1   **A kinematic analysis of *Micrurus* coral snakes reveals unexpected variation in stereotyped**

2   **anti-predator displays within a mimicry system**

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13   *locomotory motion, Peruvian Amazon, snake behaviour*

14   **Abstract**

15   1. Warning signals in chemically defended organisms are critical components of predator-prey

16   interactions, often requiring multiple coordinated display components for a signal to be effective.

17   When threatened by a predator, venomous coral snakes (genus *Micrurus*) display a vigorous,

18   non-locomotory thrashing behaviour that has been only qualitatively described. Given the high-

19   contrast and often colourful banding patterns of these snakes, this thrashing display is

20   hypothesized to be a key component of a complex aposematic signal under strong stabilizing

21   selection across species in a mimicry system.

22   2. By experimentally testing snake response across simulated predator cues, we analysed

23   variation in the presence and expression of a thrashing display across five species of South

24   American coral snakes.

25   3. Although the major features of the thrash display were conserved across species, we found

26   significant variation in the propensity to perform a display at all, the duration of thrashing, and

27   the curvature of snake bodies that was mediated by predator cue type, snake body size, and

28   species identity. We also found an interaction between curve magnitude and body location that

29   clearly shows which parts of the display vary most across individuals and species.

30   4. Our results suggest that contrary to the assumption in the literature that all species and

31   individuals perform the same display, a high degree of variation persists in thrashing behaviour

32   exhibited by *Micrurus* coral snakes despite presumably strong selection to converge on a

33   common signal. This quantitative behavioural characterization presents a new framework for

34   analysing the non-locomotory motions displayed by snakes in a broader ecological context,

35   especially for signalling systems with complex interaction across multiple modalities.

36   **Introduction**

37   Venomous prey animals often use conspicuous phenotypes to communicate their lethal

38   toxicity to potential predators (Ruxton 2018). These aposematic signals can be visual, chemical,

39   acoustic, or can involve complex interactions between multiple distinct components, such as

40   colour patterning and body motion (Rowe and Halpin 2013; Dalzeill and Welbergen). According

41   to the theory of mimicry, toxic prey animals can reinforce the aposematic signal to their

42   predators by converging on a common phenotype, even across multimodal components (*e.g.,*

43   conspicuous colour and behavioural display; Müller 1878; Wallace 1867; Sherratt 2008).

44   Coral snakes are highly venomous elapid snakes in the genus *Micrurus* that have been

45   described as a mimicry system using visual warning signals of their chemical defence (Campbell

46   and Lamar 2004). Most coral snake species are found in the Neotropics, with the highest

47   sympatric species richness in the Western Amazon Basin (Davis Rabosky et al. 2016a). Coral

48   snakes are well known for their conspicuous red and black coloration (Fig. 1A), a high-contrast

49   banding pattern that effectively creates an aposematic signal deterring avian (Smith 1975; Smith

50   1977) and potentially mammalian predators (Green and McDiarmid 1981; Savage and Slowinski

51   1992; Martins 1996; Buasso et al. 2006). In addition to their bright coloration, coral snakes

52   encountering a threat also produce a distinctive anti-predator display that includes elements such

53   as body flattening, intermittent thrashing, head hiding, and coiling of the tail, which is often

54   elevated and waved or "waggled" (Greene 1973; Greene 1979; Fig. 1A; Supp. Video 1). In some

55   species, this display is also accompanied by an auditory cloacal "popping" sound, eversion of the

56   hemipenes, and emission of cloacal musk and faeces (Greene 1973; Sazima and Augusto 1991).

57   Because such similar elements of this behavioural display have been reported across many coral

58   snake species in both Asia and the Americas (Brown et al. 2013), this anti-predator response is

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59   expected to have 1) an old origin that predates the arrival of this clade in the Western

60   Hemisphere ~35mya and 2) a significant genetic basis like other homologous traits derived from

61   shared ancestry (Wake et al. 2011). The main purpose of this display has been hypothesized as

62   inducing a surprisingly effective cognitive illusion that reduces the ability of the attacking

63   predator to identify and target the head (Roze, 1996). The intermittent, self-occluding thrashing

64   is described as the foundational display component driving this effect in all species examined.

65   However, individual-level variation in mimicry traits is also expected to exist, with

66   critical impacts on the ecological and evolutionary dynamics of mimicry over space and time.

67   Variation in the banding elements of coral snake colour pattern has been well documented (Davis

68   Rabosky et al 2016a, b) and experimentally tested using clay replicas for its effect on predator

69   deterrence (Brodie III 1993, Buasso et al. 2006, Kikuchi and Pfennig 2010). As previous authors

70   have noted, clay replica studies may not accurately reflect the true deterrence value of a given

71   phenotype because they exclude behaviour (Brodie III 1993). Behavioural movement has the

72   potential to greatly alter the perception of a colour pattern (Titcomb et al. 2013), suggesting that

73   colour pattern and non-locomotory behaviour in coral snakes may interact to produce a complex

74   anti-predator signal that is not fully explained by either individual component. However, neither

75   the drivers of variation in *Micrurus* anti-predator displays nor the relationship of behaviour to

76   colour pattern has ever been tested.

77   While snake locomotion has been quantitatively characterized in a variety of contexts

78   (Lissmann 1950; Jayne 1986; Moon and Gans 1998; Hu et al. 2009; Socha 2011, Titcomb et al.

79   2013), most studies of non-locomotory snake behaviours rely upon qualitative descriptions

80   (Arnold and Bennett 1984, Brodie III 1992). Here we present the first quantitative kinematic

81   analysis of non-locomotory anti-predator behaviours in *Micrurus* coral snakes and test for signal-

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82   reinforcing similarity within and among species, as predicted by mimicry theory. By

83   characterizing these behaviours in response to experimentally induced predator contexts, we

84   form a functional basis for understanding both the signalling mechanism of the aposematic

85   phenotype and the selective pressures shaping behavioural convergence among species in a

86   mimicry system.

