



## Patterns of head shape and scutellation in *Drymarchon couperi* (Squamata: Colubridae) reveal a single species

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Krysko *et al.* (2016a) used analyses of DNA sequence data to reveal two genetic lineages of *Drymarchon couperi*. The Atlantic lineage contained specimens from southeastern Georgia and eastern peninsular Florida, and the Gulf Coast lineage contained specimens from western and southern peninsular Florida as well as western Florida, southern Alabama, and southern Mississippi. In a second paper Krysko *et al.* (2016b) analyzed morphological variation of the two lineages, which allowed them to restrict *D. couperi* to the Atlantic lineage and to describe the Gulf Coast lineage as a new species, *Drymarchon kolpobasileus*. This taxonomic discovery was remarkable for such a large, wide-ranging species and was notable for its impact on conservation. Because of population declines, particularly in western Florida, southern Alabama, and southern Mississippi, *D. couperi* (sensu lato) was listed as Threatened under the Endangered Species Act (United States Fish and Wildlife Service 1978, 2008) and repatriation of the species to areas where it had been extirpated was listed as a priority conservation goal (United States Fish and Wildlife Service 1982, 2008). Such repatriation efforts were attempted in Alabama, Florida, Georgia, and South Carolina, starting in 1977 (Speake *et al.* 1987), but failed to create viable populations, likely because too few snakes were released at too many sites (Guyer *et al.* 2019; Folt *et al.* 2019a). A second attempt at repatriation was started in 2010 and concentrated on release of snakes at a single site in Alabama (Stiles *et al.* 2013). However, Krysko *et al.* (2016a) criticized this repatriation effort because it appeared to involve release of *D. couperi* (sensu stricto) into the geographic region occupied by *D. kolpobasileus* (as diagnosed in Krysko *et al.* 2016b).

Under the species concept implemented by Krysko *et al.* (2016b), demonstration of reciprocal monophyly coupled with discovery of unique morphological features were deemed to be sufficient to discriminate *D. couperi* (sensu lato) into two species. Elsewhere, we document that the molecular data of Krysko *et al.* (2016a) derive a mitochondrial gene tree that fails to demonstrate reciprocal monophyly of two lineages and that extant populations of Eastern Indigo Snakes are comprised of 6–8 genetic populations characterized by significant population admixture (Folt *et al.* 2019b). Nevertheless, two distinct morphological phenotypes might be present. Although Krysko *et al.* (2016b) found no differences between the two lineages for eight scale characters typically used to diagnose snakes (snout-vent length, number of ventrals, number of subcaudals, number of supralabials, number of infralabials, number of temporals + preoculars + postoculars, number of dorsal scale rows one head length posterior to the head, and number of dorsal scale rows one head length anterior to the vent), five size-free linear measurements of head morphology (head length, head height, length of a temporal scale, length of the 7th infralabial, and width of the 7th infralabial) did allow differentiation. Strongest differentiation was associated with relative head length and height and relative scale length and width of the 7th infralabial. In particular, Atlantic lineage specimens were described to have long and deep heads with long and narrow 7th infralabials, and Gulf Coast lineage specimens were described to have short and narrow heads with short and wide 7th infralabials. Additional significant differentiation was associated with temporal length, with Atlantic lineage specimens having relatively elongate temporals and Gulf Coast specimens having relatively short temporals. Thus, measures of head, temporal, and infralabial

shape were used to diagnose each lineage as a separate species (Krysko *et al.* 2016b). As indicated in Enge and Krysko (2019), these diagnoses were intended for use in museum and field settings, where bivariate shape differences typically are used to separate species rather than summaries of subtle patterns detectable only in multivariate space.

