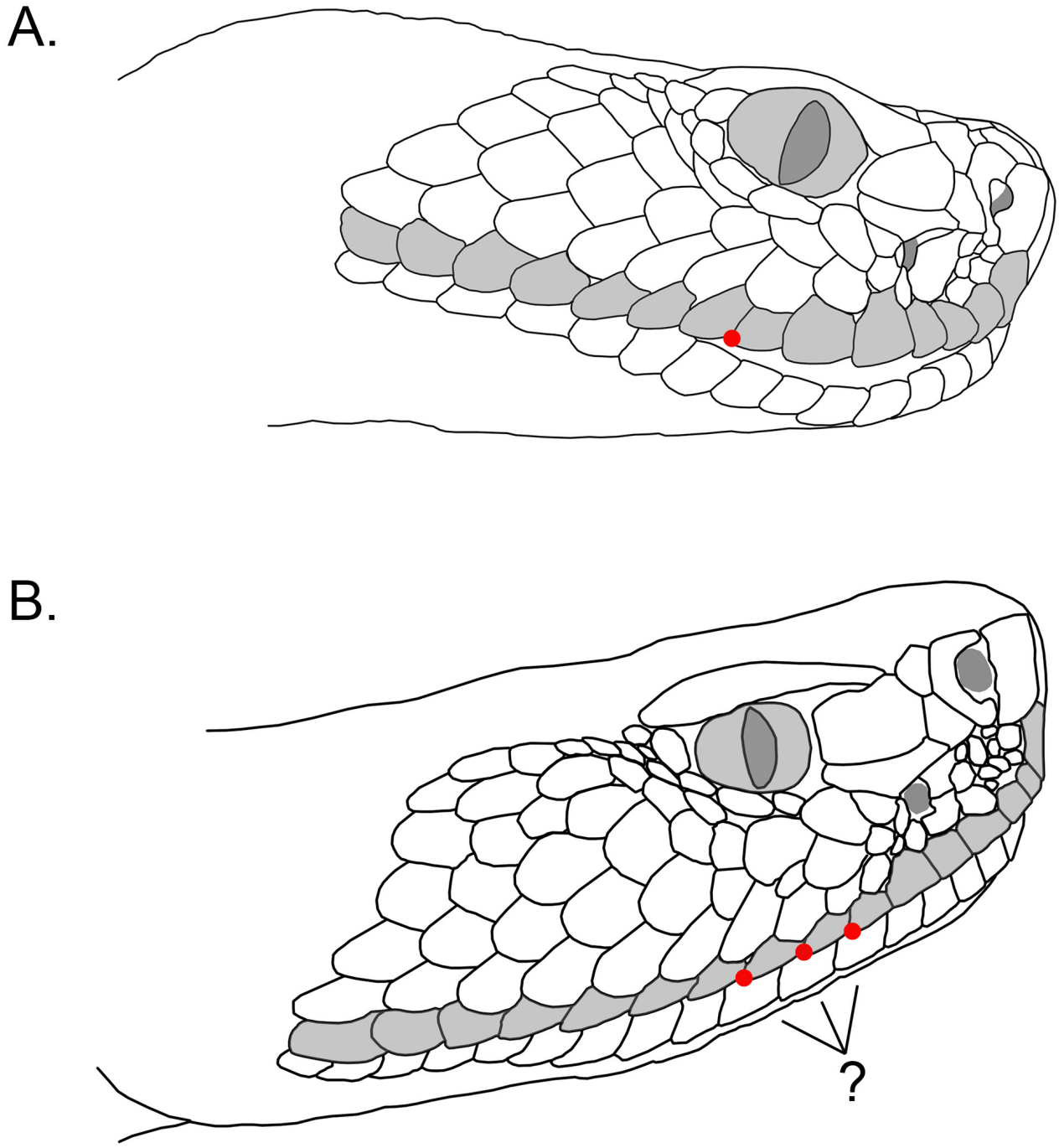

FIGURE 1. Diagrams of specimens of the *Crotalus viridis* complex with 14 (A) and 16 (B) supralabial scales, respectively (supralabial scale rows are shaded in grey). For (A), the red dot marks a fixed landmark at the center of the series, seven scales from the first and seven scales from the last supralabial scale. Placing a corresponding fixed landmark on specimen B requires that one choose between seven scales from the first supralabial, seven scales from the last supralabial, or the suture at the center of the series, providing three mutually exclusive options (red dots). Selecting any of these options changes the topological position of the landmark relative to the first, the last, or both of these scales in the series. Fixed landmarks applied to scale rows variable in count renders secondary homology intractable, regardless of whether scales are counted from a consistent anchor point.

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