

Original article

Species or population? Systematic status of *Vieja coatlicue* (Teleostei: Cichlidae)

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Pacific and Atlantic-slope rivers of the Isthmus of Tehuantepec in Mexico contain two cichlid species of the genus *Vieja* relevant to the current study, *V. zonata* and *V. coatlicue*, respectively. The Atlantic-slope species was initially considered a population of uncertain taxonomic status and recently described as a distinct species based on three putatively diagnostic morphological characters. The objective of this study was to combine new and existing genetic data, along with reanalysis of morphological characters, to test the distinctiveness of *V. coatlicue*. Little genetic variability and no geographic structuring were recovered. Additionally, putatively diagnostic morphological character states were present across both species, failing to separate the forms. The synthesis of molecular and morphological data supports the recognition of *V. coatlicue* as a junior synonym of *V. zonata*.

Keywords: Cichlid, Freshwater, Mexico, River, Taxonomy.

Dos especies del género *Vieja* se encuentran distribuidas a lo largo Istmo de Tehuantepec en México; *V. zonata* se distribuye en los ríos de la vertiente del Pacífico y *V. coatlicue* en los ríos de la vertiente del Atlántico. La especie que se distribuye en la vertiente del Atlántico era considerada como una población de estado taxonómico incierto, pero recientemente fue descrita como nueva especie en base a tres caracteres morfológicos diagnósticos. El objetivo de este estudio fue utilizar datos moleculares nuevos con datos moleculares previamente publicados en combinación con una reanálisis de los caracteres morfológicos para testear la validez taxonómica de la especie *V. coatlicue*. Nuestros análisis moleculares no recobran estructura geográfica y además muestran baja variabilidad genética. Además, los tres caracteres morfológicos diagnósticos se encontraron presentes en individuos de ambas especies, nuestro trabajo sugiere que estos caracteres morfológicos diagnósticos no proven información que permita separar a las formas que se distribuyen en la vertiente del Pacífico y la vertiente del Atlántico. Nuestra síntesis de datos moleculares y morfológicos provee evidencia para reconocer a *V. coatlicue* como una sinónima mas reciente de *V. zonata*.

Palabras clave: Agua dulce, Cíclido, México, Río, Taxonomía.

Introduction

The genus *Vieja* Fernández-Yépez, 1969 is a clade of predominantly Northern Middle American cichlids, with highest diversity and endemism occurring in southern Mexico and Guatemala. One species in the genus, *Vieja zonata* (Meek, 1905), is endemic to Mexico and found in rivers along the Isthmus of Tehuantepec from the Río Tequisistlán in Oaxaca west of Tehuantepec to Río Tapantepec near the Chiapas border likely near the town of San Pedro Tapantepec (Kullander, 2003; Miller *et al.*, 2005; Matamoros *et al.*, 2015). In addition, an

Atlantic slope population of this species exists in the Río Coatzacoalcos drainage (Conkel, 1993; Miller *et al.*, 2005) that Miller *et al.* (2005) claimed did not represent *V. zonata* but a “population of undetermined taxonomic status”. Del Moral-Flores *et al.* (2018) recognized the Río Coatzacoalcos population as a distinct species, describing it as *V. coatlicue* based on three diagnostic morphological characters. Compared to *V. zonata*, the species was diagnosed based on an elongate body, dorsal-fin origin equal or posterior to opercular border, and a separation between the ends of non-filamentous rays of the pelvic fins and the anus.

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In a molecular systematic study of *Vieja*, McMahan *et al.* (2010) recovered low genetic differentiation among individuals of sister species *V. zonata* and *V. guttulata* (Günther, 1864) in their phylogeny of the genus. Species-level analyses were beyond the scope of that study; however, Atlantic slope (Río Coatzacoalcos) sequences of *V. zonata* (currently *V. coatlucue*) were included in their molecular phylogeny and all populations were recovered in the same clade. Given the lack of genetic distinctiveness of the population in Río Coatzacoalcos, the objective of this study was to synthesize genetic data and reassess the morphological characters provided by del Moral-Flores *et al.* (2018) to test morphological distinctiveness of *V. coatlucue* in the Río Coatzacoalcos drainage.