87

88   **Methods**

89   *Data collection*

90   All animal-related procedures have been approved by the University of Michigan

91   Institutional Animal Care and Use Committee (Protocols #PRO00006234 and #PRO00008306)

92   and the Peruvian government SERFOR (Servicio Nacional Forestal y de Fauna Silvestre; permit

93   numbers: 029-2016-SERFOR-DGGSPFFS, 405-2016-SERFOR-DGGSPFFS, 116-2017-

94   SERFOR-DGGSPFFS). We collected data during five field expeditions in the Amazonian

95   lowlands of Peru from March 2016 to December 2018, at Villa Carmen, Los Amigos, Madre

96   Selva, and Santa Cruz Biological Stations (Fig. 1B). We captured snakes either in funnel traps or

97   opportunistically during transects, then transported the snakes in fabric bags secured within 20L

98   lidded buckets back to the station. During capture and handling, all trained personnel were

99   equipped with snake hooks, tongs, venom defender gloves (1-2-1 Products Ltd., Alfreton, UK),

100   and knee-high rubber boots to avoid envenomation.

101   We recorded anti-predator behaviour in a pop-up behavioural arena constructed of

102   corrugated plastic (Fig. 1C) illuminated by a string of led lights attached to the inner surface at

103   the top edge of the arena walls (see Davis Rabosky, Moore, et al. *submitted* for more details on

104   construction). We marked the inner surface of the arena with visual fiducial markings to aid in

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105   the removal of lens distortion and measurement. We covered the inner surface of the arena with

106   an adhesive transparent plastic film (Con-Tact, Rubbermaid) to facilitate thorough and rapid

107   cleansing and preserve the visual fiducial markings. Since previous research has shown that

108   snakes are physiologically affected by temperature, chemical cues, and light in an environment

109   (Schieffelin and de Quieroz 1991), it is likely that the behaviours exhibited in laboratory

110   environments and by captive individuals differ significantly from those exhibited under natural

111   conditions. Therefore, we made every effort to ensure similar experimental conditions for each

112   behavioural trial. After capture, snakes were kept undisturbed in bags for less than 24 hours

113   before behavioural trials and the inner surface of the arena was washed with unscented soap and

114   water to limit exposure to the chemical cues of previous experimental subjects.

115   We placed snakes into the arena, one at a time, and immediately began recording their

116   behaviour. After the first trial, we measured surface temperature of each individual with a Raytek

117   Raynger ST81 infrared temperature sensor. We recorded snake behaviour using either two or

118   three GoPro (San Mateo, California) Hero 4+ Black or three Hero 5+ Black cameras filming

119   from overhead and lateral views (see Fig. 1C for camera positions) at 30, 60, or 120 frames per

120   second, depending on the lighting conditions.

121   We used three different stimuli to elicit anti-predator behaviours: overhead looming,

122   pulsed vibration, and a tactile stimulus through physical contact. To simulate avian predation

123   threat, we quickly moved a piece of cloth across the top of the arena to create the visual looming

124   and pressure wave stimuli produced by a swooping bird. To simulate a large mammal predator,

125   we used the vibration produced by a cellular phone and placed it in contact with the arena. To

126   test for response after contact with a predator, we used a 1m snake hook to lightly tap the snake.

127 We randomized all treatments across individuals, and we recorded snake behaviours for up to

128 two minutes, allowing one minute of time to rest between the trials.

129 After behavioural testing, we either vouchered snakes into the University of Michigan

130 Museum of Zoology (UMMZ) or the Museo de Historia Natural in Lima, Peru (MUSM), or

131 released the snake at the point of capture. All vouchered snakes were also weighed for body

132 mass, measured for snout-vent length (SVL) and tail length, and sexed where possible. Field

133 numbers and museum accession numbers (when available) for each individual are reported in

134 Table 1.

135

136 *Video analysis*

137 We selected videos for analysis that included thrashing behaviour with minimal

138 translocation that stayed within the field of view of one camera (Fig. 1A). We used the Adobe

139 Premiere Pro (Adobe Systems, San Jose) built-in filter for GoPro Hero 4+ Wide angle to remove

140 lens distortion. We wrote custom Matlab (Mathworks, Natick, Massachusetts) code to perform a

141 projective transformation on each video frame, removing perspective to produce an image for

142 direct measurement.

143 We used QuicktimePro 7 to watch the videos frame-by-frame and recorded the first and

144 last frames that included motion as the start and end of each thrash. We used ImageJ to measure

145 the length of the snake in the video image, which was compared to measurements taken at the

146 time of vouchering. We traced the centreline of the snake body at the end of each bout of

147 thrashing with ImageJ (Fig. 2A).

148 We wrote custom Matlab code to analyse the centreline of the snake body. First, we

149 resampled the traced centreline to produce 500 evenly spaced points (Fig. 2B). We corrected for

150   noise in centreline tracing by smoothing the centreline using a moving average method with a

151   span of 30 points. Each point along this smoothed centreline can be thought of as a point on the

152   edge of a circle that provides the closest approximation to the body curvature at that point. The

153   angle of the tangent line of this circle may differ drastically from point to point in acutely curved

154   areas. To calculate the curvature at each point along the body, we measured the distance from the

155   focal point to a line formed by the tenth points to the left and right (Fig. 2C). The curvature,

156   commonly reported in radians per unit length, is a measurement of the tangent direction's

157   sensitivity among nearby points on a curve. Higher curvature values indicate more acute curves.

158   We did not compute the curvature for the anterior and posterior extrema with fewer than

159   ten points on a side (2% of body length). We used built-in Matlab functions to locate the areas of

160   local maximum curvature along the body of the snake (for example, the red dot in all panels of

161   Fig. 2). If multiple local peaks were recorded in a 30-point window, the curvatures and their

162   indices were averaged to one point.

163   To automatically determine whether a particular curvature vector was located to the right

164   or left of a snake's head, we wrote custom Matlab code to record the angle of each curvature

165   vector in a snake-centreline coordinate frame. We defined the temporary snake-centreline axis as

166   a vector pointing from the current point of interest towards its neighbouring point, in the

167   direction closer to the head of the snake. A curvature vector falling to the left of the snake-

168   centreline axis indicated a curve to the left of the snake's head (Fig. 2, red dot). With this method

169   for computing curve direction, we calculated the percentage of left or right instances of local

170   maximum curvature in each observation.