In generating diagnoses for the two species, Krysko *et al.* (2016b) described temporals in both lineages to be 2+2 and invariant on each side but noted that "...many specimens had smaller divided scales" (Krysko *et al.* 2016b: 555). The 2+2 formula specifies the presence of a pair of anterior (one dorsal and one ventral) and a pair of posterior (one dorsal and one ventral) temporals (Savage 2002; fig. 1A). This condition was illustrated in Krysko *et al.* (2016b) for *Drymarchon melanurus*, but the condition occurred in none of their figures for *D. couperi* or *D. kolpobasileus*. Instead, figures for the two species of Eastern Indigo Snake showed an extra ventral anterior temporal ( $3_v+2$  condition; Fig. 1B; likely the source of the phrase quoted above; see also fig. 255 of Powell *et al.* 2012) or an extra dorsal and ventral anterior temporal (4+2 condition; Fig. 1C). When identifying the temporal scale that they measured, Krysko *et al.* (2016b, their fig. 3) highlighted the central-most dorsal temporal of an individual expressing the 4+2 condition, but when summarizing results of their temporal scale analyses they highlighted the dorsal posterior temporal of an Atlantic lineage individual expressing the  $3_v+2$  condition and the dorsal posterior temporal of a Gulf Coast lineage individual expressing the 4+2 condition (Krysko *et al.* 2016b, their fig. 5). Thus, it is unclear which temporal scale Krysko *et al.* (2016b) measured for specimens with only two dorsal temporals, and how measures of the central dorsal temporal allowed interpretation of unmeasured posterior temporals.

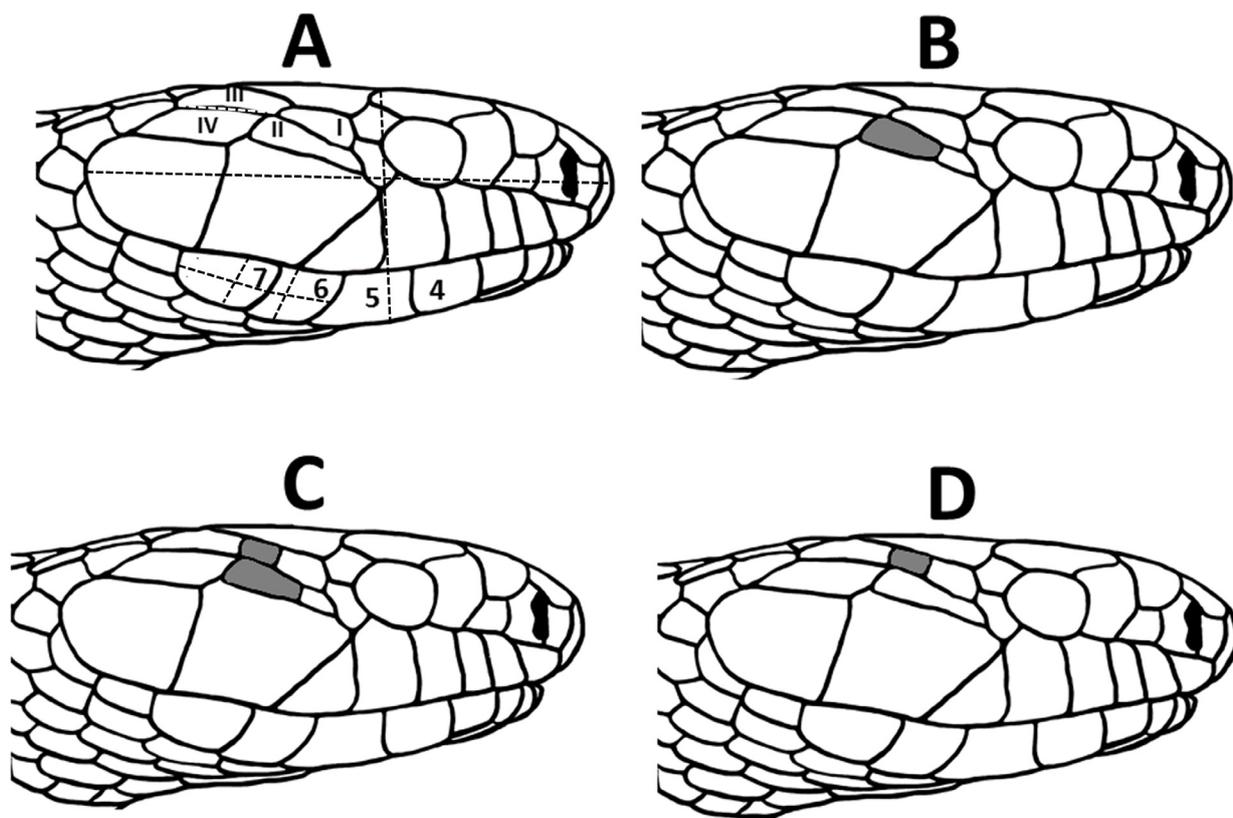
Examination of the infralabial character of Krysko *et al.* (2016b) reveals similar problems. The authors stated that the 7th infralabial was used in their morphological analyses, but they emphasized the 6th infralabial in the figure identifying this scale (excluding mental, as in fig. 60B of Palmer & Braswell 1995). This incongruity is evident because the 4th and 5th infralabials are noticeably enlarged in all specimens of *D. couperi* (sensu lato) that we have examined (see results below), a feature consistent with all figures in Krysko *et al.* (2016b), and the scale that they highlighted is adjacent to the 5th infralabial. Thus, it is unclear which scale was measured for this important character.