Material and Methods

Molecular data. Sequence data for *V. coatlucue* and several populations of *V. zonata* have been published in previous studies (e.g. McMahan *et al.*, 2010; Řičan *et al.*, 2016). We sequenced three additional individuals to provide data from other localities for both species (Fig. 1; Tab. 1). Whole genomic DNA was extracted from tissue

samples (muscle or fin clip) using the DNeasy kit (Qiagen, Inc.). The mitochondrial cytochrome *b* (cyt *b*) gene was amplified and sequenced for samples following protocols described in Perdices *et al.* (2002). Cytochrome *b* is an appropriate marker to address the main objective of this study as it has been quite useful to provide resolution at intra- and interspecific levels within Middle American cichlids (López-Fernández *et al.*, 2010; McMahan *et al.*, 2010, 2017a; Harrison *et al.*, 2014; Barrientos-Villalobos *et al.*, 2018). Additionally, various nuclear markers have failed to delineate taxa at this level (López-Fernández *et al.*, 2010; McMahan *et al.*, 2010; Řičan *et al.*, 2016). Amplification products were visualized on a 1% agarose gel, followed by purification with ExoSap-IT, and then sequenced on an ABI 3730 in the Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum of Natural History. An individual of *V. guttulata* was sequenced as an additional ingroup taxon to test monophyly of *V. zonata* and *V. coatlucue*. *Vieja fenestrata* (Günther, 1860) was used as an outgroup following phylogenetic relationships of McMahan *et al.* (2010) and Řičan *et al.* (2016). All new sequences were deposited in GenBank (Tab. 1).

Tab. 1. Specimens/tissues examined. No. refers to number by tip in phylogeny (Fig. 2). Acronyms follow Sabaj (2016).

Species	No.	Tissue No.	Voucher No.	GenBank	Drainage/Locality	Latitude	Longitude
<i>Vieja coatlucue</i>	2	-	-	KU854739	Río Coatzacoalcos	18.152194	-94.790194
<i>Vieja coatlucue</i>	5	-	-	KU854741	Río Coatzacoalcos	16.767778	-95.021806
<i>Vieja coatlucue</i>	7	SLU-TC 347	SLU 5010	FJ668647	Río Coatzacoalcos	16.77148	-95.01855
<i>Vieja coatlucue</i>	10	SLU-TC 341	SLU 5010	FJ668639	Río Coatzacoalcos	16.77148	-95.01855
<i>Vieja coatlucue</i>	11	SLU-TC 348	SLU 5010	FJ668638	Río Coatzacoalcos	16.77148	-95.01855
<i>Vieja coatlucue</i>	13	-	TNHC 29065	AY324023	Río Coatzacoalcos; Río Dos Caños	18.168106	-94.916667
<i>Vieja coatlucue</i>	15	-	TNHC 29054	AY324024	Río Coatzacoalcos; Río Sarabia	17.102197	-95.040522
<i>Vieja coatlucue</i>	16	-	-	KU854740	Río Coatzacoalcos	16.767778	-95.021806
<i>Vieja coatlucue</i>	17	-	MNCN 147114	DQ990728	Río Coatzacoalcos; Río Sarabia	17.102197	-95.040522
<i>Vieja coatlucue</i>	-	-	UNICACH 6702	-	Río Coatzacoalcos; Río Jaltepec	17.387582	-95.057214
<i>Vieja coatlucue</i>	-	-	UMMZ 184759	-	Río Coatzacoalcos; Río Almoloya	16.8333333	-95
<i>Vieja coatlucue</i>	-	-	UMMZ 178548	-	Río Coatzacoalcos; Donaji	17.386577	-95.05784
<i>Vieja fenestrata</i>	-	-	-	DQ990726	Río Coatzacoalcos	-	-
<i>Vieja guttulata</i>	-	SLU-TC 2272	SLU 8117	MK837107	Río Coatán; Tapachula	14.896442	-92.323668
<i>Vieja zonata</i>	1	-	TNHC 29059	AY324026	Río los Perros	16.557617	-95.091117
<i>Vieja zonata</i>	3	-	-	KU854738	Río Tehuantepec	16.339361	-95.241917
<i>Vieja zonata</i>	4	-	-	MK837015	Río los Perros	16.442715	-95.028022
<i>Vieja zonata</i>	6	SLU-TC 343	SLU 5014	FJ668637	Río Tehuantepec	16.377621	-95.241244
<i>Vieja zonata</i>	8	SLU-TC 344	SLU 5014	FJ668646	Río Tehuantepec	16.377621	-95.241244
<i>Vieja zonata</i>	9	SLU-TC 342	SLU 5014	FJ668642	Río Tehuantepec	16.377621	-95.241244
<i>Vieja zonata</i>	12	-	-	MK837016	Río Ostuta	16.499821	-94.432838
<i>Vieja zonata</i>	14	-	TNHC 29058	AY324025	Río los Perros	16.557617	-95.091117
<i>Vieja zonata</i>	-	-	UMMZ 178573	-	Río Tehuantepec	16.415523	-95.597819
<i>Vieja zonata</i>	-	-	UMMZ 184746	-	Río Novillero, Tapantepec	16.390642	-94.133723
<i>Vieja zonata</i>	-	-	UNICACH 6356	-	Río Ostuta	16.43171	-94.24725
<i>Vieja zonata</i>	-	-	FMNH 3776	-	Niltepec	-	-
<i>Vieja zonata</i>	-	-	FMNH 3777	-	Niltepec	-	-