171

172   *Statistical approach*

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173   To maximize our inference ability and include information from all individuals, we first

174   assessed whether snakes displayed any anti-predator behaviour (e.g. thrashing, escaping, or head

175   hiding) in every recorded trial of *Micrurus* behaviour as a binary variable (presence or absence

176   of a response). For the individuals that did respond, we then assessed the expression of that

177   response using the number of thrashing events, their durations, and the body location and

178   direction of the curvatures in each post-thrash pose. For the purpose of statistical modelling, we

179   calculated the sum of the magnitudes of curvature for each of the 500 points along the body of

180   the snake for each post-thrash pose, and we quantified each individual's preference for a left or

181   right head kink by determining the direction of the most anterior curve across multiple

182   observations in the same trial. For each of these response variables, we constructed generalized

183   linear mixed models (GLMMs) to test for effect of species, body size (SVL), sex, and stimulus

184   type on the presence or expression of a response while accounting for individual collection ID as

185   a random effect because every individual was tested more than once. For binary (Y/N) response

186   tests of stimulus, we only included the treatments that had more than 5 observations per response

187   category (contact, looming, and vibration), which removed 12 trials of 160 total. We also tested

188   for the effect of collection site in the one species (*Micrurus lemniscatus*) that was collected from

189   all localities, as there was otherwise high variability/stochasticity in which species were found at

190   each collection site. If co-occurring species affect the anti-predator displays of individuals in a

191   locality due to mimetic local adaptation, we would expect *M. lemniscatus* to have the highest

192   behavioural variation because it was found sympatrically with every other species in our dataset.

193   Finally, we tested for decay of the thrashing signal over the course of a trial by regressing thrash

194   duration and sum of body curvature over time and assessing mean slope deviation from zero. All

195   statistical models were built in R v 3.6.1 using the package 'lme4' (Bates et al. 2015) for mixed

196   modelling, and significance was assessed at α=0.05 using number of groups in each GLMM as a

197   conservative estimate of the degrees of freedom denominator.

198   To determine the thrashing frequency for each species, we divided the total number of

199   observed thrashing events by the total amount of time each snake in the species was observed

200   while encountering the experimental stimulus. The total amount of time excludes trials in which

201   no thrashing was observed. Time in which the snake left the field of view was subtracted from

202   this total time. To then compare these metrics across species, we bootstrapped data by randomly

203   sampling with replacement from a combined dataset comprised of all individuals in a given

204   species. To maintain the differences in propensity to thrash, these bootstrapped values were

205   sampled in proportion to the thrashing frequency described above.

206

207   **Results**

208   *Overall behavioural response*

209   We recorded 160 behavioural trials in total across 25 snakes: 14 trials from 3 individuals

210   of *Micrurus annellatus*, 16 trials from 2 individuals of *M. hemprichii*, 71 trials from 12

211   individuals of *M. lemniscatus*, 30 trials from 4 individuals of *M. obscurus*, and 29 trials from 4

212   individuals of *M. surinamensis* (Table 1).

213   Not all individuals responded to all predator cues in our trials. We found that the

214   probability of response depended most strongly on an additive effect of both body size (binomial

215   GLMM; $F_{1,22}$= 5.732, P=0.026) and species ($F_{4,22}$= 5.907, P=0.002), with larger individuals and

216   *M. surinamensis* and *M. hemprichii* least likely to respond (Fig. 3). We did not find a significant

217   effect of collection locality (*M. lemniscatus* only, see Methods; $F_{2,12}$= 0.099, P=0.906) on the

218   probability of response, nor significant interaction between SVL and species ($F_{4,17}$= 0.665, P=

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219   0.625). We also found no effect of sex ($F_{1,22}$= 0.780, P= 0.387) or temperature ($F_{1,25}$= 1.474, P=

220   0.236) on display probability, although we note that most of our trials were conducted across a

221   narrow range of body temperatures between 25.1-27.8°C (q20-q80). Although our ability to run

222   some higher order multiple regressions was limited by our sample sizes, the effect of stimulus

223   type was marginally significant in a single fixed effect model ($F_{2,22}$= 3.278, P=0.057) and may

224   interact with other effects. Observationally, response to a contact stimulus produced a response

225   in every individual tested, but the probability of response varied across species and body sizes in

226   looming and vibration trials.

227   When analysing the thrashing display of the individuals that did respond to a predator cue,

228   we found that thrash duration depended on body size ($F_{1,20}$= 48.075, P<0.001) and stimulus type

229   ($F_{2,20}$= 4.877, P= 0.019), with larger snakes and those presented with contact stimuli performing

230   longer thrash displays. We also found that magnitude of body curvature in the post-thrash pose

231   depended upon stimulus ($F_{2,20}$= 5.601, P= 0.012) and species ($F_{4,20}$= 5.962, P= 0.003), such that

232   *Micrurus lemniscatus* and those presented with a looming cue displayed the highest degree of

233   body curvature (Supp. Fig. 1). Sex had no effect on either magnitude ($F_{1,19}$= 0.212, P= 0.651) or

234   duration ($F_{1,19}$= 2.266, P= 0.149; one unvouchered, unsexed individual excluded). We also found

235   no effect of temperature on magnitude ($F_{1,20}$= 0.284, P= 0.600) or duration ($F_{1,20}$= 0.155, P=

236   0.697). We found no significant preference within individuals for directionality (or

237   "handedness") in local maximum curvature, as nearly all individuals turned both heads and

238   bodies in both directions during display (mean proportion of local maximum body curves to the

239   right: 0.495; mean proportion of poses with right-kinked heads: 0.430, but 17/20 individuals had

240   proportions between 0.2-0.8). We also found no significant effect of body size, species, or

241   stimulus on the number of thrashing events within trials (all P > 0.05). Additionally, we saw no

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242   significant decay of the thrashing response over the course of trials, either in duration of thrash

243   (mean slope: -0.003, range: -0.077 to 0.043) or degree of body curvature (mean slope: -0.288,

244   range: -5.23 to 5.46). We did not have enough replicates to perform a robust test of locality

245   effects.