Despite the problems outlined above, the diagnoses of Krysko *et al.* (2016b) provide some basis that the two genetic lineages of *D. couperi* are morphologically diagnosable and can be distinguished in field and museum settings from bivariate examination of the 6<sup>th</sup> infralabial (wider than tall in *D. couperi*; taller than wide in *D. kolpobasileus*), dorsal posterior temporal (smaller ratio of scale width to length in *D. couperi* than in *D. kolpobasileus*), and head shape (long and deep in *D. couperi*; short and narrow in *D. kolpobasileus*). Below, we use a sample of live and preserved specimens from areas representing both lineages to test whether head shape, dorsal posterior temporal shape, and 6th or 7th infralabial shape separate the lineages in univariate or bivariate space.

We examined snakes housed at the Orianne Center for Indigo Conservation (OCIC) that were raised as stock for repatriation efforts. Our sample included 58 individuals likely representing all three genetic populations associated with the Atlantic lineage (Atkinson, Bryan, Evans, Long, Telfair, Wayne, and Wheeler counties, Georgia, USA) and 16 individuals likely representing two of three genetic populations of the Gulf Coast lineage (Citrus, Highlands, and Marion counties, Florida, USA; lineages from Krysko *et al.* 2016a; genetic populations from Folt *et al.* 2019b). We photographed lateral or dorsolateral aspects of the head of each specimen including a millimeter ruler for scale. From the images, we categorized the condition of the temporal scales for each specimen based on four character states created by the number and position of the scales (Fig. 1). We generated a contingency table providing counts of specimens in each of the four categories for each lineage. We used Chi-square analysis to determine whether the relative proportions of temporal scale categories differed between the Atlantic and Gulf Coast lineages. Additionally, we measured total head length (posterior-most point of 8th supralabial to anterior tip of rostral;  $n = 74$ ), head height (only for photos in lateral aspect; at level of anterior-most point of parietal suture;  $n = 35$ ), and length of the dorsal posterior-most temporal (intersection of ventral posterior-most temporal, dorsal posterior-most temporal, and adjacent first dorsal scale to intersection of ventral posterior-most temporal, dorsal posterior-most temporal, and adjacent ventral temporal;  $n = 74$ ; Fig. 1A). We measured all distances using Adobe Photoshop 6.0 with reference to the photographed ruler. We used analysis of covariance (ANCOVA) to test whether the linear relationship between head length and head height differed between Atlantic and Gulf Coast lineages. We divided the length of the dorsal posterior temporal by head length to control for effects of body size and used an analysis of variance to test whether adjusted temporal length differed among the four categories of temporal scales.

We also examined 11 preserved specimens in the Auburn University Museum collections (AUM 18437, 18444, 34209, 34216, 38868–69, 40744, 40750, 40752, 40815, and 42334). Because these snakes were from southeastern Georgia, we assumed them to belong to the Atlantic lineage. For these specimens, we measured length and width of the 6th and 7th infralabial scales with dial calipers (Fig. 1A). We measured both scales because it was not clear which of these was

measured by Krysko *et al.* (2016b). Additionally, we used photos of the type specimens presented in Krysko *et al.* (2016b) to determine length and width of the 6th and 7th infralabial scales using Adobe Photoshop 6.0. A length-to-width ratio was then calculated for each specimen. Mean differences between 6th and 7th infralabials were tested as a paired t-test. Differences between our sample of Atlantic lineage snakes and the type specimens was determined by visual inspection. We used SAS v.9.4 for all morphological analyses (SAS Institute, Inc 2013) with  $\alpha = 0.05$ .

