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Evolutionary history and diversity in the ball roller beetle *Canthon cyanellus*

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To understand the evolutionary history of species, it is necessary to know the mechanisms for reproductive isolation, divergence-time between populations, and the relative action of the evolutionary forces (e.g., mutation, genetic drift, gene flow) within and between populations of the same, or closely related species. Although *Canthon* is one of the more diverse genera of neotropical beetles, insufficient research has been done to comprehend the divergent patterns that explain its speciation process. The absence of diagnostic morphological characters and the wide geographic variation of qualitative traits in Scarabaeinae obscures species delimitation, genealogical limits between populations, and its taxonomy. *Canthon cyanellus* is one of the best-known species in ecological and evolutionary aspects. It is a widely distributed species in the tropical forests of America. Also, the current deforestation has facilitated its incursion into open areas. Individuals from different populations have similar morphological characters but show wide variation in body color throughout their distribution, which makes it difficult to delimit the subspecies that comprise it. Recently, studies have been carried out to elucidate the pre- and postzygotic isolation mechanisms between populations and the historical biogeographical processes favoring cladogenesis events during the Pleistocene. Morphological variation of the male genitalia does not correspond to the phylogeographic structure. However, the morphological differences in one of the pieces of the endophallic sclerites have allowed a preliminary delimitation of some genetically differentiated clades. Finally, we consider that the joint analysis of traditional morphological taxonomy and phylogeography is important to understand the speciation process in the *C. cyanellus* complex.

KEYWORDS

aedeagus, allopatric populations, color polymorphism, genetic structure, incipient speciation, Mexican Transition Zone, reproductive barriers, taxonomic status

1. Introduction

Biological evolution is a complex hierarchically structured process encompassing microevolution, speciation, and evolution above the species level (Gould, 2002). Thus, to

understand the evolutionary history and the mechanisms involved in the formation of new species is necessary to know the historical biogeographical processes (e.g., the geographical separation between regions), which modify the distribution and population densities of the species (Mayr, 1963; Endler, 1977). Likewise, it is important to know the pre- and post-zygotic reproductive isolation mechanisms (Dobzhansky, 1970; Coyne and Orr, 1998), which are the first step toward the formation of new species.

With this knowledge, it is possible to test different biogeographic scenarios and establish their relationship with the patterns of genetic differentiation among populations and among species (Hewitt, 1996; Irwin, 2002). Reproductive isolation mechanisms are intensified when genetic drift, recurrent point mutation, and inbreeding affect populations with small effective sizes (Wright, 1982). In this regard, some studies have compared divergent patterns of characters (e.g., morphological, genetic, ecological, behavioral, niche, etc.) between populations of the same species, or between populations of phylogenetically close species founded evidence of genetic structure between populations, mechanisms of reproductive isolation, and morphological differences (e.g., in the grasshopper *Chorthippus parallelus*: Tregenza et al., 2000; in the bumblebees *Bombus ephippiatus* and *Bombus wilmattae*: Duennes et al., 2012, 2017; in the cicada *Subpsaltria yangi*: Liu et al., 2019).

Within the order Coleoptera, the coprophagous beetles Scarabaeinae Latreille, 1802 are very diverse (ca. 6,700 species; Bánki et al., 2022). Their widespread distribution, behavioral complexity, and diverse morphology make them a good model for studying the evolutionary processes that have given rise to them (Mullen and Shaw, 2014). Different analysis and phylogenetic approaches have brought advances in the systematics of Scarabaeinae dung beetles (Monaghan et al., 2007; Tarasov and Génier, 2015; Gunter et al., 2016; Tarasov and Dimitrov, 2016). Phylogenetic studies for taxonomic levels below subfamily are recent (Nolasco-Soto et al., 2017, 2020; Hensen et al., 2018, 2020, 2021; Maldaner et al., 2019; Pardo-Díaz et al., 2019; Mello et al., 2021; Moctezuma et al., 2021; Halffter et al., 2022). Those studies have explored different data sets (e.g., molecular, morphometric, behavioral, ecological).

The Scarabaeinae Deltachilini Lacordaire, 1856 is one of the oldest tribes along with the Dichotomiini (Davis et al., 2002). *Canthon* Hoffmannsseg, 1817 is the most speciose lineage of neotropical Deltachilini (Halffter and Martínez, 1977). The phylogenetic relationships established within *Canthon* and close related genera from morphological characters indicate that it is a polyphyletic group (Medina et al., 2003). These data led to the creation of putative subgenera; however, it is difficult to set clear boundaries between species and subspecies due to the absence of diagnostic morphological characters (Cupello and Vaz-de-Mello, 2018; Vieira et al., 2019; Nunes et al., 2020; Vaz-de-Mello et al., 2020). In addition, many Scarabaeinae species exhibit intraspecific geographic variation in body color (e.g., *Silvicanthon obscurus* Schmidt, 1920, *Phanaeus tridens* Castelnau, 1840, and *Canthon*

quinquemaculatus Castelnau, 1840). Therefore, some authors have designated subspecies based on the patterns of coloration in some species (e.g., *Canthon rutilans* Harold, 1868; *Canthon latipes* Blanchard, 1845; *Phanaeus pyrois* Bates, 1887; Arnaud, 2002, Medina et al., 2003).