246

247   *Intraspecific variation in M. lemniscatus*

248   We compared the location and magnitude of body curvatures of the post-thrash pose

249   among all individuals of each species. The variation among nine individuals of *M. lemniscatus*

250   (Fig. 4) is emblematic of the intraspecific variation found in each of the other species as well (see

251   Supp. Figs. 2-5, available online).

252   Overall, there is strikingly high consistency in thrash duration (Fig. 4A) and location and

253   degree of body curvature (Fig. 4B) both within and among individuals of this species. Areas of

254   highest curvature (highest peaks) are concentrated towards the anterior portion in each snake,

255   demonstrating the stereotypical "neck kinking" mentioned in qualitative descriptions of this

256   display. In most individuals, both sides of the body are used fairly equivalently in body curving

257   (Fig. 4B). However, within the bounds of this stereotyped display, the ability of snakes to

258   dynamically adjust display components across cues and ontogeny contributed to substantial

259   intraspecific variation across all metrics. The longest duration of thrash occurred in RAB 3574

260   (Fig. 4 A, seventh row), which was a generally active and moderately sized individual from Los

261   Amigos. The most consistent and short durations of thrash were displayed by RAB 2415 (Fig. 4

262   A, second row), which was generally inactive, and the smallest snake captured of this species

263   from Santa Cruz (overall SVL effect on thrash duration also shown in Supp. Fig. 1). As seen in

264   the second row of Fig. 4B, this individual predominantly thrashed with the anterior portion of the

265   body while keeping the rest of the body relatively stationary. There also appears to be substantial

266   variation in how much of the anterior body displays the acute curves most associated with the

267   neck, as well as how much of the posterior body displays the acute curves associated with tail

268   coiling (not analysed here; see in particular RAB 3573 and 3333). Overall, there appears to be a

269   similar amount of variation in behaviour as there is in coloration (precise colour variation shown

270   in Fig. 4B, see legend), even though both traits are expected to be under exceptionally strong

271   stabilizing selection.

272

273   *Interspecific variation*

274   We found a similarly high consistency across species in the major features of the thrash

275   display: the duration of thrashing tended to be relatively short (median value below 0.5 seconds

276   for all species; Fig. 5A) and all species tended to have the largest magnitude of curvature

277   towards the anterior portion of the body, irrespective of substantial variation in typical coloration

278   and patterning (Fig. 5B; see also Supp. Figs 2-5). Beyond these major features, however, we

279   again found variation in multiple components. The difference between the mean curvature at

280   midbody and the mean curvature at the head was highest in *M. lemniscatus* and *M. obscurus*, and

281   lowest in *M. hemprichii* and *M. surinamensis*. Similarly, the region of higher curvature extended

282   posteriorly from the head much further in *M. lemniscatus* and *M. obscurus* than in *M. hemprichii*,

283   *M. surinamensis*, and *M. annellatus*. However, posterior body curving near the tail was highest

284   in *M. obscurus*, which happens to be the species with the shortest relative tail length in our

285   dataset. This pattern may indicate compensation for a shorter tail by involving more of the body

286   in the thrashing display. Additionally, the variance in thrashing durations was surprising, as

287   longer bouts of thrashing were not equally likely for all species. *Micrurus annellatus* was the

288   least likely to display for more than 1 second, *M. surinamensis* and *M. hemprichii* always

289   displayed for less than 2 seconds, while *M. lemniscatus* and *M. obscurus* thrashing lasted up to 3

290   seconds (Fig. 5B).

291

292   **Discussion**

293   By analysing the kinematics of non-locomotory coral snake anti-predator behaviours, we

294   provide a new approach for quantitative comparison of critical signalling elements both within

295   and among species. These results have important implications for testing theoretical expectations

296   of mimicry systems and comparing results to other mimicry systems, such as butterflies, in

297   which all players are chemically defended. Following theoretical predictions of these Müllerian

298   systems, we expected to find greater behavioural similarity among sympatric species than

299   intraspecific variation across multiple sites or even just high similarity across all individuals

300   irrespective of species or locality. Contrary to this expectation, we found surprising diversity in

301   1) propensity to display at all, 2) duration of thrashing, and 3) dynamics of body positions and

302   kinematics during the display, with no clear relationship to patterns of species sympatry. In

303   particular, we found that this signal can be dynamically adjusted across ecological contexts, such

304   that small snakes and those under physical contact by a predator are the most likely to respond

305   and produce the most vigorous responses.

306

307   *Ecologically relevant variation in signal construction: what matters?*

308   Our superficially paradoxical results add to mounting evidence that the drivers of trait

309   variation in mimicry systems are not well understood (Joron and Mallet 1998; Mallet and Joron

310   1999; Cox and Davis Rabosky 2013; Davis Rabosky et al. 2016a). Although one interpretation

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311 could be that South American coral snakes are not part of a mimicry system, a more likely

312 possibility is that the lethal levels of toxicity allow for greater trait variation due to the higher

313 cost to predators that erroneously identify prey (Pough 1988). In particular, our results suggest

314 that some aspects of the behavioural display – a short thrash duration and an acutely kinked neck

315 – may be more important for effective signalling to predators than other traits, such as which

316 direction the body curves or the degree of curvature beyond the neck. Additionally, our

317 behavioural results provide an interesting comparison to variation in colour pattern, which is

318 fairly extreme among species (Fig. 5B) but considered the most important trait for

319 communicating the potential for chemical defence. More broadly, our ability to use a single

320 method to analyse anti-predator display across a clade with a divergence date of at least 10

321 million years (Pyron and Burbrink 2014) gives some appreciation for how long the main

322 thrashing element of this display has been maintained within the coral snake lineage. However,

323 our results suggest that simplistic models of signal canalization in mimicry systems may benefit

324 from expansion that accounts for the ways in which signals can and do still vary within and

325 among species.