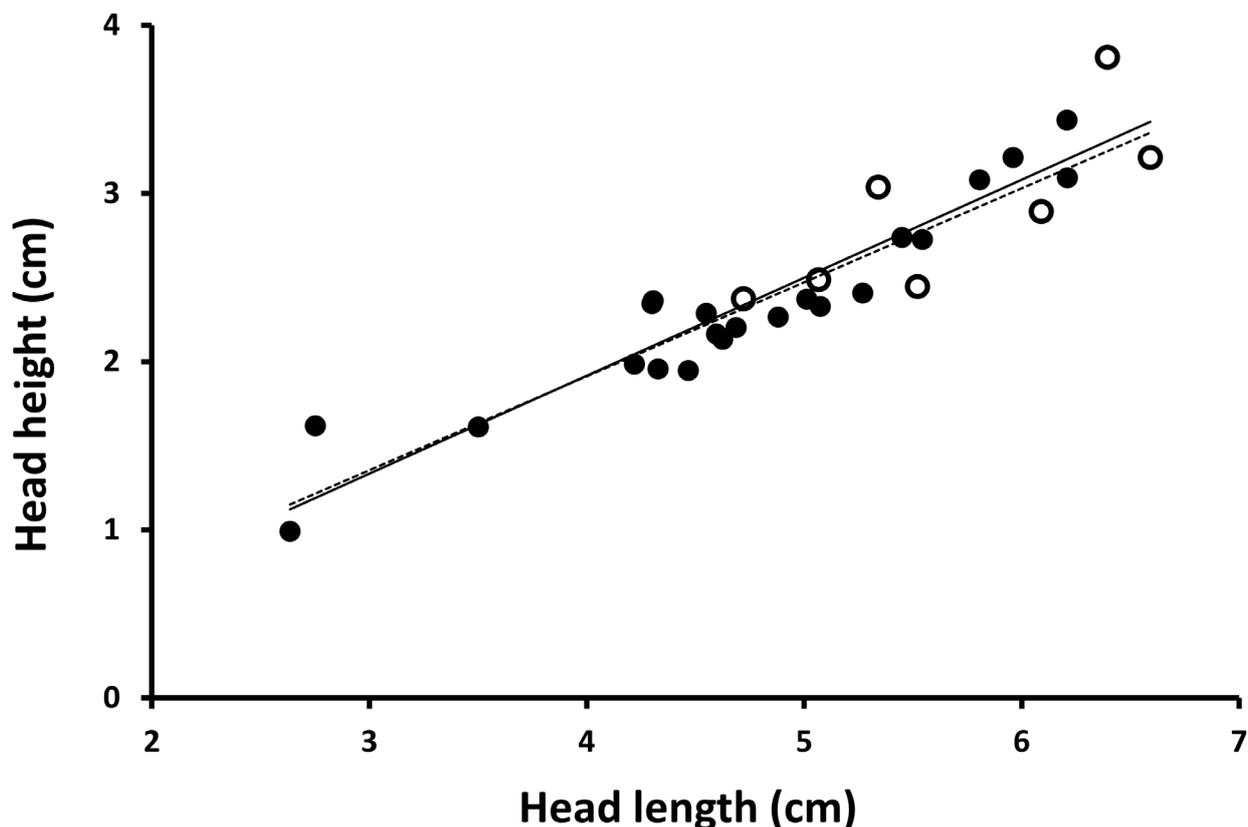


**FIGURE 1.** Head scale patterns in Eastern Indigo Snakes (*Drymarchon couperi*). A) 2+2 condition of temporals (I = dorsal anterior temporal; II = ventral anterior temporal; III = dorsal posterior temporal; IV = ventral posterior temporal) and position of 4th, 5th, 6th, and 7th infralabials; dashed lines represent linear measurements described in the text; B)  $3_v+2$  condition of temporals (extra ventral temporal shaded); C)  $4+2$  condition of temporals (extra dorsal and ventral temporal shaded); D)  $3_d+2$  condition of temporals (extra dorsal temporal shaded).