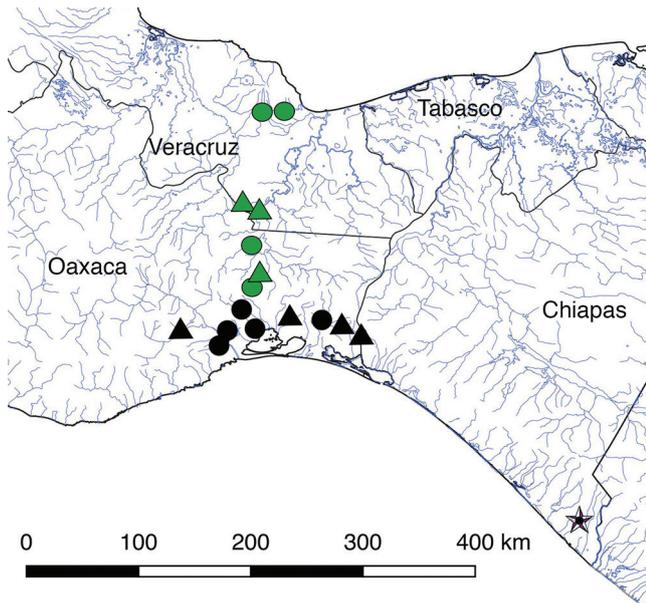


Fig. 1. Map of Mexico displaying localities for specimens (triangles) examined and tissue samples (circles) for *Vieja coatlucue* (green) and *Vieja zonata* (black) used in this study. Star indicates locality for *Vieja guttulata* sample.

Sequence data were visually inspected and aligned using the software Geneious v.9.1 (Kearse *et al.*, 2012). A multiple sequence alignment was generated using the Muscle algorithm (Edgar, 2004) implemented in Geneious using default parameters. The final alignment consisted of 1,050 base pairs. Partition scheme and model selection were performed using the software PartitionFinder (Lanfear *et al.*, 2012) using a greedy search. The best partition scheme and substitution model were selected using the Akaike Information Criterion (AIC).

Phylogenetic hypotheses were inferred under a Bayesian framework using the software Mr. Bayes v.3.2.6 (Huelsbeck *et al.*, 2001) implemented in the CIPRES portal (Miller *et al.*, 2010). Three independent analyses of 12,000,000 generations of two replicated runs each were executed, with tree sampling every 6,000 generations. Sampling stationarity was assessed via visual inspection in Tracer v.1.5 (Rambaut *et al.*, 2018) and observed average standard deviations of split frequencies (<0.01). The initial 25% of sampled trees were discarded as burn-in. Bayesian posterior probabilities were calculated using the post burn-in trees, and the three independent runs were compared to evaluate topological congruence.

Due to missing sequence data in one sample (KU854740), estimation of genetic diversity and reconstruction of a haplotype network relied on a slightly reduced dataset (1,011 base pairs). Nucleotide (π ; Nei, Li, 1979), genetic (θ_w ; Watterson, 1975), and haplotype (Hd) diversity indices were calculated in the software DnaSP v.5.10 (Librado, Rozas, 2009). Matrilineal haplotypes were reconstructed using statistical parsimony analysis in the software TCS (Templeton *et al.*, 1992) with a 95% confidence limit for

connected haplotypes. The recovered network with TCS was used to infer gene genealogies using a median-joining network (Bandelt *et al.*, 1999) implemented in the software POPART (Leigh, Bryant, 2015).