Canthon cyanellus LeConte, 1859 is a necrophagous roller beetle from the Neotropics (Favila, 2001), which provides valuable ecosystem services similar to other Scarabaeinae (e.g., nutrient recycling, fly control, carrion removal, secondary seed dispersal, soil aeration; Nichols et al., 2008). It is a well-studied species in ecological and evolutionary aspects related to reproductive behavior (Favila, 2001; Favila et al., 2005; Chamorro-Florescano and Favila, 2008, 2009, 2016; Chamorro-Florescano et al., 2011, 2017), larval development (Hernández-Martínez and Martínez, 2003; Martínez, 2005), chemical communication (Bellés and Favila, 1983; Ortiz-Domínguez et al., 2006a,b; Cortez et al., 2012, 2015; Favila et al., 2012), population genetics and phylogeography (Ortiz-Domínguez et al., 2010; Nolasco-Soto et al., 2017; Arce-Valdés et al., 2021), among others. Traditionally, the taxonomy of *C. cyanellus* was based in non-informative morphological characters like variations in body color (Halffter, 1961). This has created uncertainty about genealogical limits among populations, which has led some authors not to recognize several of the subspecies (i.e., *Canthon cyanellus cyanellus* LeConte, 1859; *C. c. sallei* Harold, 1863, and *C. c. violetae* Halffter, 1961; Solís and Kohlmann, 2002, 2012).

Here, we will discuss the historical (e.g., cladogenesis, genetic structure) and contemporary processes (e.g., sexual recognition) that explain the evolutionary history and diversity of the *C. cyanellus* complex in its distribution range. This will allow a better understanding of the historical processes and mechanisms that have contributed to the formation of new species.

2. Evolutionary history of *Canthon*

The Scarabaeinae are of Gondwanan origin (Gunter et al., 2018). Depending on the author 12 (Smith, 2006) or 11 (Bouchard et al., 2011) tribal names have been proposed. Currently, the tribe Deltachilini encompasses over 100 genera, but according to Tarasov and Dimitrov (2016) it should comprise only 22 genera. *Canthon* belongs to the rolling beetle's group, characterized by long middle and hind legs and a head without any tubercles, keels, or horns (Halffter, 1961). Nearly 180 species are included within this genus (Bánki et al., 2022). Recently, Cupello and Vaz-de-Mello (2018) based on a taxonomic review for '*Canthon sensu lato*' concluded that at least 22 species should be considered *incertae sedis* as they do not belong to any of the subgenera currently recognized.

Although *Canthon* is mainly Neotropical, several species have Nearctic distribution (Kohlmann and Halffter, 1990). It is found from southern Canada to Argentina occupying a great diversity of ecosystems in an extremely rugged topography (Medina et al., 2003). It is absent in Chile and on the United States Pacific coast

(Halffter, 1961). The hypothesis about the origin of *Canthon* indicates that the great richness of species that occurs in the north of South America, associated with its absence in Chile and the Patagonian region, suggests a possible center of origin in the Arquibrasil massif (Halffter, 1962). However, there is no hard data that corroborates this hypothesis.

Since 1963, Halffter has made a series of contributions to the biogeography of the Mexican entomofauna, mainly from the beetles Scarabaeidae, which propose hypotheses about its evolutionary history. These contributions suggest the existence of the biogeographic zone known as the Mexican Transition Zone (MTZ), which was invaded by different lineages (i.e., cenocrons) that evolved in the Nearctic and Neotropic zones (Morrone, 2015; Halffter, 2017; Halffter and Morrone, 2017; Morrone, 2020). Apparently, *Canthon* colonized the neotropical part of the MTZ during different migratory events that occurred from South to North America. One migratory movement occurred before or during the Miocene, which included lineages that diversified widely in the Mexican Altiplano and the north of USA (e.g., *Canthon obliquus* Horn, 1894; *humectus* Say, 1932 and *pilularius* Linnaeus, 1758 species groups; Halffter, 1964; Kohlmann and Halffter, 1990; Halffter et al., 2022). A second ongoing migratory movement started after the reconstitution of the Isthmus of Panama (e.g., *Canthon viridis* Palisot de Beauvois, 1805, *C. cyanellus* and *Canthon indigaceus* LeConte, 1866; Halffter, 2017; Halffter and Morrone, 2017; Nolasco-Soto et al., 2017). Also, a current migratory movement associated with the expansion of rainforests in the last 10,000 years (e.g., *Canthon subhyalinus* Harold, 1867; *Canthon euryscelis* Bates, 1887, *Canthon angustatus* Harold, 1867) (Kohlmann and Halffter, 1990; Halffter and Morrone, 2017).

2.1. *Canthon cyanellus*

The mating of *Canthon cyanellus* takes place during the rainy season. This species uses small vertebrate carcasses to feed and reproduce (Halffter et al., 1983; Favila and Díaz, 1996). Sexual recognition occurs in the food source through cuticular compounds (Ortiz-Domínguez et al., 2006b). Sexually mature pairs cooperate in building and rolling a food ball; then, both cooperate in nest construction. At a distance of 2 to 3 m from the carcasses, the male buries the ball on which the female is mounted. On the nest, the pair copulates, and the female makes 2–6 brood balls (Favila and Díaz, 1996). One fertilized egg is laid in each brood ball; the larval development takes place in the ball until the adult hatches nearly 25 days later (Favila, 1993). The male remains in the nest with the female protecting the brood balls with chemical compounds that prevent the development of fungi and bacteria (Cortez and Favila, 2007). During his stay, the male also prevents the female from copulating with other males (Favila et al., 2005).

Canthon cyanellus has a wide distribution in the Neotropics (Figure 1): Mexico, Guatemala, Honduras, Nicaragua, Costa Rica,

Panama, Trinidad, Colombia, Venezuela, Brasil, Ecuador, and Peru (Solís and Kohlmann, 2002). At its northern limit distribution, it reaches the Nearctic region to the eastern side of the United States in Texas (Halffter, 1961). Different subspecies are recognized according to the variation in body coloration (Robinson, 1948). Halffter (1961), following the ideas of Robinson (1948), places the different color morphs in a single species (i.e., *C. cyanellus*), but recognizes three subspecies: (1) *Canthon cyanellus cyanellus* LeConte, 1859 (Figure 2A) with monochromatic green or blue color; this is distributed from Texas and part of the tropical region in Mexico; it is considered synonymous with *Canthon speciosus* Harold, 1868 and *Canthon spinosus* Harold, 1863 (Howden, 1966); (2) *Canthon cyanellus violeetae* Halffter, 1961 (Figure 2B) with the elytra dark metallic green, the pronotum, and pygidium disc, except lateral–posterior margins, are orange–reddish; it is found in southwestern Mexico. Howden (1966) suggests that *C. c. violeetae* may be synonymous with *C. c. sallei* given the great similarity with individuals from Nicaragua, the type locality of *C. c. sallei*; and (3) *Canthon cyanellus sallei* Harold, 1863 (Figure 2C) has the dorsal surface largely reddish brown; its distribution ranges from Guatemala to Peru (Blackwelder, 1944). This is considered a synonym with *C. sallei triangulatus* Schmidt, 1920 and *C. sallei gutticollis* Schmidt, 1920 (Howden, 1966).

Canthon cyanellus is part of the entomofauna that was established in the MTZ during the Plio-Pleistocene, following a dispersion path from South America to southern and southeastern Mexico through the Gulf slope of Mexico and the Pacific Ocean (Kohlmann and Halffter, 1990; Morrone, 2015; Halffter and Morrone, 2017). This distribution corresponds to a Typical Neotropical pattern (*sensu* Halffter, 1962) that is associated with tropical dry forests, cloud forests, and tropical forests with an altitudinal distribution from 0 to 2,000 m (Halffter and Morrone, 2017).

Nolasco-Soto et al. (2017, 2020) concluded that at the MTZ *C. cyanellus* has gone throughout at least seven cladogenetic events (i.e., GF, ChaCal, Ixt, SEM, NGM, Hua, SPS+SGM; Table 1 and Figure 3) during the Pleistocene (Figure 4). The ancestor of *C. cyanellus* gets in the MTZ during the Plio-Pleistocene when the main mountain systems had already formed. The most likely ancestral area for *C. cyanellus* in Mexico was on the Pacific slope, later the ancestor could follow an invasion route through the foothills of the Trans Mexican Volcanic Belt and then to northern and southern Gulf of Mexico (Nolasco-Soto et al., 2017). Subsequently, cladogenesis might have occurred in a north–south spatial sequence, initially separating the Chamela populations in Jalisco (Cha clade) and Gómez-Farías (GF clade) in the Sierra Madre Oriental from the rest of the southern populations (Nolasco-Soto et al., 2017). The GF population could be a relict at “El Cielo” Biosphere Reserve in Tamaulipas (Nunes, 2019). The next cladogenetic event is dated at ca. 1.43 Myr splitting the northern Gulf of Mexico populations (NGM clade), and the populations in southern Mexico. The Huatulco populations (Hua clade) split from the remaining southern ones (SGM+SPS clades) around 1.26 Myr. One more cladogenetic event

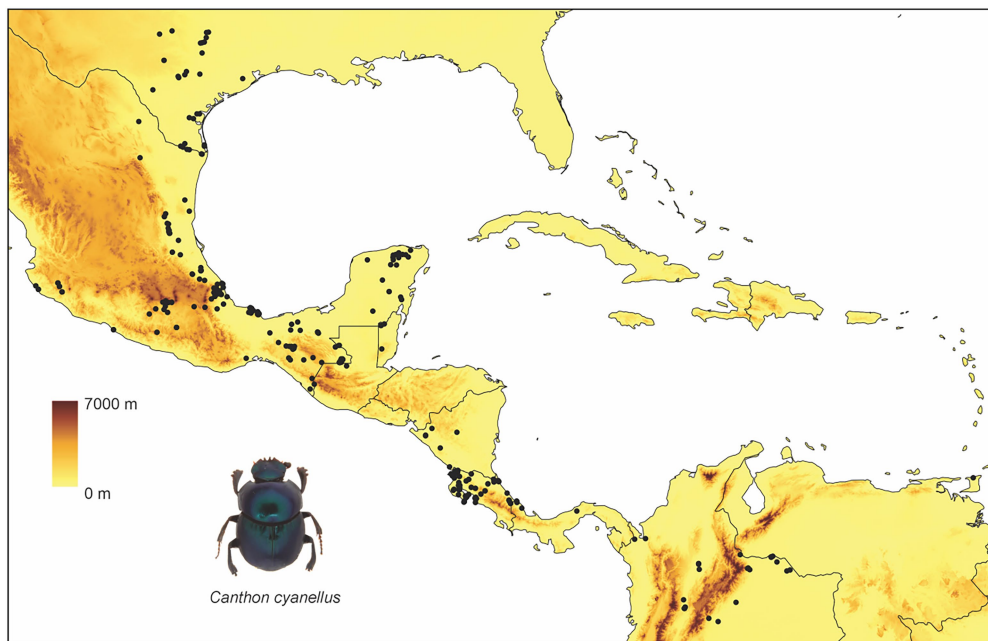


FIGURE 1
Distribution of *Canthon cyanellus*. This is how GBIF suggests citing records from different datasets: <https://www.gbif.org/occurrence/download>, Global Biodiversity Information Facility (2020).

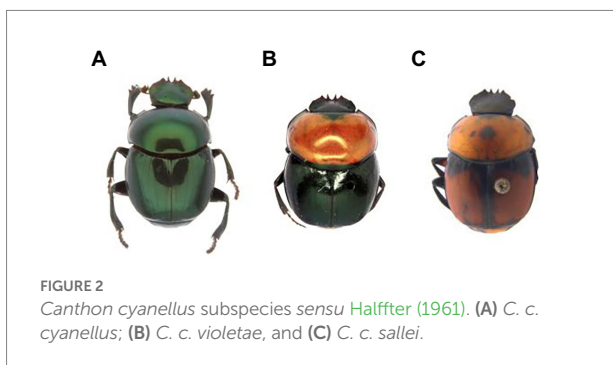


FIGURE 2
Canthon cyanellus subspecies sensu Halffter (1961). (A) *C. c. cyanellus*; (B) *C. c. violetae*, and (C) *C. c. sallei*.

that occurred at *ca.* 0.97 Myr separated the Chamela and GF clades. Finally, the estimated divergence time between the SGM and SPS clades occurred at *ca.* 0.91 Myr.

Those Cladogenetic events could be related to the contraction and expansion of tropical forests associated with glacial and interglacial cycles during the Pleistocene. A cladogenetic event that separated the NGM and SGM clades might be associated with the geological activity that occurred *ca.* 1.5 Myr east of the Trans Mexican Volcanic Belt in the state of Veracruz (Ferrari et al., 2005). Similarly, Ornelas et al. (2013) concluded that several cloud forest taxa distributed in Mexico diverge during the Pleistocene. These divergences suggest that the genetic differentiation of those species can be explained by the dynamics of the forests influenced by the climatic fluctuations of the Quaternary. On the other hand, the Isthmus of Tehuantepec may represent a biological corridor for *C. cyanellus* similarly as what has been observed for other taxa (e.g.,

fruit flies *Anastrepha*: Antonio-Hernández et al., 2018; *Sturnira hondurensis* bats: Torres-Morales, 2019).

The demographic history of the *C. cyanellus* populations in the MTZ indicates that they remained in stasis during most of the Pleistocene (Nolasco-Soto et al., 2017). Postglacial demographic expansions began *ca.* 180,000–20,000 years and continue to the present (Figure 4). Currently, these expansions can be associated with landscape changes due to tropical deforestation and intensive farming, favoring their incursion into open areas such as grasslands (Arellano et al., 2008; Salomão et al., 2018).

3. Incipient speciation

3.1. Reproductive barriers

During the speciation process, prezygotic (e.g., ecological, behavioral, reproductive seasonality, gametic incompatibility) or postzygotic (e.g., sterility or hybrid unviability) barriers are formed which restrict gene flow between populations, which leads to their differentiation (Tregenza et al., 2000; Sánchez-Guillén et al., 2012). During the divergence process between sister species, it is possible to evaluate the selection forces that act on the reproductive barriers because they are acquired during this process (grey area sensu de Queiroz, 2007).

Cuticular compounds (CHCs) mediate recognition and sexual attraction in several insect species (Chung and Carroll, 2015). In *C. cyanellus*, the composition of CHCs varies between sexes and allopatric populations distributed along the Gulf slope of Mexico

TABLE 1 Localities of *Canthon cyanellus* from Mexico and Colombia.

State/ Locality	Locality code	Clade code	Location (Latitude, Longitude)	Elevation (m.a.s.l.)
Tamaulipas, Gómez- Farías	GF	GF	23.0480, −99.1433	379
Veracruz, Tuxpan	Tp	NGM	20.9544, −97.4661	52
Veracruz, Papantla	Pap	NGM	20.4167, −97.4500	200
Veracruz, La Mancha	Man	SGM	19.5688, −96.4092	194
Veracruz, Jalcomulco	Jal	SGM	19.3286, −96.7469	370
Veracruz, Los Tuxtlas	Tx	SGM	18.5833, −95.0667	120
Chiapas, Raymundo Enríquez	Raye	SPS	14.8642, −92.3005	99
Chiapas, El Vergel	Ver	SPS	14.7029, −92.2672	22
Chiapas, Palenque	Nbet	SEM	17.2815, −91.6466	140
Campeche, Calakmul	Cal	SEM	18.3302, −89.8227	225
Oaxaca, Los Chimalapas	Chim	SEM	16.9122, −94.6697	212
Oaxaca, Huautulco	Hua	Hua	15.7800, −96.0900	30
Jalisco, Chamela	Cha	ChaCal	19.4997, −105.0229	90
Guerrero, Ixtapa	Ixt	Ixt	17.6583, −101.5752	120
Natagaima, Tolima, Colombia	Col	Col	3.6483, −74.9997	328

(Ortiz-Domínguez et al., 2006a, 2006b). In the beetle populations of Gómez-Farías such variation has been associated with a change in food preferences for Diplopoda carcasses (Ortiz-Domínguez et al., 2006a). The diet affects the quantity and quality of CHCs preventing sexual recognition and mate selection (Chung and Carroll, 2015). In *C. cyanellus*, the differences in the composition of CHCs are not strong enough to prevent interpopulation mating. Aggressive behavior does occur during sexual recognition at least under laboratory conditions. Interpopulation crosses had low fecundity and fertility, being more evident in couples from populations separated by ≥ 600 km (Ortiz-Domínguez et al., 2006a). This suggested that the populations throughout its distribution are in an incipient speciation process. Similarly,

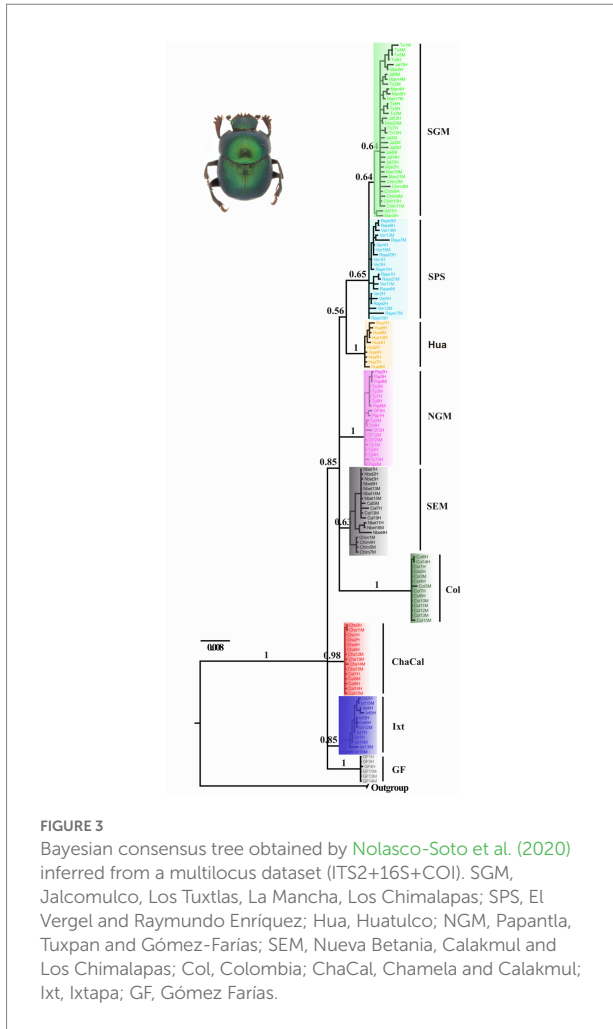
Grimaldi et al. (1992) observed in heterospecific experimental crosses of allopatric populations of the *Drosophila testacea* that reproductive isolation is asymmetric between some crosses. This is determined by differences in sexual recognition, the lack of transfer of sperm, and hybrid unviability.

In damselflies of the genus *Ischnura* (Sánchez-Guillén et al., 2012, 2014), *Drosophila* flies (Kamimura and Mitsumoto, 2012), and Carabidae beetles (Ishikawa, 1987; Kubota and Sota, 1998), reproductive isolation it is determined by mechanical prezygotic barriers, which prevent the formation of hybrids. Even though the populations of *C. cyanellus* are genetically structured (Nolasco-Soto et al., 2020), the morphology of the aedeagus is similar between individuals and populations (Figures 5, 6). Instead, the morphological differences in the peripheral medial sclerite allowed a possible separation between the different subspecies of *C. cyanellus* (Nunes, 2019; Figure 7). In contrast, in species of beetles: *Phanaeus tridens* group, the morphology of the phallobase, parameres, and endophallite copulatrix are attributes that apparently are in a state of evolutionary stasis (Moctezuma et al., 2021). In *Onthopagus taurus* Schreber, 1759 morphology variation in four of the five endophallic sclerites influenced male's fertilization success in the context of sperm competition (House and Simmons, 2003). In *C. cyanellus*, sperm competition influences paternity success (Favila et al., 2005). Therefore, it is possible, that in *C. cyanellus* the endophallic sclerites may be subject to sexual selection.

3.2. Diversity and genetic structure

Ortiz-Domínguez et al. (2010) quantified the diversity and genetic structure of five allopatric populations of *C. cyanellus* from tropical-forests located on the slope of the Gulf of Mexico. Those populations were structured in two groups, one to the north and one to the south. According to Nolasco-Soto et al. (2017), these groups correspond to the NGM and SGM clades. This might explain the problems of sexual recognition, low fecundity, and fertility, as well as the aggressive behavior, observed between couples from different populations (Ortiz-Domínguez et al., 2006a, 2006b). This genetic structure could be explained by isolation by distance, which restricts gene flow between them (Nolasco-Soto et al., 2017; Figure 8).

Similarly, in the species *Canthon staigi* Pereira, 1953 with wide distribution in the Atlantic forests in Brazil, Paraguay, and Argentina, the effect of habitat loss and fragmentation decreases the genetic diversity of its populations. However, they do not have a fragmented genetic structure due to a high gene flow between populations (Ferreira-Neto et al., 2017). This suggests that the current diversity and genetic structure of neotropical Scarabaeinae populations may be influenced by changes in the landscape caused by anthropic activities. Other factors that can lead to speciation are related to the size of the occupied area, the type of habitat, population demography, life history attributes of the species, as well as historical processes (Nolasco-Soto et al., 2017; Maldaner et al., 2019).



Microsatellite markers indicated that populations of *Canthon staigi*, which have been altered by anthropic activities, presented inbreeding depression and less allelic richness compared to populations in conserved areas (Cruz et al., 2017). Currently, 14 polymorphic microsatellites have been developed (2–16 alleles each) for the populations of *C. cyanellus* that belong to the different clades identified by Nolasco-Soto et al. (2017, 2020). These microsatellites contain private alleles that suggest their potential use to assess the genetic structure and more precise geographic delimitation of clades within *C. cyanellus* (Arce-Valdés et al., 2021).

4. Chromatic variation and cryptic species

In *C. cyanellus* the cuticular color variation does not match with the genetic structure of the populations or with the taxonomic division *sensu* Halffter (1961) (Nolasco-Soto et al., 2017, 2020; Figure 9). Populations with genetically differentiated metallic green individuals along the Pacific slope and the Gulf of Mexico reveal cryptic species in allopatry. The only genetically

differentiated populations, which are distinguished by cuticular color, are in the region of Tuxpan and Papantla (i.e., very dark blue and green), and in Gómez-Farías (i.e., bright light green). In the state of Chiapas, there are populations with dark green individuals genetically differentiated from other populations in which individuals with polychromatic and monochromatic colors coexist (Nolasco-Soto et al., 2017, 2020). Solís and Kohlmann (2002) carried out a detailed study for *C. cyanellus* in various locations in Costa Rica, demonstrating the coexistence of several cuticular colors, being an example of a polytypic species, and suggesting genetic differences between populations. Based on that fact, Solís and Kohlmann (2002) do not recognize subspecies. The same coloration patterns as those described by Solís and Kohlmann (2002) can be found in beetles from Chiapas, Mexico. The same can be observed in Nicaragua and Honduras for *C. c. sallei*, but light and dark colors also occur in South American populations (Vaz-de-Mello and Cupello, 2018).

Similarly, in the beetle *Coprophanaeus encifer* Germar, 1821, color variation is not related to cryptic speciation or genetic structure throughout its distribution in South America (Maldaner et al., 2019). On the other hand, *Canthon rutilans* different color morphs were assigned to the subspecies *C. r. rutilans* Castelnau, 1840 and *C. r. cyanescens* Harold, 1868; furthermore, recent studies found that these two subspecies diverge in thermal adaptations (Hensen et al., 2018), reproductive behavior (Hensen et al., 2020), and ecological niche (Hensen et al., 2021). This species indicates that the geographical variation of cuticular color in some species of Scarabaeinae might be related to cryptic speciation or species divergence, nonetheless the assignment of subspecies considering color as the only differentiation character (Solís and Kohlmann, 2002; Cupello et al., 2021).

In some species of Scarabaeinae, the color has been related to the period of activity (Hernández, 2002), thermoregulation (Amore et al., 2017; Alves et al., 2018; Cuesta and Lobo, 2019), phenotypic plasticity (Stanbrook et al., 2021), and Batesian mimicry (Alves et al., 2018). In the geotrupid beetle, *Phelotrupes auratus* local selection affects individuals of different colors that are regulated by multiple loci and correlated with barriers to gene flow (Araki and Sota, 2021). In *C. cyanellus* the metallic green color has a genetic basis (Favila et al., 2000), but it is possible that environmental factors also determine color variation throughout its distribution as has been suggested for other Scarabaeinae (Davis et al., 2008; Scholtz, 2009). Therefore, we do not rule out that stochastic processes such as genetic drift and genetic control by multiple loci may influence geographic color variation in this species.

5. Discussion

The evolutionary scenario that explains the diversification of *C. cyanellus* is associated with historical isolation by distance, which restricted the genetic flow between populations, causing their speciation in allopatry during the Pleistocene. The eight

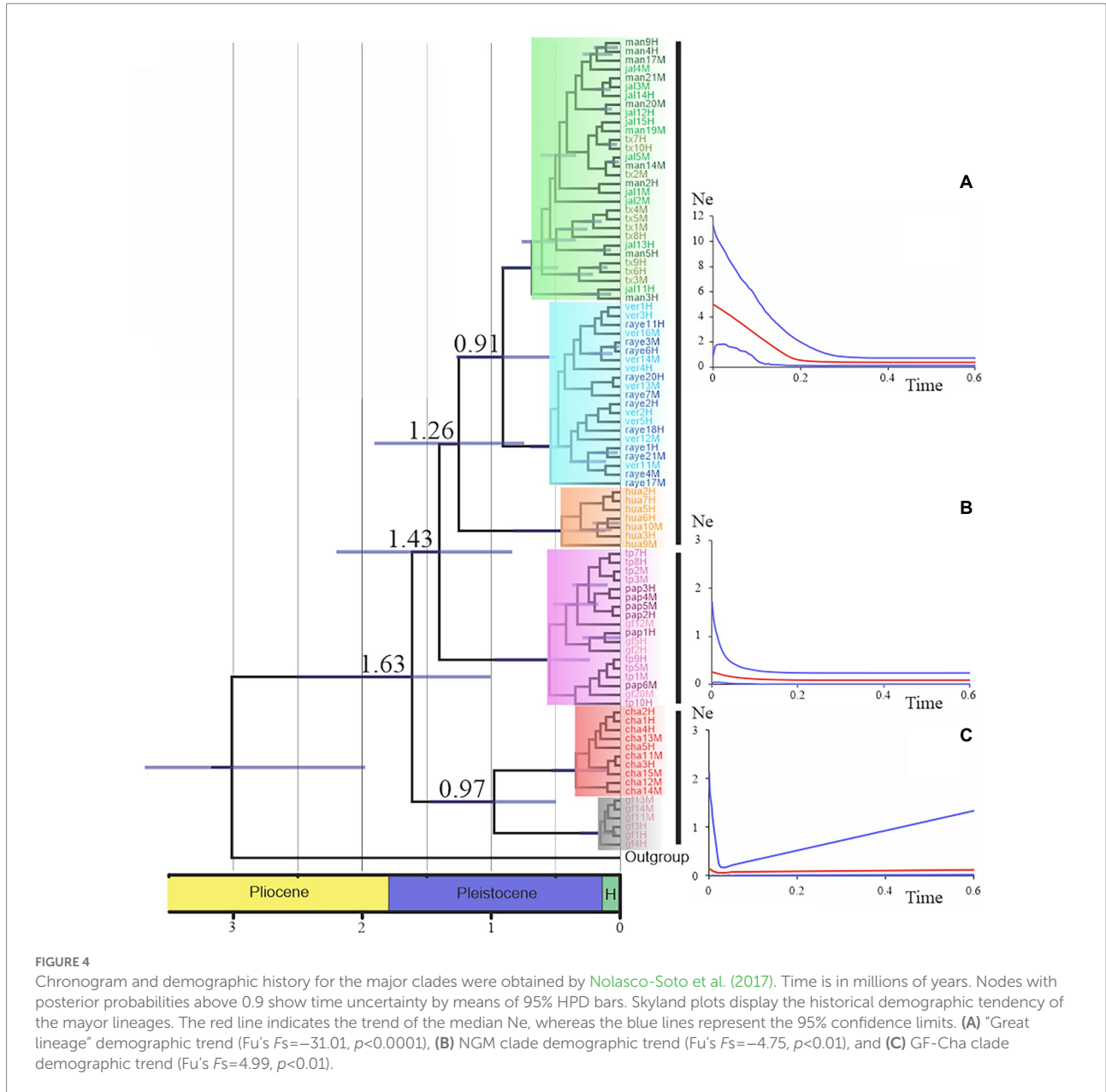


FIGURE 4

Chronogram and demographic history for the major clades were obtained by Nolasco-Soto et al. (2017). Time is in millions of years. Nodes with posterior probabilities above 0.9 show time uncertainty by means of 95% HPD bars. Skyland plots display the historical demographic tendency of the mayor lineages. The red line indicates the trend of the median N_e , whereas the blue lines represent the 95% confidence limits. (A) "Great lineage" demographic trend (F_u 's $F_s = -31.01$, $p < 0.0001$), (B) NGM clade demographic trend (F_u 's $F_s = -4.75$, $p < 0.01$), and (C) GF-Cha clade demographic trend (F_u 's $F_s = 4.99$, $p < 0.01$).

historically separate units recovered by Nolasco-Soto et al. (2020) can be identified by nucleotide synapomorphies. Traditional morphological taxonomy for this group was based on the middle peripheral sclerite character. However, based on a phylogenetic species concept, those eight units can be recognized as different species, in which pre-and postzygotic reproductive barriers are developing.

The observed geographic variation in the aedeagus morphology and the body color of *C. cyanellus* is not related to the inferred genetic structure of the populations nor the current taxonomic classification. The latitudinal distribution of color variation in Mexico tends toward monochromatic green. Although the adaptive significance of color variation is unclear, this may suggest some thermoregulatory function or selective

pressure imposed by different local environmental conditions (Amore et al., 2017; Alves et al., 2018; Cuesta and Lobo, 2019). It is possible that different evolutionary mechanisms interact to produce geographic variation in body color in this group of beetles (Araki and Sota, 2021).

Studies carried out with microsatellite molecular markers, suggest that the diversity and current genetic structure of populations can be shaped by changes in the landscape caused by human activities (Cruz et al., 2017). This may occur due to habitat fragmentation, favoring in some cases the possibility of allopatric speciation due to geographic isolation and the effect of genetic drift. It is necessary to evaluate to what degree the current genetic structure of *C. cyanellus* populations might be shaped by the effects of fragmentation, extensive use of

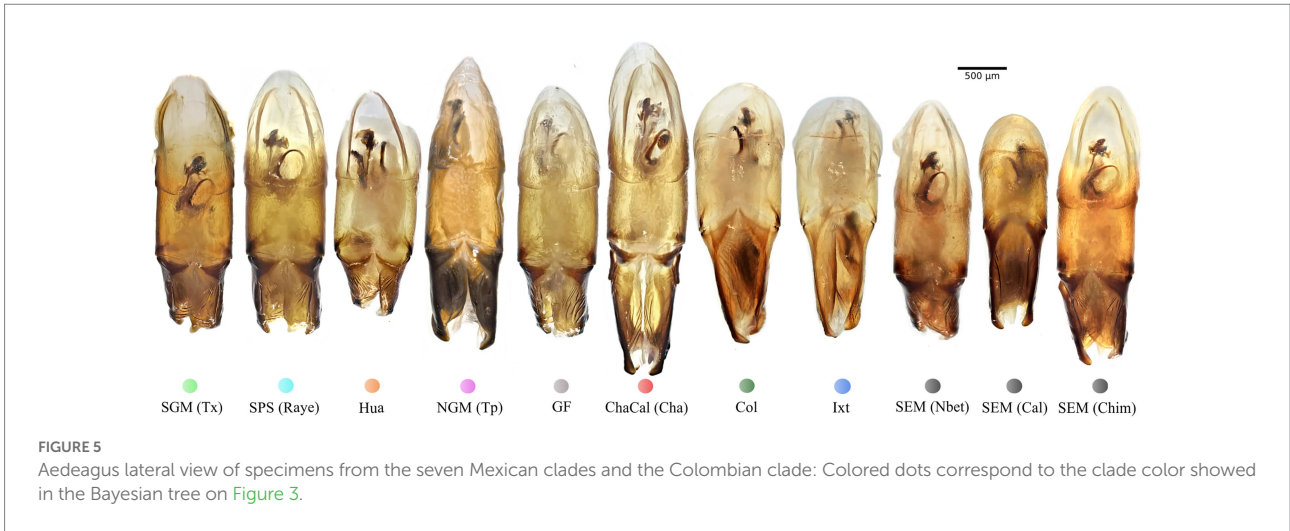


FIGURE 5
Aedeagus lateral view of specimens from the seven Mexican clades and the Colombian clade: Colored dots correspond to the clade color showed in the Bayesian tree on Figure 3.

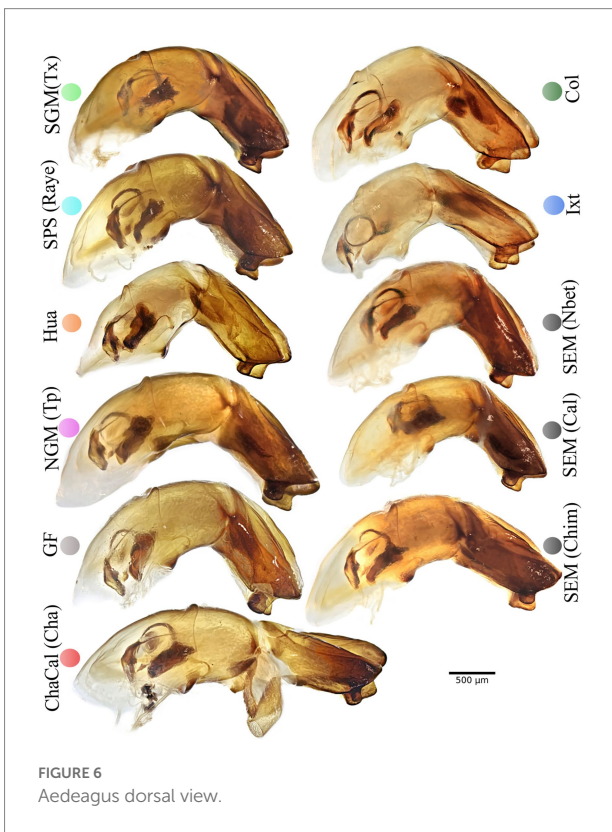


FIGURE 6
Aedeagus dorsal view.

well as to explore the importance of pre- and postzygotic reproductive barriers in this species.

6. Taxonomic status

Key to the determination of the subspecies of *Canthon cyanellus* (*sensu* Halffter, 1961).

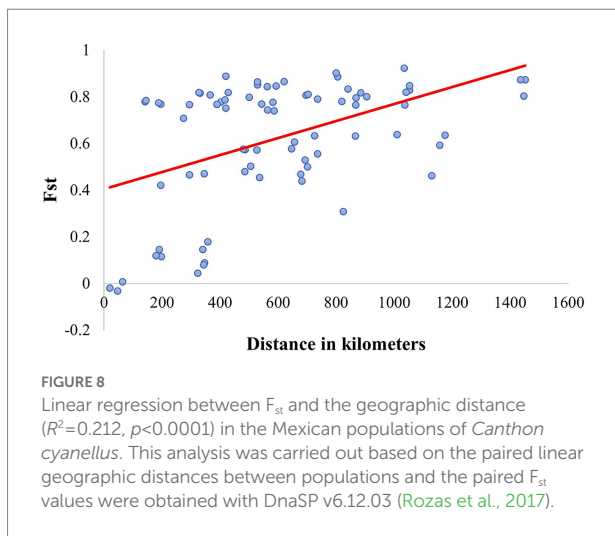
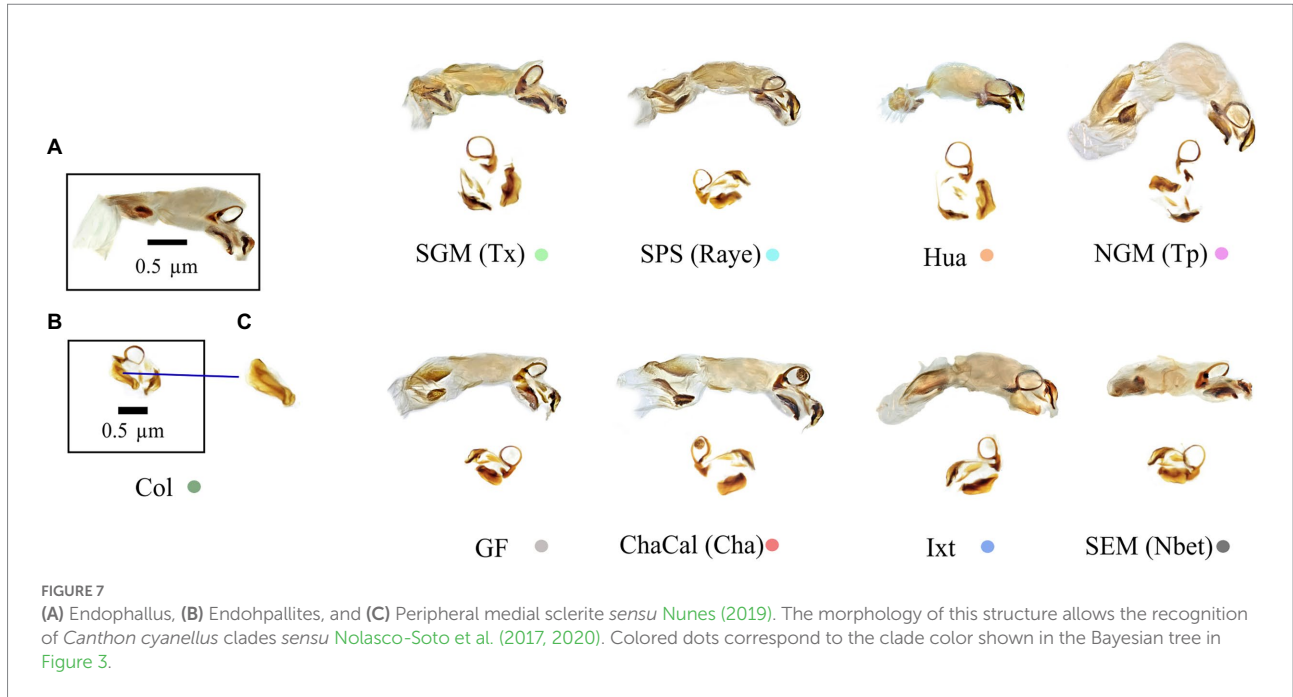
1. Quadridentate clypeus. Hind tibiae markedly arched. The separation between proepisternae and proepimera only with an indication of a keel, limited to the internal part, close to the coxa. Teeth of the anterior tibiae, especially the two most apical ones, notably directed forwards, obliquely: the three tibial teeth are grouped in the most apical part of the piece *C. cyanellus* LeConte.
2. Dorsal surface green, blackish-green or dark blue. Pronotum with fine to evident and dense punctuation. With antescutelar impression. Hind tibiae markedly arched, forming an angle with the point of inflection toward the middle of the tibia. Texas and Mexico. *C. cyanellus cyanellus* LeConte.

Head and periphery of the pronotum dark green, the pronotum, and pygidium disc, except the latero-posterior margins, orange-reddish.

3. With antescutelar impression. