Microgeographic variation in body condition of three Mexican garter snakes in central Mexico

Erika Valencia-Flores [1] , Crystian S Venegas-Barrera [2] , Victor Fajardo [3] , Javier Manjarrez [Corresp. 1]

[1] Facultad de Ciencias, Universidad Autónoma del Estado de México, Toluca, Estado de México, México

[2] División de Estudios de Posgrado e Investigación, Instituto Tecnológico de Ciudad Victoria, Ciudad Victoria, Tamaulipas, México

[3] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico

Corresponding Author: Javier Manjarrez
Email address: jsilva@uaemex.mx

Background. Geographic variation in body size and condition can reveal differential local adaptation to resource availability or climatic factors. Body size and condition are related to fitness in garter snakes (*Thamnophis*), thus good body condition may increase survival, fecundity in females, and mating success in males. Phylogenetically related species in sympatry are predicted to exhibit similar body condition when they experience similar environmental conditions. We focused on interspecific and geographical variation in body size and condition in three sympatric Mexican garter snakes from the highlands of Central Mexico. **Methods.** We assessed SVL, mass, and body condition (obtained from Major axis linear regression of ln-transformed body mass on ln-transformed SVL) in adults and juveniles of both sexes of *Thamnophis eques*, *T. melanogaster*, and *T. scalaris* sampled at different locations and ranges from 3-11 years over a 20-year period. **Results.** We provide a heterogeneous pattern of sexual and ontogenic reproductive status variations of body size and condition among local populations. Each garter snake species shows locations with good and poor body condition; juvenile snakes show similar body condition between populations, adults show varying body condition between populations, and adults also show sex differences in body condition. We discuss variations in body condition as possibly related to the snakes' life cycle differences.

MICROGEOGRAPHIC VARIATION IN BODY CONDITION OF THREE MEXICAN GARTER SNAKES IN CENTRAL MEXICO

6 Erika Valencia-Flores[1], Crystian S. Venegas-Barrera[2], Victor Fajardo[3] and Javier Manjarrez[1]

8 [1] Facultad de Ciencias, Universidad Autónoma del Estado de México, Toluca, Estado de México,

9 México

10 [2] División de Estudios de Posgrado e Investigación, Instituto Tecnológico de Ciudad Victoria,

11 Ciudad Victoria, Tamaulipas, México

12 [3] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México,

13 Toluca, Estado de México, México

15 Corresponding Author:

16 Javier Manjarrez[1]

17 Instituto Literario 100, Centro, Toluca, Estado de México, CP 50000, México

18 Email address: jsilva@uaemex.mx

ABSTRACT

21   **Background.** Geographic variation in body size and condition can reveal differential local

22   adaptation to resource availability or climatic factors. Body size and condition are related to

23   fitness in garter snakes (*Thamnophis*), thus good body condition may increase survival, fecundity

24   in females, and mating success in males. Phylogenetically related species in sympatry are

25   predicted to exhibit similar body condition when they experience similar environmental

26   conditions. We focused on interspecific and geographical variation in body size and condition in

27   three sympatric Mexican garter snakes from the highlands of Central Mexico.

28   **Methods.** We assessed SVL, mass, and body condition (obtained from Major axis linear

29   regression of ln-transformed body mass on ln-transformed SVL) in adults and juveniles of both

30   sexes of *Thamnophis eques*, *T. melanogaster*, and *T. scalaris* sampled at different locations and

31   ranges from 3-11 years over a 20-year period.

32   **Results.** We provide a heterogeneous pattern of sexual and ontogenic reproductive status

33   variations of body size and condition among local populations. Each garter snake species shows

34   locations with good and poor body condition; juvenile snakes show similar body condition

35   between populations, adults show varying body condition between populations, and adults also

36   show sex differences in body condition. We discuss variations in body condition as possibly

37   related to the snakes' life cycle differences.

39   INTRODUCTION

40   Organisms usually respond to differences in environmental conditions by exhibiting local

41   adaptation in phenotypic traits. Geographic variation in phenotypic traits associated with body

42   size and condition can reveal differential adaptation of local populations to local biotic and

43   abiotic fluctuations as presence of related species, resource availability, or climatic factors

44   (Bronikowski & Arnold 1999, Bronikowski 2000, Miller et al. 2011). Also, geographic variation

45   in body size and body condition can reveal fundamental variation in selective pressures,

46   especially in reptiles such as snakes (Bronikowski & Arnold 1999, Miller et al. 2011). Thus,

47   analyses of geographic variation in body size and condition are important to explain locally

48   variable adaptations that produce morphological diversity in snake species. Geographic variation

49   in body condition comes from many causes, including phenotypic plasticity (Krause, Burghardt

50   & Gillingham 2003) or microevolutionary change among natural populations (Bronikowski

51   2000). These population differences may arise from geographic variation in food resources

52   (Bronikowski & Arnold 1999), climate (Ashton 2001), or intra-inter species interactions (e.g.

53   Kurzava & Morin 1994).

54   Body condition is an expression of weight and length (size-adjusted body mass), and it is

55   correlated with body reserves (Hayes & Shonkwiler 2001), especially with energy stores in the

56   liver, muscle, and fat of snakes (Bonnet et al. 1998, Falk, Snow & Reed 2017). During periods of

57   low resource availability, starvation and low body reserves are a good predictor of mortality

58   (Shine et al. 2001, Kissner & Weatherhead 2005), decreased reproductive status (Naulleau &

59   Bonnet 1996, Lind & Beaupre 2015, Catherine, LeMaster & Lutterschmidt 2018), and low

60   growth rates in snakes (Bronikowski 2000).

61   Thus, there is a relation between body size and condition with fitness, but in different

62   ways for the two sexes, especially with reproductive status of snakes. For example, a good body

63   condition may be associated with enhanced survival of both sexes of garter snakes, greater

64   fecundity in female garter snakes, and increased mating success for males (Naulleau & Bonnet

65   1996); thereby, a reduction in body condition may reduce reproductive capacity (Lind & Beaupre

66   2015). Conversely, adult female snakes in poor condition that are carrying eggs experience

67   greater mortality (Madsen & Shine 1993, Brown & Weatherhead 1997, Shine et al. 2001).

68   Additionally, phylogenetically related species in sympatry are predicted to exhibit similar

69   body condition when they have similar ecology, because they share similar evolutive history,

70   interspecific interactions and selective pressures (i.e. Yom-Tov & Geffen 2006, Koyama et al.