Morphological data. Material examined covered four localities for *V. zonata* (N= 29) along Pacific drainages in the Isthmus of Tehuantepec, including the holotype and paratype, and three populations of *V. coatlucue* (N= 22) in the Río Coatzacoalcos (Tab. 1; Fig. 1). Our goal was to reassess the putative morphological distinctiveness of *V. coatlucue* relative to *V. zonata*; thus, characters examined herein focused on the three described by del Moral-Flores *et al.* (2018) to diagnose *V. coatlucue* as a distinct species from *V. zonata*. Standard length (SL) and maximum body depth (BD) were measured for each specimen. The ratio of SL:BD, also known as the fineness ratio, was calculated and used as a measure of body elongation, with greater values indicating more elongate bodies (Aguirre *et al.*, 2016). Additionally, data on anterior insertion of the dorsal fin relative to posterior of the operculum (anterior/posterior to or in-line with) and presence/absence of a space between non-filamentous portions of the pelvic rays and the anus were recorded. These data were assessed to determine if the proposed diagnostic characters could separate the two species and if morphological differences were congruent with any observed patterns of genetic differentiation.

Results

Molecular data. A total of 10 individuals of *V. coatlucue* (four localities) and seven individuals of *V. zonata* (five localities) were analyzed based on new and previously published sequences. The best partition scheme was by codon with independent substitution models (1st codon: HKY, 2nd codon: F81, 3rd codon: GTR+gamma). A single clade containing *V. zonata* and *V. coatlucue* was recovered based on phylogenetic analysis of *cyt b* data, with an average 0.2% sequence divergence observed across all individuals of both species. We recovered no geographic structuring within this clade (Fig. 2). *Vieja guttulata* was recovered as sister to the clade containing *V. zonata* and *V. coatlucue*, with an average sequence divergence of 2.2%. This result is consistent with McMahan *et al.* (2015) and Řičan *et al.* (2016). Sequence divergence between the outgroup (*V. fenestrata*) and ingroup was 2.7%.

We recovered four haplotypes in the network generated by statistical parsimony (Fig. 3). One haplotype was common and widely distributed across the Coatzacoalcos and Tehuantepec river basins and shared between individuals of *V. coatlucue* and *V. zonata* (Fig. 3). Finally, all samples from the clade containing *V. coatlucue* and *V. zonata* showed low levels of nucleotide ($\pi=0.493$, standard deviation 0.131), genetic ($\theta_w=0.0016$, standard deviation 0.0078), and haplotype (Hd=0.0031, standard deviation 0.00139) diversity across populations and river basins.

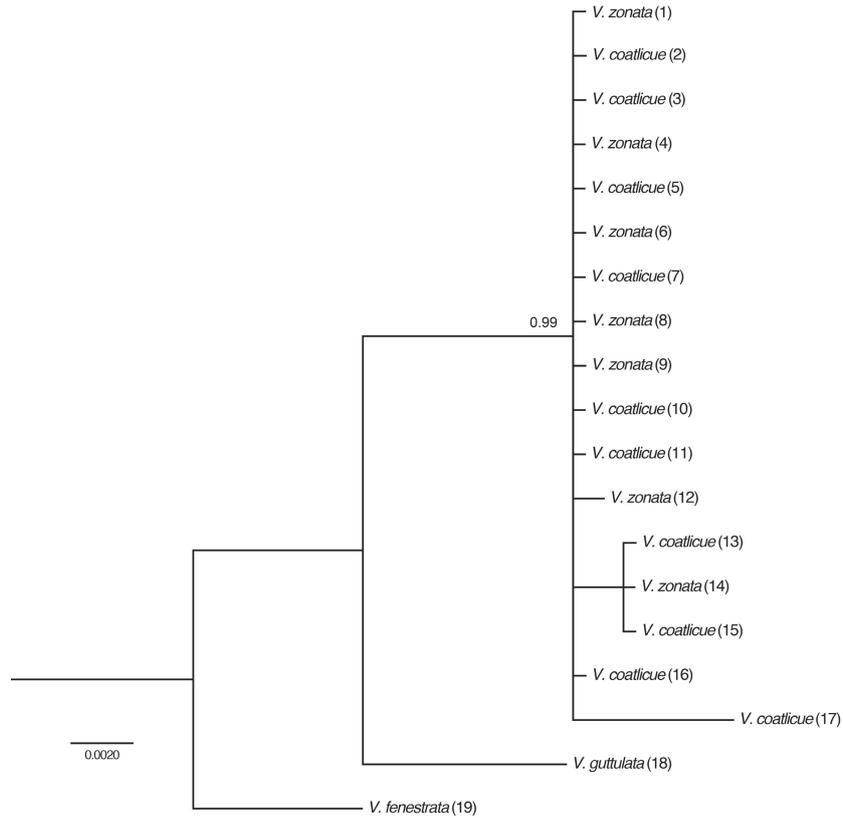


Fig. 2. Bayesian phylogeny based on cytochrome *b* sequence data. Number above clades is posterior probability.

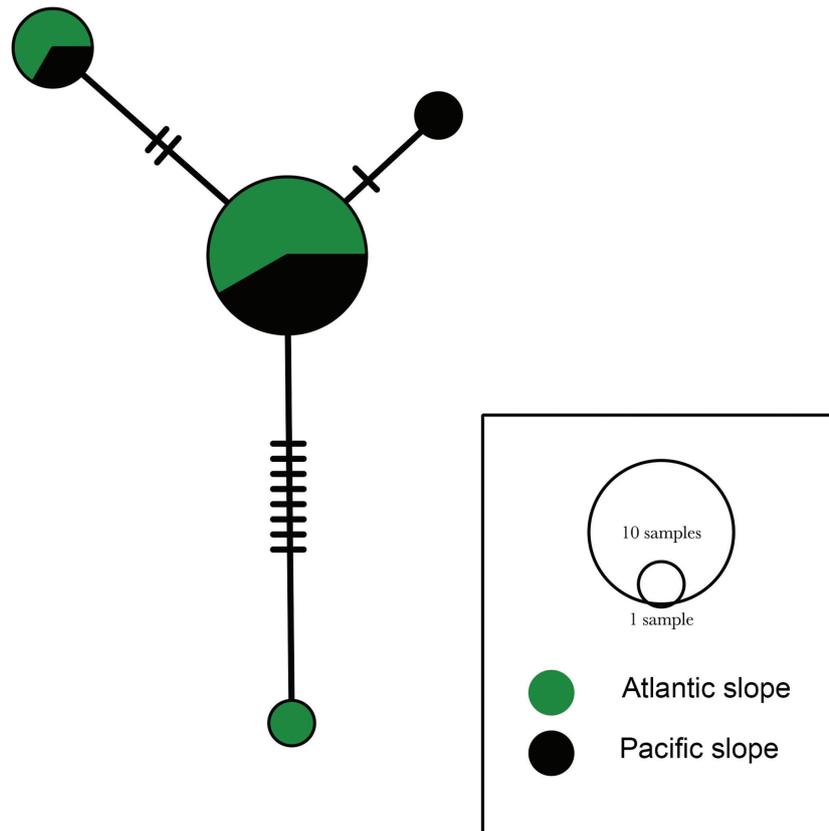


Fig. 3. Haplotype network recovered from analysis of the *cyt b* dataset for populations of *Vieja coaticue* (Atlantic) and *Vieja zonata* (Pacific). Dashed lines represent mutational steps.

Morphological data. The average fineness ratio for *V. coatlicue* was 2.11 (N=22, standard deviation 0.11, minimum 1.87, maximum 2.31), compared to 2.28 (N=29, standard deviation 0.1, minimum 2.04, maximum 2.45) for *V. zonata* (Tab. 2). This reveals a slightly more streamlined or elongate overall body shape in *V. zonata*, inconsistent with the purported elongate body diagnostic of *V. coatlicue*. Across both species we found that most individuals possessed a dorsal-fin origin marginally anterior to- or in-line with the posterior margin of the operculum, although a moderately higher number of individuals of *V. zonata* exhibited a dorsal-fin origin slightly posterior to the operculum (Tab. 3; Fig. 4). Dorsal-fin origin was only vaguely anterior to the posterior end of the operculum in individuals with this character state and could easily be considered in-line with posterior of the operculum. Additionally, we observed these character states to be skewed in bent specimens, depending on if gathering data from the right or left sides. Absence of a space between non-filamentous rays of the pelvic fin and the anus was observed across the majority of individuals of both *V. coatlicue* and *V. zonata* (Tab. 3; Fig. 5).

Tab. 2. Fineness ratios (SL:BD) for *Vieja coatlicue* and *Vieja zonata*. SD = standard deviation.

	N	Mean	SD	Minimum	Maximum
<i>V. coatlicue</i>	22	2.11	0.11	1.87	2.31
<i>V. zonata</i>	29	2.28	0.10	2.04	2.45

Tab. 3. Number of individuals of *Vieja coatlicue* and *Vieja zonata* with examined character states dorsal-fin origin relative to operculum and presence/absence of space between non-filamentous ends of the pelvic fins and anus.

	Dorsal-fin origin relative to operculum		
	anterior	equal	posterior
<i>V. coatlicue</i>	10	8	4
<i>V. zonata</i>	9	9	11

	Separation between pelvic fin and anus	
	absent	present
<i>V. coatlicue</i>	18	4
<i>V. zonata</i>	25	4



Fig. 4. Dorsal-fin origin relative to operculum in *Vieja coatlicue* (top; UMMZ 178548) and *Vieja zonata* (bottom; FMNH 3776 (holotype), UMMZ 184746), illustrating variability within and between species. Scale bar equals 1cm.



Fig. 5. Pelvic fin relative to anus in *Vieja coatlicue* (left of vertical line; UMMZ 178548) and *Vieja zonata* (right of vertical line; FMNH 3776 (holotype), UMMZ 184746), illustrating variability within and between species. Asterisk indicates holotype of *V. zonata*.

Discussion

We found a lack of monophyly for *V. zonata* and *V. coatlucue*, as well as absence of any reasonable level of genetic divergence across species or populations. Additionally, reanalysis of putatively diagnostic morphological characters fails to unambiguously separate the two species, with all character states being observed across the range of the two species. Fineness ratios show the opposite trend from description of *V. coatlucue*, with slightly more elongate bodies in populations of *V. zonata*. The origin of the dorsal fin relative to posterior of the operculum exhibits variability between the two species; however, in nearly all specimens the origin is very close to in-line with the posterior edge of the operculum. Few individuals, across both species, exhibit a clearly anterior or posterior condition. We found that specimens of either species rarely possess a clearly defined space or gap between tips of the (non-filamentous) pelvic fin and the anus. High levels of variability were consistently observed for this character across all material examined. Indeed, the holotype of *V. zonata* possesses the most evident space between the non-filamentous pelvic rays and the anus (Fig. 5). The variability in these morphological characters is high and consistent across populations of both species. Thus, the combination of morphological and genetic data supports the recognition of *V. coatlucue* as a junior synonym of *V. zonata*.

Many studies have demonstrated high levels of intraspecific morphological variability within cichlid fishes (McMahan *et al.*, 2017b; Barrientos-Villalobos *et al.*, 2018; Gómez-González *et al.*, 2018). While genetic data are certainly not a required component of detection and description of new species, these independent sources of data can offer additional ways to test hypotheses regarding species delineation. While McMahan *et al.* (2010) provided sequence data from populations of *V. zonata* from the Río Tehuantepec and Río Coatzacoalcos, admittedly the authors did not discuss this in text given it was outside the scope of that study. However, locality data and sequences for these specimens were published and deposited in GenBank. Řičan *et al.* (2016) later found the same lack of genetic distinctiveness between *V. zonata* from both slopes of the Isthmus of Tehuantepec. Anzueto-Calvo *et al.* (2016) additionally report the occurrence of *V. zonata* in the Río Grijalva, which should be studied in future analyses of this and related species.

The Isthmus of Tehuantepec represents the narrowest geographic point between the Pacific and Atlantic basins in the northern portion of Middle America and has been demonstrated to be an important region for organismal dispersal across Mexico, permitting taxa to expand between rivers along both slopes (Quiroz-Martínez *et al.*, 2014). This hypothesis is additionally supported by occurrence of a population of *V. zonata* in the Atlantic Río Coatzacoalcos. Thus, *V. zonata* in the Atlantic represent a recently dispersed population and not a distinct lineage.

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