Our sample of OCIC specimens recovered four categories of temporal scales from both Atlantic and Gulf Coast lineages (Table 1). In 24% of specimens, temporals conformed to the 2+2 formula that Krysko *et al.* (2016b) described as being invariant (Fig. 1A), with 38% of specimens exhibiting an extra ventral temporal (Fig. 1B), 23% of specimens having extra dorsal and ventral temporals (Fig. 1C), and 15% of specimens exhibiting an extra dorsal temporal (Fig. 1D). The frequency with which these four categories occurred differed between Atlantic and Gulf Coast lineage specimens (Table 1;  $\chi^2 = 12.11$ ;  $df = 3$ ;  $p < 0.01$ ), with Atlantic lineage snakes tending to have conditions with two dorsal temporals and Gulf Coast lineage snakes tending to have conditions with three dorsal temporals. Head shape, based on ANCOVA of head height on head length, did not differ between Atlantic and Gulf Coast lineages in either slope (Fig. 2;  $F = 0.07$ ;  $df = 1$ ;  $p = 0.79$ ) or elevation ( $F = 0.48$ ;  $df = 1$ ;  $p = 0.49$ ). Length of the dorsal posterior-most temporal, expressed as a proportion of head length, differed significantly among temporal categories ( $F = 18.34$ ;  $df = 3$ ;  $p < 0.0001$ ), with the dorsal posterior-most temporal being proportionately shorter when three dorsal temporal scales are present relative to when two dorsal temporal scales are present (Fig. 3). When the length and width of the 6th and 7th infralabial scales were converted to a length-to-width ratio, the distribution of our sample of scales from Atlantic lineage snakes encompassed values for both type specimens for each scale (Fig. 4). Length-to-width ratios differed between 6th and 7th infralabials ( $t = 8.07$ ;  $df = 12$ ,  $p < 0.0001$ ), with 7th infralabials being more elongate than 6th infralabials.

**TABLE 1.** Frequency of occurrence of four character states for temporal scales in the Atlantic and Gulf lineages of Eastern Indigo Snakes.

Mitochondrial Clade	Scale Condition			
	2+2	3 <sub>v</sub> +2	3 <sub>d</sub> +2	4+2
Atlantic	13	26	10	9
Gulf	5	2	1	8



**FIGURE 2.** Bivariate plot of head height on head length. Values from Atlantic lineage indicated by solid circles and solid line; values from Gulf Coast lineage indicated by open circles and dashed line.

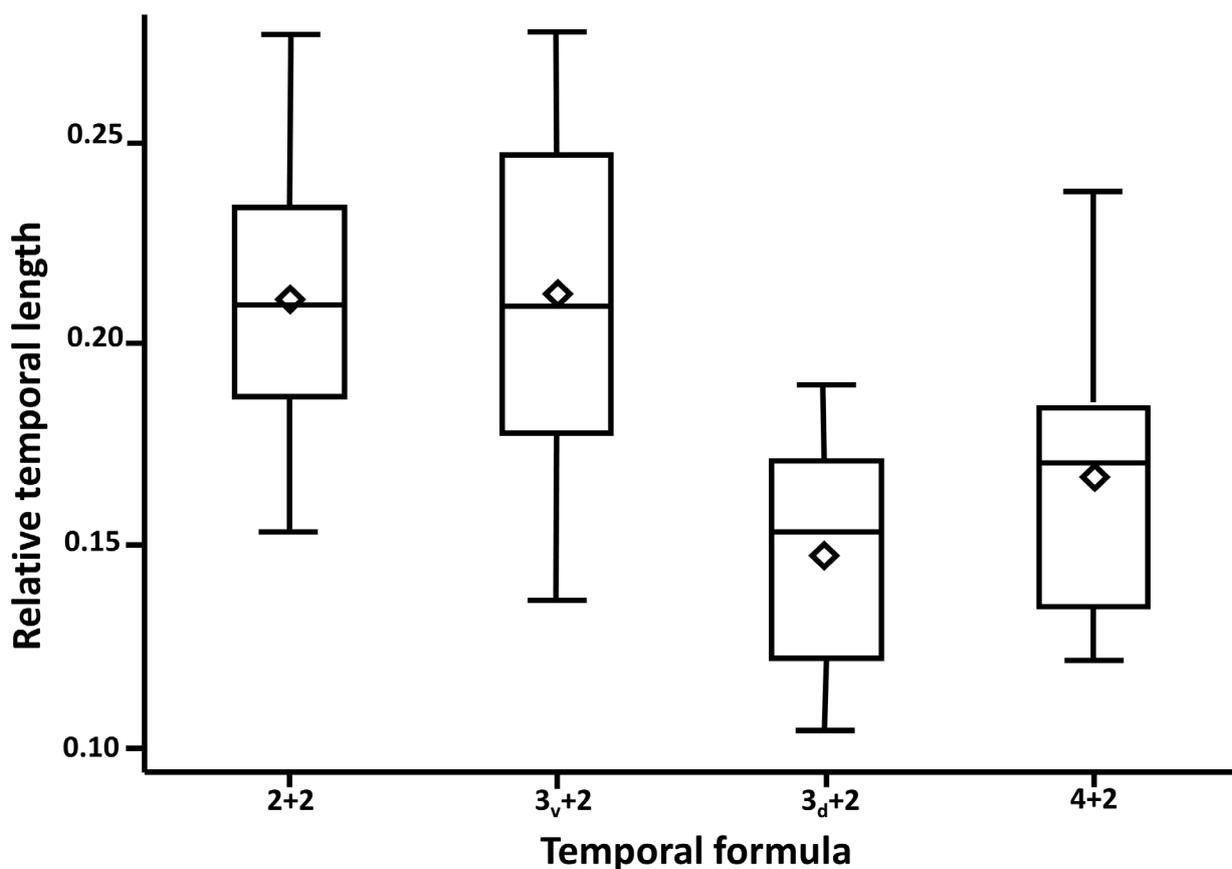
Our results reject the hypothesis that the Atlantic and Gulf Coast lineages of *D. couperi* (Krysko *et al.* 2016a) are identifiable on the basis of aforementioned phenotypic characters. We reach this conclusion after examining the variables used by Krysko *et al.* (2016b) to diagnose each lineage. Of the disparities that emerge between our analyses and theirs, the conformation of the infralabials is the most problematic. The figures presented by Krysko *et al.* (2016b) and Enge and Krysko (2019) for the 6th infralabial show great promise for diagnosing lineages. However, we were struck by how dissimilar the 6th infralabial of Atlantic specimens appeared to be from the long and thin scale shape ascribed to them by Krysko *et al.* (2016b, fig. 5A). Our analyses demonstrate that the 6th and 7th infralabials differ in shape, that the shape of the 7th infralabial conforms to the shape ascribed to the Atlantic lineage, and that the shape of the 6th infralabial conforms to that ascribed to the Gulf Coast lineage. Given that it is unclear which of these scales was measured by Krysko *et al.* (2016b) and that the range of variation of each scale within a sample of Atlantic lineage snakes encompasses both type specimens, we raise the possibility that Krysko *et al.* (2016b) intended to measure the 7th infralabial but inadvertently measured the 6th for Gulf Coast lineage specimens and the 7th for Atlantic lineage specimens. If the mental scale was included in the count for one lineage but not the other, this would provide a plausible explanation for their strong separation of the two lineages based on this scale and an inability for this to translate into a useful difference in character states in our analysis.

Our results for the temporal scale reveal great variation in the number of these scales present in Eastern Indigo Snakes. The four categories that characterize this variation are found in both Atlantic and Gulf Coast lineage snakes, in-

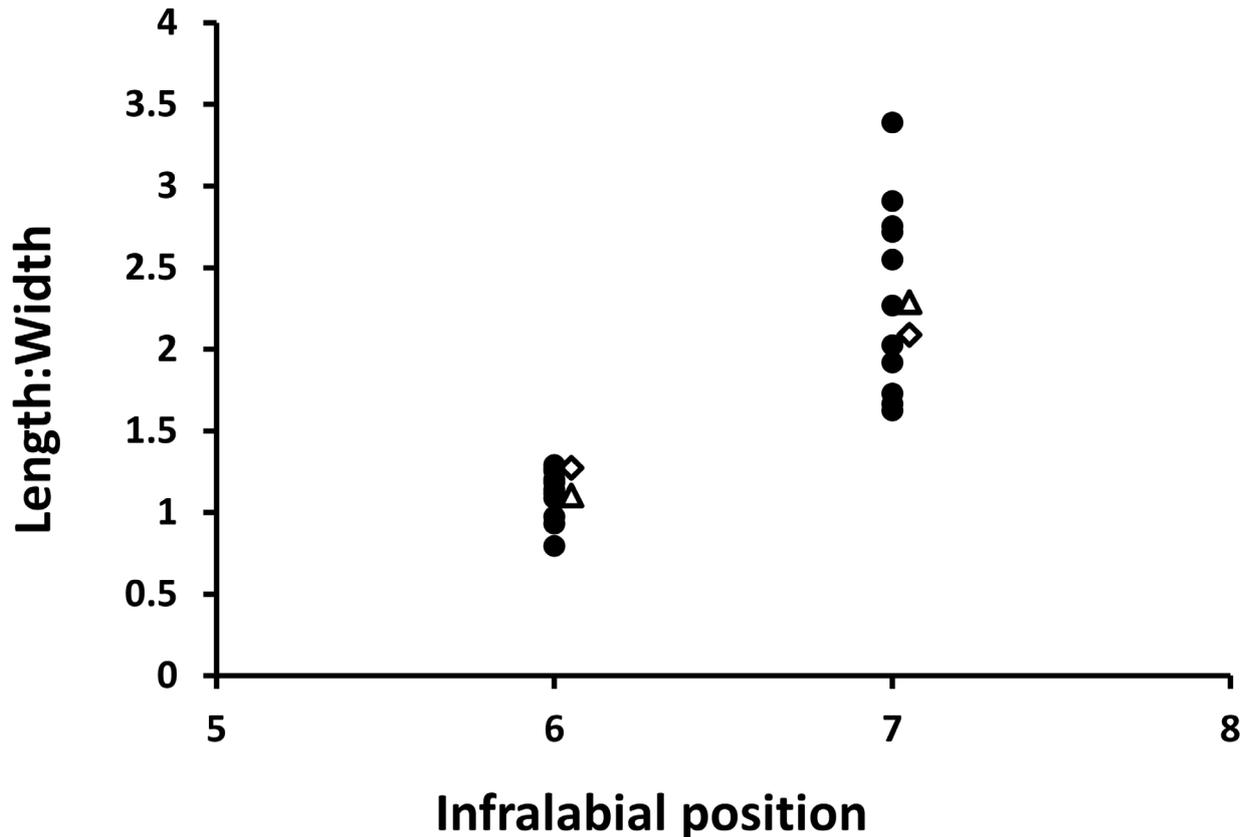
dicating that this feature is not diagnostic. Nevertheless, Atlantic lineage snakes tend to have two dorsal temporals while Gulf Coast lineage snakes tend to have three. We assume that Krysko *et al.* (2016b) intended to measure the dorsal posterior-most temporal and, therefore, we focused our attention on this scale. Our data indicate that the length of the dorsal posterior-most temporal, relative to head length, becomes shortened if three dorsal temporals are present and becomes elongate if two dorsal temporals are present. This finding indicates that the scale shapes revealed by Krysko *et al.* (2016b) represent distinguishable groups, but that the groups are created by numbers of temporal scales present rather than representing two species. We speculate that the different morphologies of the dorsal posterior-most temporal result because, during embryonic development of some individuals, the dorsal anterior temporal divides, limiting space for development of the dorsal posterior-most temporal. Because these two conditions of the dorsal temporals are present in both Atlantic and Gulf Coast lineages the two lineages cannot be diagnosed by relative lengths of temporal scales.

Krysko *et al.* (2016b) also used head shape to diagnose the two lineages, with Atlantic lineage snakes having an elongate deep head and Gulf Coast lineage snakes having a short narrow head. Our bivariate examination of head length and height revealed no difference in head shape between the two lineages. We have no convenient explanation for this disparity, except to note that head height is difficult to measure consistently. Specimens preserved with mouths open are likely to have larger values for head height than those with mouths closed. If the relative frequency of open-mouthed versus closed-mouthed specimens (or any other preservation artifact) differed between lineages, this might yield a spurious association of head shape with lineage. Our measurements were made from live specimens with closed mouths, which we infer reduces measurement error. If the lineages differed in a way that would allow separation of them, then our ANCOVA should have revealed this difference.

Along with evidence of extensive population genetic admixture and gene flow between the Atlantic and Gulf Coast lineages (see Folt *et al.* 2019b), our evaluation of morphological features finds further support for not recognizing *D. kolpobasileus* as a distinct species. Therefore, we formally propose here *Drymarchon kolpobasileus* to be placed in the synonymy of *Drymarchon couperi*.



**FIGURE 3.** Box and whiskers plot of distribution of ratio of dorsal posterior-most temporal scale length to head length in four categories of temporal scales (see Figure 1). Vertical lines indicate range; box indicates interquartile, horizontal line indicates median; open diamond indicates mean.



**FIGURE 4.** Distribution of length-to-width ratio of 6th and 7th infralabials in 11 Atlantic lineage specimens of *Drymarchon couperi* (dark spots). Open triangles indicate ratios from type specimen of *D. couperi* (Atlantic lineage); open diamonds indicate ratios from type specimen of *D. kolpobasileus* (Gulf Coast lineage).

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