71   2015, Sivan et al. 2015). For example, closely related species of garter snakes with highly

72   overlapping ranges in Mexico, *Thamnophis melanogaster* and *T. eques*, show similar patterns of

73   neonate body condition as a function of date of birth (Manjarrez & San-Roman-Apolonio 2015).

74   To understand the complex evolution of body condition, we studied interspecific and

75   geographical variation in traits known to be associated with body condition in three sympatric

76   Mexican garter snakes (*Thamnophis* sp.) inhabiting five sites from the highlands of Central

77   Mexico. Given that the geographic distribution of these three garter snakes comprises a range of

78   different environmental conditions, we hypothesized that traits associated with body condition

79   of snakes would potentially reveal a pattern of geographical variation among local populations

80   that could be influenced by dietary differences, ontogenic reproductive status (juvenile, adult),

81   and sex of snakes. We predicted that the geographic variation in body condition in garter snakes

82   is influenced by diet differences among populations, such that body condition would vary among

83   populations. We discuss possible body condition differences as they are related to life cycle

84   differences.

85   In this study we assessed snout-vent length (SVL), mass, and body condition in adults

86   and juveniles of both sexes from three sympatric garter snakes in the Central Mexican Highlands

87   (Fig. 1); Mexican garter snake (*Thamnophis eques*), Mexican Black-bellied garter snake (*T.

88   melanogaster*), and Longtail Alpine garter snake (*T. scalaris*). They are grouped within the well-

89   supported clade of garter snakes composed of species found mostly in Mexico (de Queiroz,

90   Lawson & Lemos-Espinal 2002, Guo et al. 2012, McVay & Carstens 2013). Garter snakes are

91   the most abundant snake genus in Central Mexico (Flores-Villela, Canseco-Marquez & Ochoa-

92   Ochoa 2010). Garter snakes exhibit substantial variation within and among species in certain

93   aspects of morphology, behavior and physiology, a pattern specially demonstrated in two North

94   American species recognized as examples of evolution (Burghardt & Schwartz, 1999). There is

95   considerable intra-interspecific variation in color patterns, body size, diet, habitat, resistance to

96   toxic prey, reproductive characteristcs and behavior that the patterns of the constraints may vary

97   among poplations (Burghardt & Schwartz 1999, Rossman, Ford & Seigel 1996). In general,

98   garter snakes are sexually dimorphic in body size (Shine 1993) with females regularly larger than

99   males (Shine 1994). Almost all studies comparing the body condition of garter snake species

100   were conducted separately for each sex and rarely have been combined in a single study;

101   therefore, there is scarce information of possible sex differences in garter snake body condition,

102   but see (Rollings et al. 2017).

103   We chose the species *T. eques*, *T. melanogaster*, and *T. scalaris* that occur in Central

104   Mexico because there are no studies that describe the body condition or its possible interspecific

105   or spatial variations under natural conditions for these three species. Only one study of *T.*

106   *melanogaster* and *T. eques* detected body condition patterns in offspring born from females

107   caught in the wild (Manjarrez & San-Roman-Apolonio 2015). For both species, body condition

108   of neonates differed by being lower in the early season and higher in the late season. Snout-vent

109   length of neonates and mean mass of neonates per litter did not change throughout the birth

110   season (Manjarrez & San-Roman-Apolonio 2015).

111   *Thamnophis eques* is widely distributed from Central Mexico to southern New Mexico

112   and Arizona in the United States (Rossman, Ford & Seigel 1996). It is a generalist snake because

113   it preys on both terrestrial and aquatic prey such as frogs, fish and tadpoles, and occasionally,

114   mice and lizards (Drummond & Macías García 1989, Manjarrez 1998, Manjarrez, Pacheco-

115   Tinoco & Venegas-Barrera 2017). *Thamnophis melanogaster* is endemic to the Central Mexican

116   Plateau. It is a semiaquatic snake present at the edge of water bodies and preys mostly on

117   tadpoles, fish, and leeches (Rossman, Ford & Seigel 1996, Manjarrez, Macías García &

118   Drummond 2013). *Thamnophis scalaris* is also endemic to Central Mexico (Rossman, Ford &

119   Seigel 1996). It inhabits forests and grasslands, where it specializes on earthworms, although it

120   can eat vertebrates such as lizards and mice (Manjarrez, Venegas-Barrera & García-Guadarrama

121   2007).

122

123   MATERIALS & METHODS

124   In Central Mexico, we irregularly sampled garter snakes at eight different locations in the Rio

125   Lerma drainage (Fig. 1A) over a period of 20 years, however, we selected only those five

126   populations (Fig. 1B) with more than 24 records of snakes, which allowed us to make spatial and

127   sex comparisons. We selected the records of snakes collected over three different years for *T.*

128   *scalaris* (2003, 2005, and 2010) at three locations; seven years for *T. melanogaster* (2005–2011),

129   at two locations, and eleven years for *T. eques* (2000–2003, 2005–2011) at three locations (Table

130   1). Locations are separated by 92.6 Km of mean distance (SD = 80 Km, range 9.5–215 km).

131   Among the five sites, mean annual temperature ranged from 13.7˚–18.1˚C and mean annual

132   precipitation ranged from 116 mm–755.8 mm (Table 1). *Thamnophis eques* were captured

133   between March and November, *T. melanogaster* between January and December, and *T. scalaris*

134   between June and November.

135   We found snakes by searching under rocks and tree trunks, and some were found simply

136   basking on the ground. All snakes were captured by hand. Adult females were carefully

137   examined for the presence of embryos, and those identified as gravid were excluded from

138   analysis. Measurements of captured snakes included sex (visual inspection of tail-base breadth or

139   by everting the male hemipenes in small snakes), snout-vent length (SVL), and mass (measured

140   on an electronic scale [±0.1 g]). Dietary differences among the localities were examined by

141   analysis of stomach contents from *T. eques* and *T. melanogaster*. We obtained stomach contents

142   by making the snakes regurgitate by abdominal palpation (Fitch 1987). For *T. scalaris*, no

143   stomach contents were recorded. Immediately after processing, snakes were released where they

144   had been captured.