326

327 *Challenges of studying non-locomotory behaviour in venomous coral snakes*

328   The non-locomotory movements of snakes present several unique challenges to

329 quantitative biomechanical analyses. While many forms of snake locomotion involve either

330 linear or roughly sinusoidal body shapes (Lissmann 1950; Jayne 1986; Moon and Gans 1998; Hu

331 et al. 2009; Socha 2011, Titcomb et al. 2013), non-locomotory behaviours are less mechanically

332 constrained and therefore result in a high frequency of self-occlusion. The elongate body form

333 and high degrees of freedom conferred by the highly articulated musculoskeletal system result in

15

334 more acute curves than observed in other model organisms with elongate body forms (e.g.

335 *Caenorhabditis elegans*, Padmanabhan 2012; Brown 2013). Such extreme self-occlusions make

336 it difficult to use automated tools for passively tracing the snake centreline.

337 Furthermore, the banded colour patterns and self-mimicry by the tail of the head (Greene

338 1973) make it difficult to identify the morphological features of a snake from an isolated video

339 frame without context. These features likely contribute to snake fitness by making it difficult for

340 a predator to target an attack towards the head (Brodie III 1993) and have resulted in a

341 particularly challenging dataset for existing computer vision tools.

342 While fiduciary markers placed on the snake can aid in collecting precise kinematic data

343 (Gart 2019), the handling required to affix such markers likely alters the behavioural response of

344 the snake. Furthermore, the highly toxic venom of these snakes, together with impressively

345 adhesive-resistant skin lipids (Torri et al. 2014), makes it impractical and unsafe to attempt such

346 marking.

347 The smooth surface of the arena required for thorough cleansing resulted in lower

348 substrate friction for the snake. Although some slipping was observed, recent studies suggest that

349 snake motor control is independent of surface friction (Schiebel et al. 2019). Thus, we argue that

350 our results likely reflect snake behavioural variation on more natural substrates.

351

352 *Future directions*

353 We demonstrate that a video-based approach can be feasibly applied to quantitatively

354 examine the non-locomotory behaviours of snakes under semi-natural conditions. This approach

355 facilitates ecologically relevant biomechanical inquiry with strong evolutionary impact. While

356 we recognize that our stimuli, especially the vibratory stimulus, may not be a perfect match to

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357   those provided by potential predators, at least one individual responded to every category of

358   stimulus with an anti-predator display.

359   In addition to venomous coral snakes, distantly related snakes with varying toxicity have

360   independently converged several times on these conspicuous colour patterns and thrashing

361   displays (Greene 1979; Davis Rabosky et al. 2016b). Just as previous studies have leveraged a

362   quantitative analysis of colour pattern to measure convergence and experimentally examine the

363   effect on predation rates (Brodie III 1993; Buasso 2006), the analysis presented above enables

364   more precise measurement of convergence and an experimental approach for determining the

365   effect of behaviour on predation rates. Since snakes of all sizes, and consequently all ages,

366   display vigorous thrashing behaviour in the absence of any parental care (Shine 1988), this anti-

367   predator response has likely evolved as an innate response, with little learning over the course of

368   a lifetime. In this case, quantitative characterization of anti-predator behaviours can be modelled

369   as phenotypic traits to gain insight into the evolutionary processes underlying the patterns of

370   behaviour observed in nature.

371   **Author contributions**

372   TYM, JGL, and ARDR designed the experiment and collected the data. TYM, ARDR,

373   and SMD analysed the data. TYM and ARDR wrote the main draft of the manuscript, and all

374   authors contributed to manuscript editing and revision.

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385   illustrations. The authors declare no conflicts of interest.

386   **Data accessibility**

387   Videos will be archived and freely available at https://deepblue.lib.umich.edu/data. Code

388   to compute the curvature of the snakes is posted in a GitHub repository.

389

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487

488   **Tables**

Species	Catalog Number	Field Number	Sex	SVL (mm)	Mass (g)	Locality	Thrashing?
Micrurus annellatus	MUSM 39056	RAB2815	F	410	17.5	Los Amigos	No
Micrurus annellatus	UMMZ 246856	RAB1144	F	422	12.0	Los Amigos	Yes
Micrurus annellatus	UMMZ 248451	RAB3275	F	497	18.1	Los Amigos	Yes
Micrurus hemprichii	UMMZ 246857	RAB1810	M	740	86.0	Madre Selva	Yes
Micrurus hemprichii	MUSM 37347	RAB2035	M	617	52.5	Madre Selva	Yes
Micrurus lemniscatus	NA	Released5	NA	NA	NA	Villa Carmen	No
Micrurus lemniscatus	NA	Released9	NA	848*	NA	Villa Carmen	Yes
Micrurus lemniscatus	NA	Released12	NA	NA	NA	Villa Carmen	Not analyzed (out of frame)
Micrurus lemniscatus	UMMZ 246858	RAB1993	F	632	23.4	Madre Selva	Yes
Micrurus lemniscatus	MUSM 37348	RAB2415	F	372	6.1	Santa Cruz	Yes
Micrurus lemniscatus	MUSM 39057	RAB2706	M	816	68.9	Los Amigos	Not analyzed (snake hook interference)
Micrurus lemniscatus	UMMZ 248456	RAB2915	F	688	34.6	Los Amigos	Yes
Micrurus lemniscatus	UMMZ 248457	RAB3333	M	595	35.0	Los Amigos	Yes
Micrurus lemniscatus	UMMZ 248452	RAB3487	F	632	28.4	Los Amigos	Yes
Micrurus lemniscatus	MUSM 39853	RAB3573	M	570	25.7	Los Amigos	Yes
Micrurus lemniscatus	MUSM 39854	RAB3574	M	590	25.5	Los Amigos	Yes
Micrurus lemniscatus	MUSM 39855	RAB3578	M	453	14.1	Los Amigos	Yes
Micrurus obscurus	UMMZ 246859	RAB0665	F	261	5.2	Villa Carmen	Yes
Micrurus obscurus	MUSM 37350	RAB0698	F	237	5.1	Villa Carmen	Yes
Micrurus obscurus	UMMZ 246860	RAB1054	M	775	81.0	Los Amigos	Yes
Micrurus obscurus	UMMZ 248458	RAB3570	F	766	86.0	Los Amigos	Yes
Micrurus surinamensis	UMMZ 246861	RAB1099	F	657	100.0	Los Amigos	No
Micrurus surinamensis	MUSM 37352	RAB1100	F	391	25.2	Los Amigos	Yes
Micrurus surinamensis	MUSM 37353	RAB1101	M	421	32.5	Los Amigos	Yes
Micrurus surinamensis	UMMZ 246862	RAB1511	F	948	240.0	Madre Selva	No

489

490   **Table 1:** Morphological and geographical information for the snakes examined in this study.

491   Note that the Snout-Vent Length (SVL) for *Micrurus lemniscatus* Released9 was estimated from
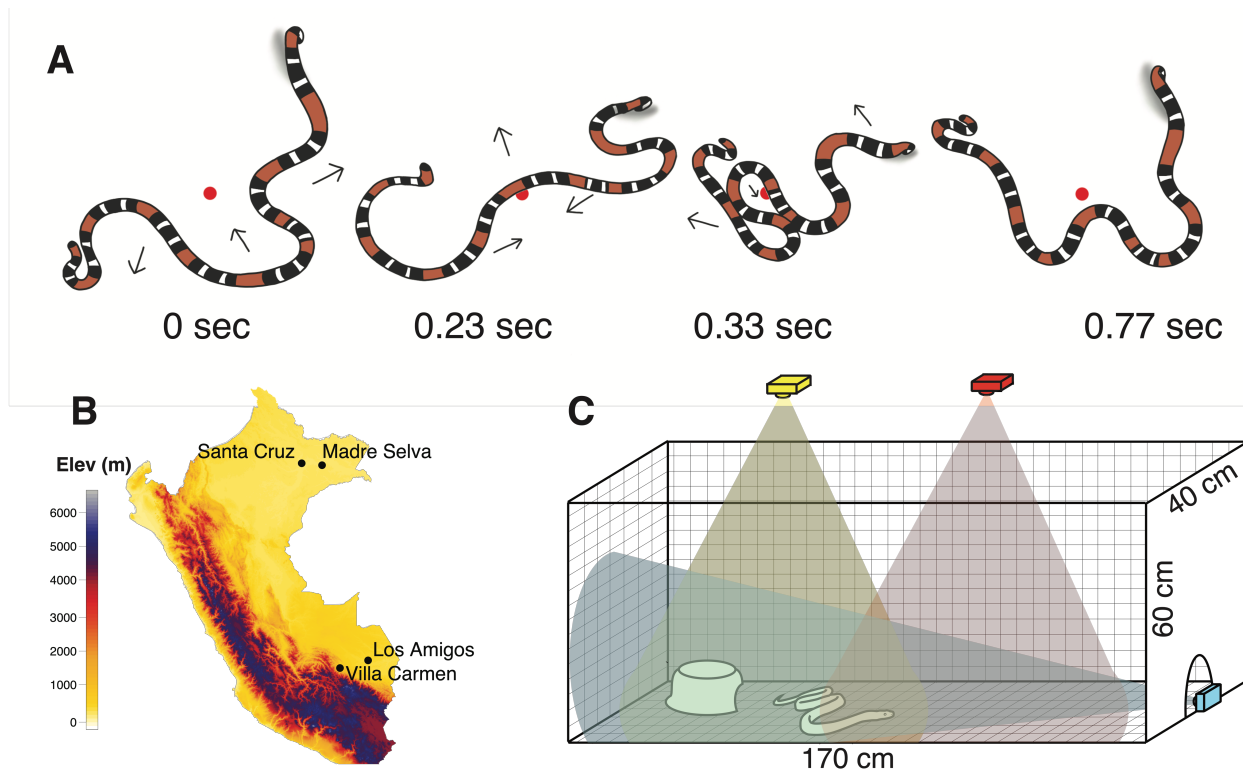
492   a still video frame. As snake morphometrics are taken at the time of vouchering, SVL, mass, and

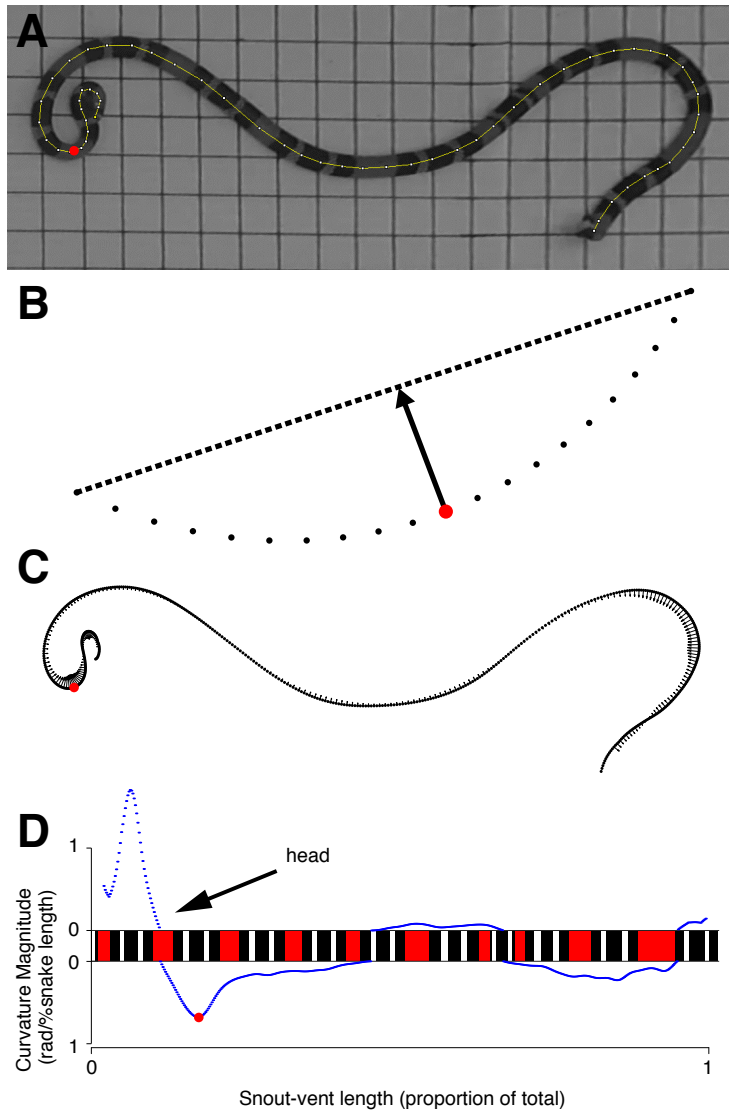493   sex were not directly measured for released individuals.

494

495

24

496 **Figures**

497
498 **Figure 1:** (A) An illustration of a coral snake thrashing display. Each of the four illustrations

499 depict an individual coral snake progressing through different phases of a thrashing display

500 (based on data in this study). The snake position with respect to the red dot shows that there is

501 negligible translocation of the snake, despite vigorous movements. The snake head is on the left

502 side of each illustration, and the coiled tail is elevated throughout the display. (B) A map of Peru

503 showing our four field sites, all in lowland Amazonian rainforest. (C) A schematic of the pop-up

504 behavioural arena. The bottom and sides of the arena are constructed from corrugated plastic and

505 connected using brass fasteners. Lines are drawn 2cm apart using permanent ink and covered

506 with a clear adhesive plastic (Con-Tact). Three Go-Pro cameras were positioned so that their

507 fields of view partially overlapped and included the entire floor-space of the arena. An opaque

508 plastic bowl with a portion removed was placed in one end of the arena to provide a refuge for

509 the snake.

25

A

B

C

D

Curvature Magnitude
(rad/%snake length)

head

Snout-vent length (proportion of total)

510

511   **Figure 2:** Methods for calculating the curvature of a snake. (A) Undistorted and rectified video

512   frames at the conclusion of each bout of thrashing were traced by hand in ImageJ. (B) Curvature

513   vectors are shown along each sampled point of the snake centreline as arrows. (C) For each focal

514   point in the snake centreline (the red focal point in B corresponds to the red focal point in B, the

515   curvature was calculated as the distance from the point to a line connecting the tenth points on

516   the left and right. (D) The corresponding curvature magnitudes plotted to the right and left of a

517   straightened depiction of a coral snake. The colour pattern of the coral snake reflects the

518   observed colour pattern of this individual.

26

519
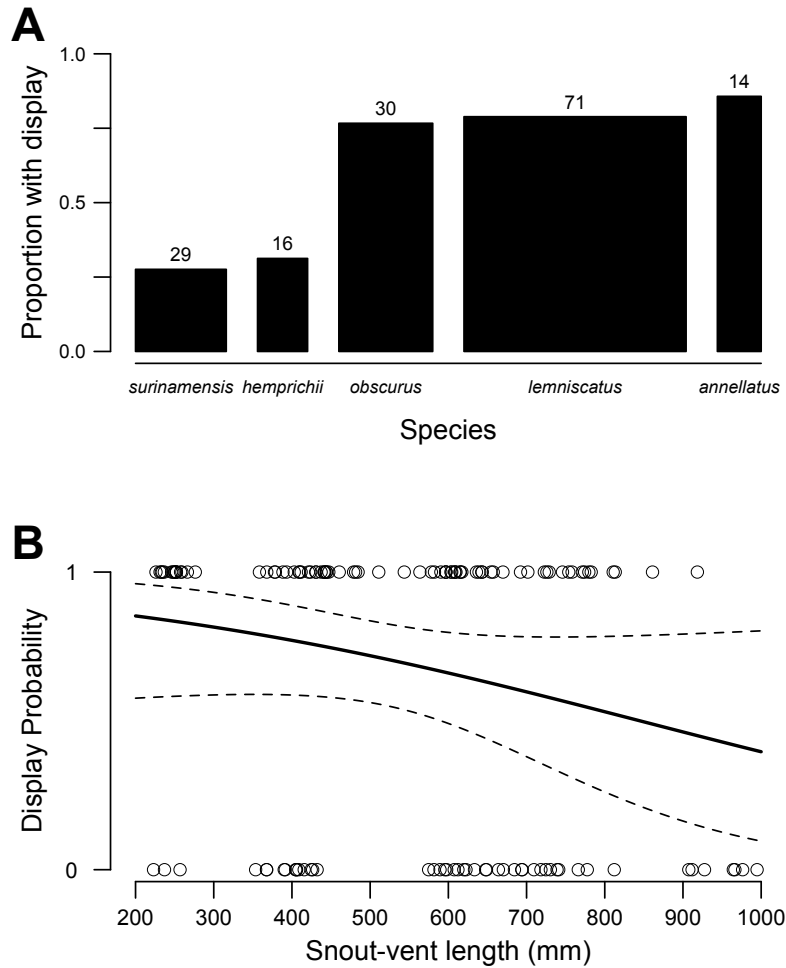
Figure 3. Not all individuals performed a thrashing display in response to all cues. Display

probability depended most strongly upon (A) species identity (binomial GLMM: $F_{4,22}$= 5.907,

P=0.002) and (B) body size as measured by snout-vent length (SVL; $F_{1,22}$= 5.732, P=0.026).

Fitted effect of SVL is predicted from a model that includes collection ID as a random factor, as

all individuals were tested more than once.
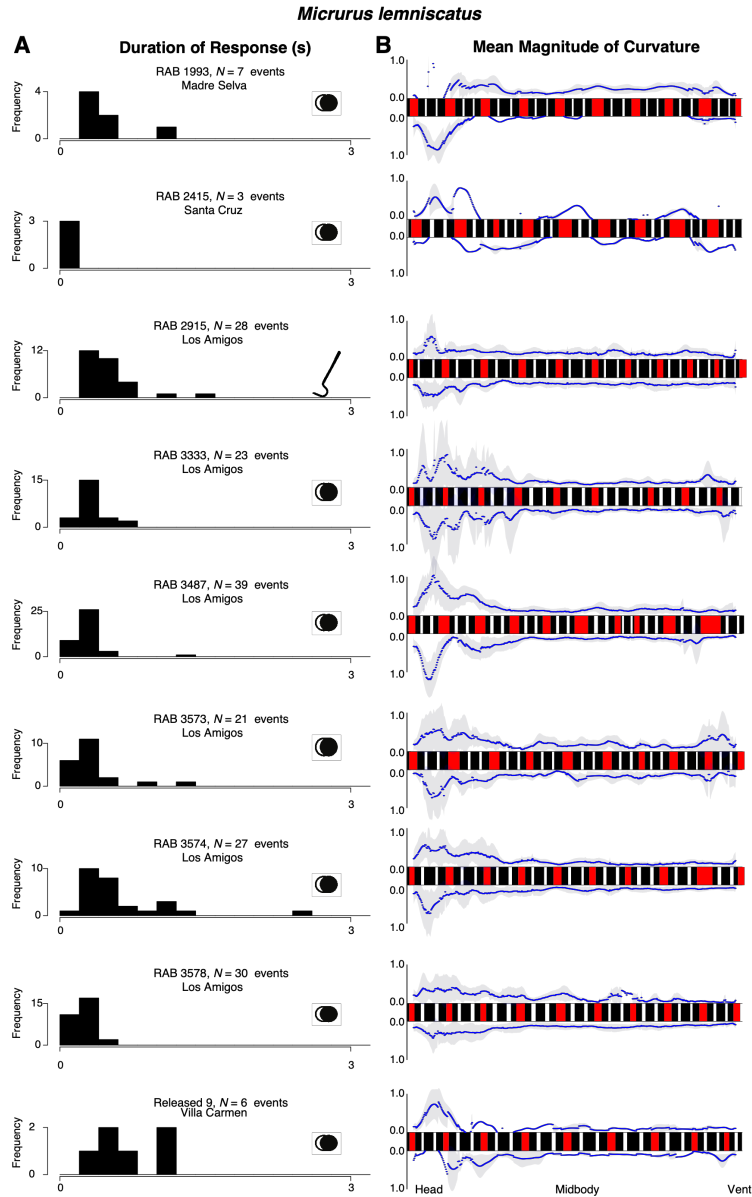
Figure 4: Intraspecific variation in thrash duration (A) and curvature (B) among individuals of *Micrurus lemniscatus.* For each row of plots, the histogram of thrash durations on the left and the mean magnitude of curvature plot on the right represent the same trial. The stimulus used in each trial is depicted by the icon on the right side of the histogram: two overlapping circles denote a looming stimulus and a snake hook denotes a tactile stimulus. Colour patterns of each snake reflect the observed colour patterns of each individual measured to sub-millimetre resolution. Snake patterns are arranged such that the anterior portion is towards the left.

533

534
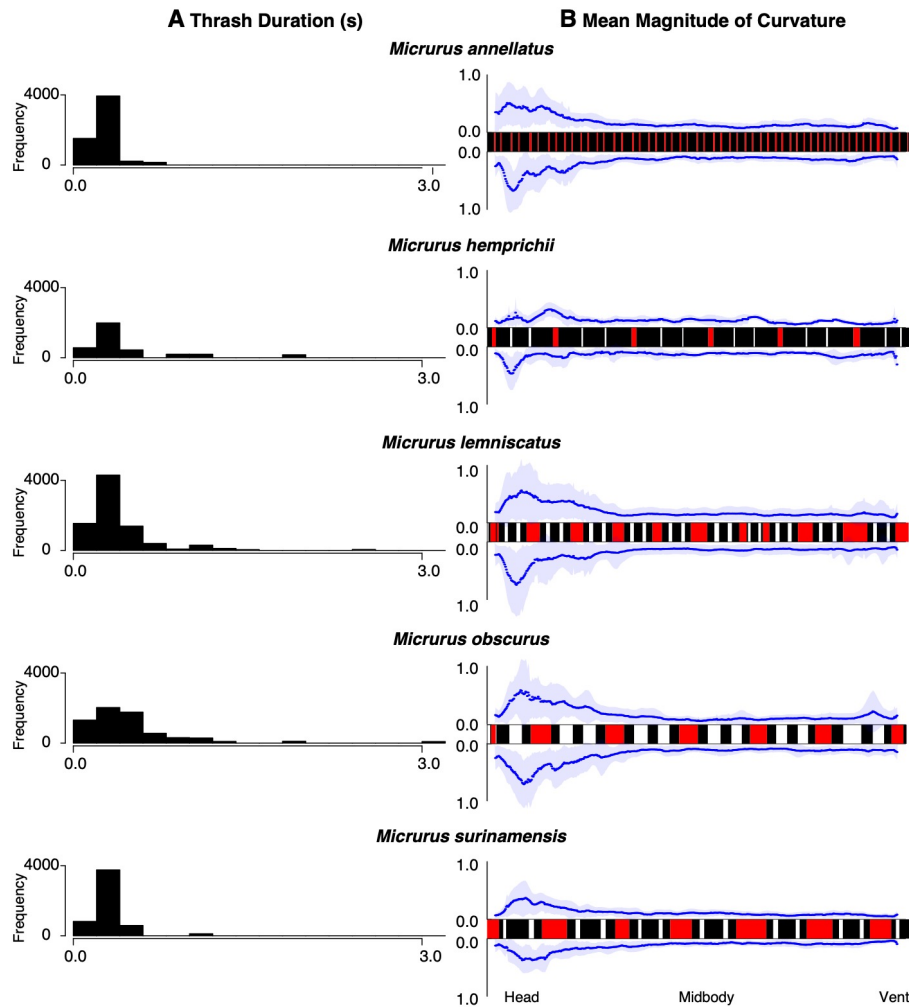
Figure 5: Interspecific variation in thrash duration (A) and curvature (B). Thrash durations for each species on the left are sampled with replacement proportionally by the ratio of events to total observed frames of video and plotted as histograms. On the right, the mean magnitude of curvature at each point along the body is plotted for one trial for each species in response to looming stimuli, with the exception of *Micrurus surinamensis*, which responded to a vibratory stimulus. Colour patterns of each snake reflect the observed colour patterns of each individual measured to sub-millimetre resolution. Snake patterns are arranged such that the left side is anterior.