145   **Analysis**

146   Individual body condition was calculated using residuals from the Major axis (MA) linear

147   regression of ln-transformed body mass on ln-transformed SVL. This MA residual index is

148   considered an excellent estimator of true snake body condition because it shows a strong

149   association with body fat mass but not SVL (Falk, Snow & Reed 2017). The condition that the

150   MA linear regression is unbiased in with respect to size is considerable for hypothesis testing,

151   because an absence of correlation with size permit to compare MA residual index across

152   individuals of different size ranges. Particularly, only 2% of the variation in the MA residual

153   index is associated with SVL (Falk, Snow & Reed 2017). This regression was significant for all

154   species (*T. eques*, $r = 0.90$, $P < 0.0001$; *T. melanogaster*, $r = 0.93$, $P < 0.0001$; *T. scalaris*, $r =$

155   0.95, $P < 0.0001$). Residuals were used to categorize body condition, with positive residuals

156   corresponding to individuals with good body condition and negative residuals corresponding to

157   individuals with poor body condition (Weatherhead & Brown 1996, Falk, Snow & Reed 2017).

158   In this way, the average condition by location is interpreted as good or bad condition by location

159   for each species.

160   Because the optimal body condition should approximate the true body condition of the

161   snakes and should be unbiased with respect to body size, we evaluated this relationship with

162   Kendall rank correlation coefficient to test for a correlation between body condition and ln-

163   transformed SVL as a measure of size and estimated the percent variation in body condition.

164   Also, with the coefficient of determination (R^2), we estimated the percent variation in body

165   condition and mass that can be explained by SVL.

166   **Geographic comparison.**

167   The SVL and mass of snakes were transformed with natural logarithms prior to analyses. We

168   utilized one-way analyses of variance (ANOVA) to compare body condition, SVL, and mass as

169   dependent variables among populations of each species. In these analyses, we pooled male and

170   female snakes because a three-way ANOVA (locality, year, and sex) indicated that body

171   condition, SVL and mass within each species did not differ between sexes, but did differ among

172   locality and between years (Table 2). We used a Chi-square goodness-of-fit test to determine if

173   sex ratio among species was different than 1:1 (Table 3). Statistical significance was assessed at

174   $\alpha = 0.05$. All data are reported as means $\pm$ 1 *SD*.

175   **Sexual and size status comparison**

176   Each snake was assigned an ontogenic reproductive status (juvenile, adult) according to size at

177   capture (adult snakes > 39.0, 33.0, and 34 cm SVL for *T. eques*, *T. melanogaster* and *T. scalaris*,

178   respectively; Manjarrez 1998, Manjarrez, Venegas-Barrera & García-Guadarrama 2007). We

179   performed a discriminant function analysis (DFA) for testing intraspecific differences (between

180   location, sex, and size category) according to the mean of the exploratory variables (SVL, mass,

181   and body condition) and for generating linear combinations that classify snakes as a function of

182   their morphological traits associated with snake body condition. The grouping variables were

183   location, sex (male, female) and ontogenic reproductive status. DFA is an inferential, descriptive

184   multivariate procedure for testing differences between groups according to the mean of all

185   variables and for generating linear combinations that classify objects as a function of their

186   characteristics (Statistica, ver. 12; StatSoft 2012).

187   The objective of DFA was to test differences between groups and identify which

188   variables discriminate between two or more groups. Comparisons between groups were

189   performed under the null hypothesis that morphological traits between categories of grouping

190   variables were similar, and the estimated value was contrasted with the theoretical value of the F-

191   distribution. We employed a probability of 0.05 to test the hypothesis, where P values lower than

192   0.05 were associated with groups of snakes showing different morphological traits, whereas

193   values greater than or equal to 0.05 were associated with groups with similar morphological

194   traits. The canonical average of the observations from each category (centroid) for the significant

195   roots (canonical scores) was plotted, which reflects morphological variations between categories

196   of grouping variables. The position of the centroids was interpreted using the variables that

197   contributed most to discriminating between groups.

198   We chose those variables that exhibited a coefficient of the factor structure higher than

199   0.5 or lower than -0.5. The coefficients represent the correlation between the original variables

200   and the roots. We applied one-way ANOVAs or Student-t with Statistica software (ver. 8.0

201   StatSoft, Tulsa, Oklahoma, USA) when only one morphological variable exhibited a coefficient

202   of the factor structure higher than 0.5 or lower than –0.5.

203   This study received the approval of field permit (Secretaria del Medio Ambiente y

204   Recursos Naturales #07164) and the ethics committee of the Universidad Autónoma del Estado

205   de México (Number 4047/2016SF). All subjects were treated humanely on the basis of

206   guidelines outlined by the American Society of Ichthyologists and Herpetologists (ASIH, 2004).

207

208   **RESULTS**

209   The biggest species of garter snake was *T. eques* with a mean body size of SVL 43.43 ± 17.57

210   cm (range 12.51–81.30), mass of 55.62 ± 60.56 g (range 1.40–335.86, *n* = 253). *Thamnophis*

211   *melanogaster* was slightly larger than *T. scalaris* (*T. melanogaster*: SVL 29.17 ± 41 cm [range

212   14.40–66.0], mass 19.10 ± 23.3 g [range 1.62–196.0], *n* = 686; *T. scalaris*: SVL 28.70 ± 9.21 cm

213   [range 12.10–53.0], mass 16.44 ± 12.59 g [range 1.30–60.70], *n* = 80).

214   The number of males and females collected was independent of locations sampled for *T.*

215   *melanogaster* (χ^2 = 0.001, *df* = 1, *P* = 0.97), and *T. scalaris* (χ^2 = 3.69, *df* = 2, *P* = 0.15), but

216   dependent on location for *T. eques* (χ^2 = 10.4, *df* = 2, *P* = 0.006). Considering all individuals

217   collected, the sex ratio was biased toward females. For *T. eques* and *T. scalaris*, the sex ratio was

218   skewed toward females in two or three locations analyzed (Table 3), whereas the sex ratio for *T.*

219   *melanogaster* was biased toward females in Cuitzeo but not in Lerma (Table 3). For *T. scalaris*

220   the female bias was very distinct, especially Zempoala where no males were found (Table 3).

221   Both body condition (residuals from MA linear regression of ln-transformed body mass

222   on ln-transformed SVL) and body mass were related to ln-SVL in each garter snake (Table 4).

223   The R^2 values suggest that more than 80% of the variation in body mass is explained by SVL,

224   and less than 12% of the variation in body condition is explained by SVL (Table 4).

225   **Geographic comparison**

226   *Thamnophis eques.* For the three locations that we analyzed for *T. eques* (Lerma, Cerrillo and

227   Cuitzeo), we observed a difference in mean body condition. *Thamnophis eques* from Lerma

228   showed a mean poor body condition that was the lowest of the three populations ($F_{2,250} = 10.7$, P

229   < 0.0001; Fig. 2), although snakes in this location were significantly larger than in the other two

230   (ln-SVL $F_{2,250} = 6.7$, $P = 0.001$). Conversely, *T. eques* from Cuitzeo showed the best body

231   condition, but the shortest length (Fig. 2). Mean body mass was not different between locations

232   of *T. eques* (ln-mass $F_{2,250} = 2.2$, $P = 0.11$).

233   *Thamnophis melanogaster.* For *T. melanogaster*, the statistical test did not detect a significant

234   difference in mean body condition between the two locations, Lerma and Cuitzeo ($F_{1,684} = 3.1$, P

235   $= 0.07$). However, the Lerma snakes were significantly larger (ln-SVL $F_{1,684} = 42.3$, $P < 0.0001$),

236   and heavier than those collected in Cuitzeo (ln-mass $F_{1,684} = 56.4$, $P < 0.0001$; Fig. 2).

237   *Thamnophis scalaris.* In this species the mean SVL and mass showed no differences among the

238   three locations analyzed (Lerma, S. Morelos and Zempoala, ln-SVL $F_{2,77} = 1.55$, $P = 0.21$; ln-

239   mass $F_{2,77} = 0.58$, $P = 0.56$), however, mean body condition was good in the individuals from S.

240   Morelos and poor for those from Zempoala ($F_{2,77} = 20.9$, $P < 0.0001$; Fig. 2).

241   **Sexual and size status comparison**

242   The results of DFA showed that each garter snake had a unique pattern of intraspecific

243   differences. The body condition of juveniles *T. eques* was better than adults ($t_{227} = 43.3$, $P <$

244   0.0001). For *T. melanogaster* and *T. scalaris* the body condition of juveniles and adults was

245   similar ($t_{684} = 0.01$, $P < 0.99$; $t_{78} = 1.5$, $P = 0.13$, respectively).

246   *Thamnophis eques.* Juvenile females of Cuitzeo had a better body condition than juvenile

247   females of Lerma (t_{36} = 2.17, P = 0.03), but body size (SVL and mass) were similar between

248   Juvenile females of both locations ($F_{2,35}$ = 2.9, P = 0.06). Juvenile males *T. eques* have similar

249   body size and body condition between Lerma and Cuitzeo.

250   Adult males *T. eques* of Cuitzeo had a higher mass (140 ± 130.1 g) than adult males of

251   Lerma (57.2 ± 32.7 g) and Cerrillo (49.7 ± 18.7 g, ANOVA $F_{2,37}$ = 8.2, P < 0.0001). Adult

252   female *T. eques* of Lerma presented greater body size (SVL 59.0 ± 9.3 cm; mass 104.0 ± 68.1 g)

253   than adult females of Cerrillo (SVL 49.7 ± 8.5 cm; mass 71.0 ± 41.3 g; DFA $F_{4,158}$ = 3.51, P =

254   0.008, Fig. 3).

255   *Thamnophis melanogaster.* Juvenile male *T. melanogaster* showed that body size traits and the

256   body condition were similar between Lerma and Cuitzeo ($F_{1, 165}$ = 1.3, P = 0.25). In the case of

257   juvenile female *T. melanogaster*, SVL was greater in Lerma (26.2 ± 4.8 cm) than Cuitzeo (23.3 ±

258   4.7 cm), and body condition was similar between both locations ($F_{1, 260}$ = 5.06, P = 0.02).

259   Adult *T. melanogaster* of both sexes presented a similar pattern. A better body condition

260   in Lerma than Cuitzeo (males: 0.09 ± 0.29 vs. −0.04 ± 0.24; females 0.18 ± 0.34 vs. −0.07 ±

261   0.33), and similar body size (SVL and mass) between Lerma and Cuitzeo (males: $F_{1,86}$ = 4.9, P =

262   0.02; females: $F_{2,98}$ = 8.07, P = 0.0006).

263   *Thamnophis scalaris.* Only females *T. scalaris* (juvenile and adult) were enough to make

264   comparisons between locations. Juvenile female *T. scalaris* of Zempoala were significantly

265   longer, lighter, and had poor body condition than other locations. Lerma snakes showed lower

266   SVL, mass, and average body condition, while snakes from Cerrillo and S. Morelos presented a

267   better body condition, average SVL, and higher mass ($F_{1,165}$ = 1.3, P = 0.25, Fig. 4A).

268   Adult female *T. scalaris* of Lerma and Zempoala had poorer body condition than those of

269   Cerrillo and S. Morelos ($F_{6,62} = 8.4$, $P < 0.0001$, Fig. 4C).

270   **Stomach contents**

271   Of the total snakes collected by species in this study, 17.4% (44 *T. eques*) and 13.3% (91 *T.*

272   *melanogaster*), had some prey in the stomach. The diet of *T. eques* in Lerma and Cerrillo

273   included aquatic prey (leeches, fish and tadpoles) and amphibious prey (frogs). The terrestrial

274   prey (earthworms and mice) were only ingested by *T. eques* at Cerrillo (Table 5). At Cuitzeo, *T.*

275   *eques* consumed mainly fish and only some leeches (Table 5).

276   The diet of *T. melanogaster* included more prey items at Lerma than in Cuitzeo. At

277   Lerma, the fish were the main prey and leech and axolotl were ingested in similar proportion

278   (Table. 5). At Cuitzeo, *T. melanogaster* snakes contained only fishes.

279

280   **DISCUSSION**

281   In this study, we provide a heterogeneous pattern of sexual and ontogenic reproductive status

282   variations in body size and condition among populations of three sympatric garter snakes

283   collected in the Central Mexico Highlands over several years. We found: (1) each garter snake

284   species shows good and poor body condition in a variety of locations, (2) juvenile garter snakes

285   show similar body condition between populations, (3) adults show different body conditions

286   between populations, and (4) adults also show sex differences in body condition. Thus,

287   geographical differences in body condition were present in juvenile female *T. eques*, both sexes

288   of adult *T. melanogaster*, and juvenile and adult females of *T. scalaris*.

289   Addittionally, dietary differences were checked to associated them with body condition

290   differences between the localities. *Thamnophis eques* snakes where fish were the predominant

291   prey (Cuitzeo) had significantly better body condition than snakes that fed on fish and other

292   aquatic, terrestrial and amphibious prey (Lerma and Cerrillo). Overall, although *T. eques* snakes

293   from Lerma and Cerrillo have greater body size than Cuitzeo snakes, adult females were

294   significantly heavier than females from Cuitzeo. On the other hand, *T. melanogaster* snakes

295   where more prey items were ingested (Lerma), had significantly greater body size (SVL and

296   mass) and better body condition (adult males and females) than *T. melanogaster* snakes where

297   fish were the only prey (Cuitzeo).

298   We hypothesized that body condition of garter snakes would reveal a pattern of

299   geographical variation influenced by ontogenic reproductive status (juvenile, adult), sex, and diet

300   differences among populations. Several problems may confound these inter- and intraspecific

301   patterns of differences in body condition because each responds to complex interactions between

302   sexual and ontogenic reproductive status with local environmental variables and local resource

303   availability (Congdon 1989, Shine et al. 2001). Thus, the differences in body condition between

304   sites may result from differences in local prey availability, dietary quality, or predation

305   efficiency (Britt, Hicks & Bennett 2006), or a complex spatio-temporal interaction that is

306   reflected in micro-geographic diet variation, a pattern common in garter snakes (Seigel 1996).

307   The body condition differences among years and localitions within species would be evidence

308   that the patterns found are likely just based on prey availability or climatic constraints on feeding

309   as temporarily fluctuating assimilation rates.

310   Morphological plasticity induced by diet is extensively documented, especially for

311   natricine snakes (e.g. Krause, Burghardt & Gillingham 2003, Vincent et al. 2009, Hampton

312   2013), and some involve comparisons of snake populations separated by geographic distances. In

313   this study, location and diet were a significant overall factor influencing body size and body

314   condition in garter snakes. The diet has differential effects on *T. eques* and *T. melanogaster*.

315   Both snakes eating fish (Cuitzeo populations) have shorter or lighter relative body sizes, but they

316   respond differentially in their body condition to the piscivorous diet. The generalist *T. eques*

317   have relative best body condition at Cuitzeo, while the specialist *T. melanogaster* apparently

318   does not present significant differences in body condition between the piscivorous population

319   versus other prey. The differences across the two localities may not be strictly due to diet, as is

320   suggested by the fact that juveniles and adult males and females in the two sites, show particular

321   differences in any measure of body size (SVL and mass).

322   Sympatric and closely related species are expected to exhibit a similar body condition

323   due to the ecological similarities that impose common selective pressures, as suggested by the

324   study in closely related and sympatric garter snakes *T. melanogaster* and *T. eques* with similar

325   patterns of neonate body condition (Manjarrez & San-Roman-Apolonio 2015). However, we

326   cannot assume that the garter snakes we studied make similar use of local energy supplies, which

327   may vary according to intra-interspecific competition and available resources (Congdon 1989),

328   especially on prey availability (Krause, Burghardt & Gillingham 2003).

329   Growth and body condition in snakes may reflect intraspecific competition intensity that

330   would correspond to availability and allocation of energy (Bronikowski 2000, Bronikowski &

331   Arnold 1999, Blouin-Demers, Prior & Weatherhead 2002). This is especially applicable for

332   female garter snakes because they are generally heavier bodied and have greater reproductive

333   energy demands than males (Naulleau & Bonnet 1996, Shine et al. 2001, Blouin-Demers &

334   Weatherhead 2007).

335   For most of the locations in this study, the sex ratio was biased towards females, a

336   common pattern in other species of *Thamnophis* (Parker and Plummer 1987); however, the basic

337   question is whether the variation is true, displaying actual population structure, or is false,

338   reflecting different sexual behavioral traits that can influence catchability of males and females

339   at some locations (Parker and Plummer 1987). The sex ratio of the present locations of the garter

340   snakes studied may not be accurate, in this sense, our conclusions about the geographical

341   differences of the corporal condition should be considered with caution due to the sexual

342   variability of the body condition between locations.

343   Ontogenic differences in body condition can result from differential resource use. For

344   example, studies on *T. melanogaster*, *T. eques*, and *T. scalaris* have reported intraspecific

345   differences in the diet of snakes, such as the changing of aquatic invertebrate to terrestrial

346   vertebrate prey between small and large snakes (Macias-Garcia & Drummond 1988, Manjarrez,

347   Venegas-Barrera & García-Guadarrama 2007, Manjarrez, Macías García & Drummond 2013,

348   Manjarrez, Pacheco-Tinoco & Venegas-Barrera 2017). This suggests different trade-off

349   strategies between growth rate and body mass for resource allocation among sites, according to

350   sex (King 1989, 1997, Krause, Burghardt & Gillingham 2003) and ontogenic reproductive status

351   (Naulleau & Bonnet 1996, Lind & Beaupre 2015). This trade-off has been sparsely studied in

352   neonate snakes (i.e *Nerodia sipedon* and *Elaphe obsolete*; Weatherhead et al. 1999, Blouin-

353   Demers & Weatherhead 2007).

354   Another reason for geographic variation in the body condition of juvenile and adult

355   snakes includes geographic variation in the percentage of juveniles and adults in the population.

356   For *T. melanogaster*, 94% of juveniles and 76% of adults were collected from Cuitzeo; while for

357   *T. eques* 70% of juveniles and 77% of adults were collected Lerma. In *T. scalaris* locations, this

358   age bias was less evident, with collection percentages of juveniles ranging from 17% to 31% by

359   location, and 15% to 27% for adults.

360   According to our results, the models propose different paths for population fitness of each

361   garter snake species assuming the current body condition. In this way, the future scenario

362   responds according to the local geographic variation of each population, however, this prediction

363   is difficult to rely upon because environmental fluctuations can be unpredictable, and changes in

364   the climate, vegetation, topography, and land use variables will reduce the future potential

365   distribution of these three garter snakes, as has been predicted in Gonzalez et al. (2018).

366   Another important pattern in this study is the interspecific difference of body condition

367   within the same location. For example, in Cuitzeo, the body condition of *T. eques* is good, and in

368   Lerma it is poor, while in *T. melanogaster* the body condition is inverse; poor in Cuitzeo and

369   good in Lerma. This difference could be explained by interspecific differences in resource use

370   and its differential microdistribution. In this sense, *T. eques* is a generalist in its diet, ingesting

371   aquatic and terrestrial prey, while *T. melanogaster* is a specialist ingesting only aquatic prey. The

372   majority of specialist-generalist trade-offs are related with wide ecological traits that result in

373   distinct performance between specialists and generalists (Drummond 1983, Futuyma & Moreno

374   1988). If these species exploit different foraging environments, it is likely that they are exposed

375   to different environmental conditions. For example, Cuitzeo is a permanent lake that offers a

376   constant aquatic foraging environment for the aquatic specialist *T. melanogaster*, while Lerma is

377   a wetland environment, more suitable for the aquatic-terrestrial *T. eques*, a differential pattern

378   that is reflected in the interspecific differential body condition within both locations. In this

379   sense, the interspecific differences of the body condition can be a reflection of the phenotypic

380   plasticity of both garter snakes, because the geographical difference in the diet is reflected in the

381   local differences of body condition.

382   The morphological differences found in these studies reflected phenotypic plasticity,

383   rather than genotypic differences, although the relative function of genotype, ontogeny, and sex

384   in the presence of this plasticity could only be inquired through future studies. Also, further

385   exploration including a larger sample size by local diet, is requirement.

386 CONCLUSIONS

387   In conclusion, our analyses suggest that traits associated with body condition of sympatric

388   Mexican garter snakes *T. eques*, *T. melanogaster*, and *T. scalaris* in the Central Mexico

389   Highlands, reveal a pattern of microgeographical variation among local populations that differ

390   little by ontogenic reproductive status, and therefore, sex has little or no influence on body

391   condition in these garter snakes. The diet has differential effects on *T. eques* and *T. melanogaster*

392   in traits associated with body condition.

393

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401

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539   9.

Capture locations of *T. eques, T. melanogaster* and *T. scalaris* in Central Mexico.

1   TABLE 1. Capture locations of *T. eques, T. melanogaster* and *T. scalaris* in Central Mexico.

Locality	Garter snake present	Coordinates N, W (Datum WGS84)	Elevation (m)	Mean annual temperature (°C)	Mean annual precipitation (mm)
Lerma, Estado de México	*T. eques, T. melanogaster, T. scalaris*	19°14'28.73", 99°29'41.14"	2573	15.8	158.7
Cerrillo, Estado de México	*T. eques*	19°24'20.86", 99°41'41.05"	2550	13.7	116
S. Morelos, Estado de México	*T. scalaris*	19°18'49.58", 99°41'29.07"	2750	13.8	746.9
Cuitzeo, Michoacan	*T. eques, T. melanogaster,*	19°55'32.83", 101°08'26.78"	1837	18.1	755.8
Zempoala, Morelos	*T. scalaris*	19°02'53.40", 99°18'44.54"	2800	14.2	514

ANOVA of ln-SVL and ln-mass as dependent variables among locations, years and sex for each garter snake species.

1   TABLE 2. ANOVA of ln-SVL and ln-mass as dependent variables among locations, years and

2   sex for each garter snake species.

	Location	Year	Sex
T. melanogaster			
body condition	1.25	3.76**	1.54
SVL	21.58***	6.56***	0.18
mass	29.21***	5.50***	0.00
T. eques			
body condition	5.59*	2.56*	0.21
SVL	12.08***	22.75***	0.99
mass	7.47**	20.82***	1.66
T. scalaris			
body condition	14.06***	14.32***	0.23
SVL	7.12**	0.34	0.67
mass	2.42	3.73*	1.10

3   * *P* < 0.05
4   ** *P* < 0.001
5   *** *P* < 0.0001

Sex ratio (male:female) of *T. eques*, *T. melanogaster*, and *T. scalaris* for each population collected from Central Mexican Highlands (*df* = 1 for all tests).

1   Table 3. Sex ratio (male:female) of *T. eques*, *T. melanogaster*, and *T. scalaris* for each
2   population collected from Central Mexican Highlands (*df* = 1 for all tests).

	T. eques		*T. melanogaster*		*T. scalaris*	
	Sex Ratio	χ^2 test (*P*)	Sex Ratio	χ^2 test (*P*)	Sex Ratio	χ^2 test (*P*)
Lerma	1:1	0.45 (0.49)	1:1	2.0 (0.15)	1:1.5	12.46 (0.0004)
Cuitzeo	1:2	4.33 (0.03)	1:1.4	14.9 (0.0001)		
Cerrillo	1:3	6.76 (0.009)				
S. Morelos					1:1.7	9.94 (0.001)
Zempoala					0:23	23.0 (<0.0001)

Kendall rank correlation and R^2 coefficients of ln-mass and body condition on ln-SVL of *T. eques*, *T. melanogaster* and *T. scalaris*.

1   Table 4. Kendall rank correlation and R^2 coefficients of ln-mass and body condition on ln-SVL

2   of *T. eques*, *T. melanogaster* and *T. scalaris*.

	T. eques *n = 253*	R^2	*T. melanogaster* *n = 686*	R^2	*T. scalaris* *n = 80*	R^2
Ln-mass	0.77*	0.84	0.81*	0.88	0.80*	0.92
Body condition	−0.25*	0.12	−0.19*	0.08	−0.21*	0.05

3   * $P < .0001$

Number of stomachs containing each prey taxon ingested by *T. eques* and *T. melanogaster* in the Lerma, Cerrillo and Cuitzeo locations. Percentages by location in parentheses.

1 TABLE 5. Number of stomachs containing each prey taxon ingested by *T. eques* and *T.*

2 *melanogaster* in the Lerma, Cerrillo and Cuitzeo locations. Percentages by location in

3 parentheses.

Prey	Lerma	El Cerrillo	Cuitzeo
Thamnophis eques			
Fish	2 (22.2)	3 (12.5)	10 (90.9)
Leech	4 (44.4)	0	1 (9.1)
Tadpole	1 (11.1)	3 (12.5)	0
Earthworm	0	7 (29.2)	0
Frog	2 (22.2)	5 (20.8)	0
Mouse	0	6 (25)	0
Thamnophis melanogaster			
Fish	30 (88.2)		54 (100)
Leech	2 (5.9)		0
Tadpole	0		0
Earthworm	0		0
Axolotl	2 (5.9)		0

Figure 1

(A) Geographic distribution of *T. eques, T. melanogaster* and *T. scalaris* in Central Mexico (digitalized from Rossman et al. 1996) and (B) study locations in the Rio Lerma drainage.
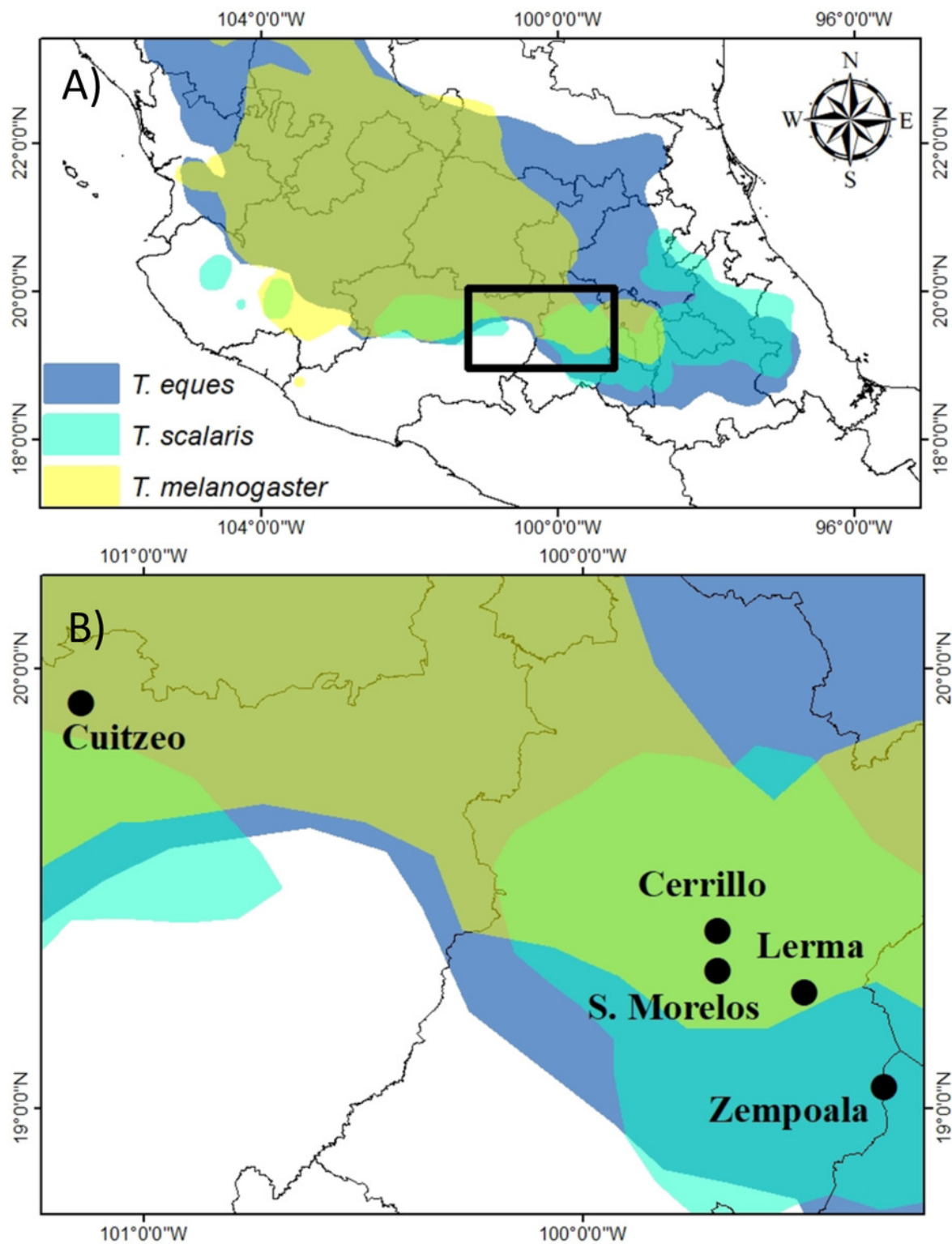
Figure 2

Body condition, SVL and mass (mean ± 1SE) of wild-caught snakes *T. eques* (A-C), *T. melanogaster* (D-F), and *T. scalaris* (G-I).

Snakes collected from locations in the Central Mexican Highlands over a period of 20 years. Body condition obtained of residuals from MA linear regression of ln-transformed body mass on ln-transformed SVL.
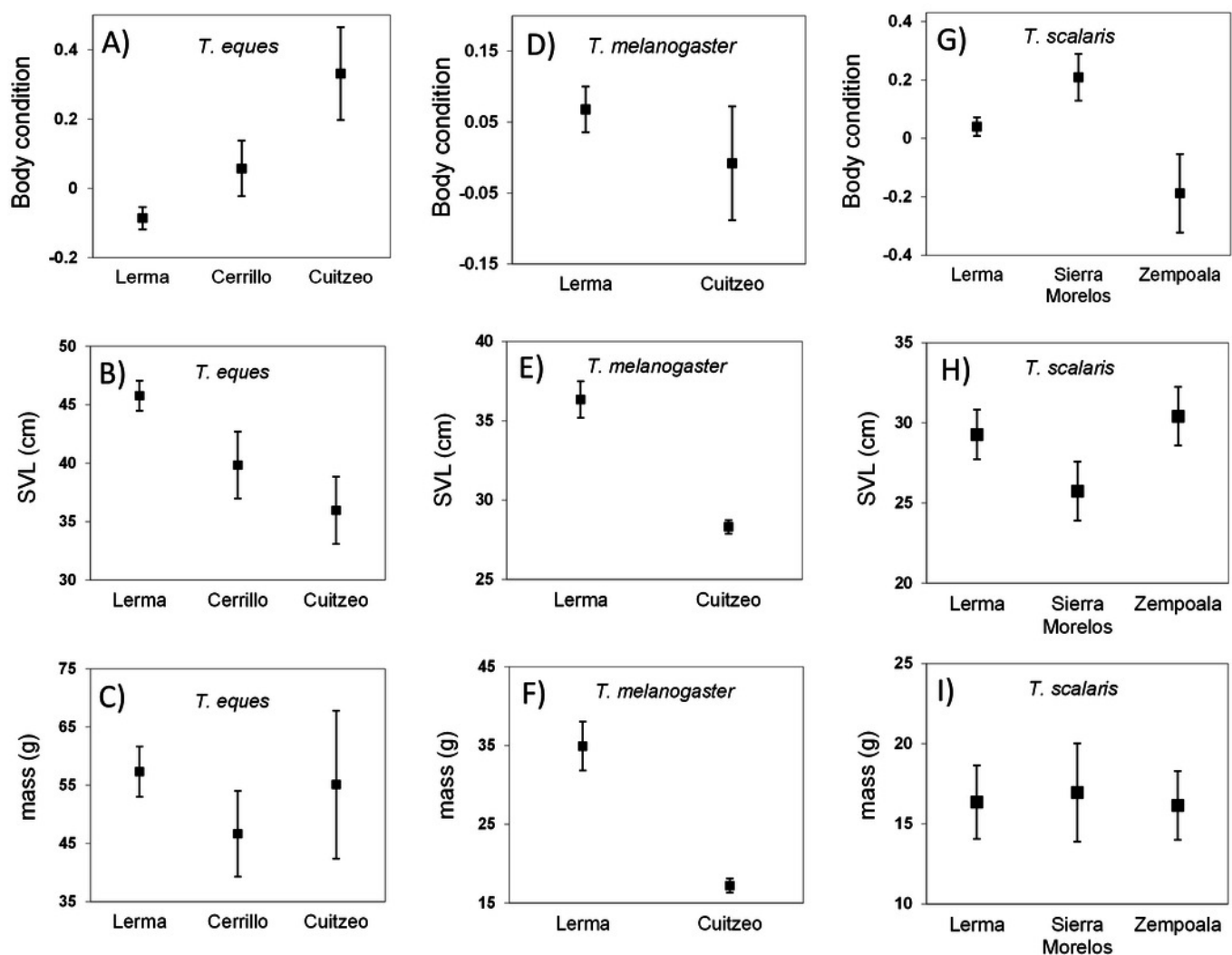
Figure 3

Average canonical position (centroid) for Lerma, Cerrillo and Cuitzeo, obtained from a discriminant function analysis of body condition traits in adult female *T. eques* and factor structure.

Isoclines represent variation on SVL of snakes in Lerma, Cerrillo and Cuitzeo.

N/A
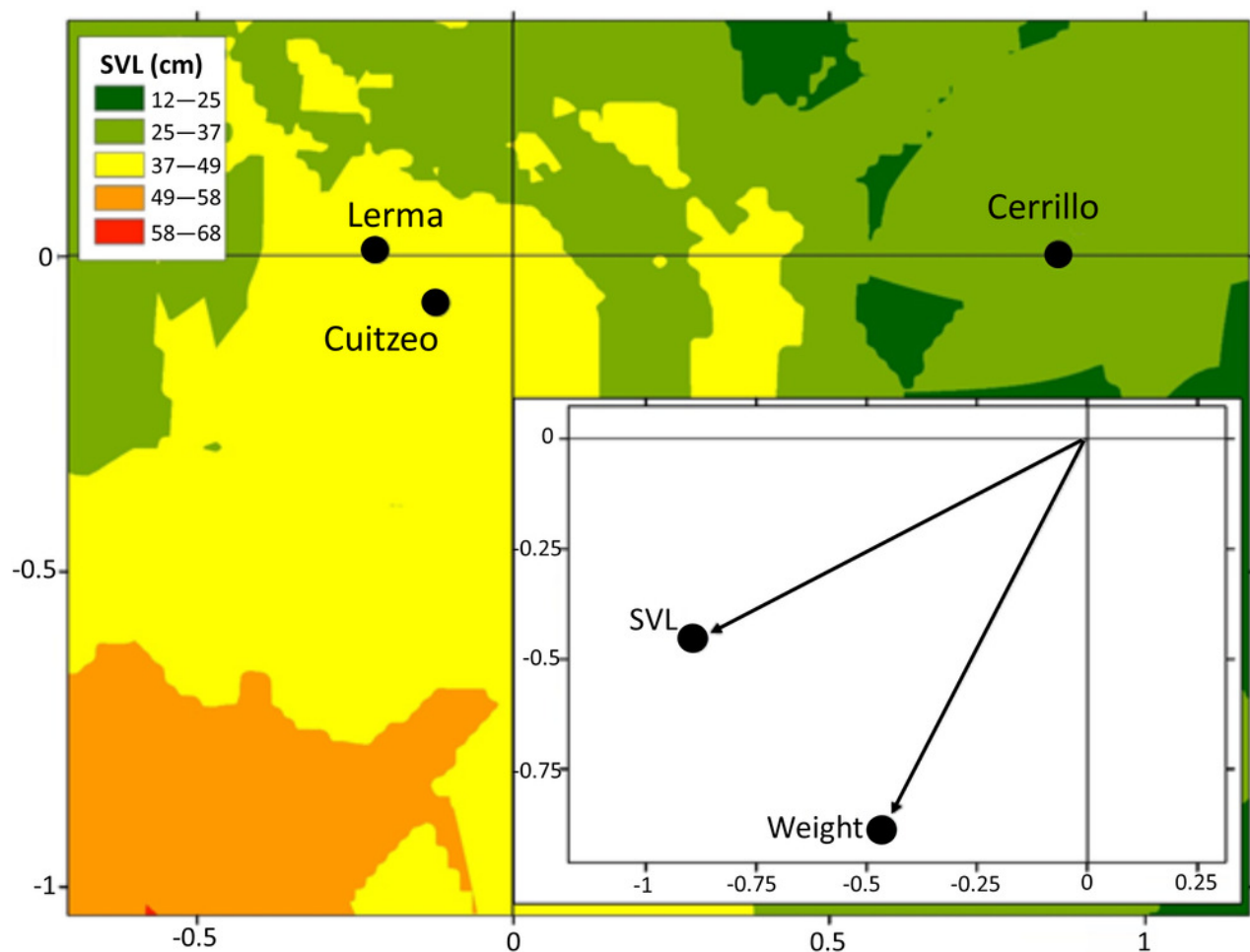
Figure 4

Canonical position of the centroids of juvenile (A) and adult (B) females of garter snakes *T. scalaris* captured from Lerma, Cerrillo, S. Morelos and Zempoala.

Centroids obtained from a discriminant function analysis and the variables with the greatest discrimination between locations (C and D). Isoclines represent variation of body conditions of snakes in Lerma, Cerrillo, S. Morelos and Zempoala.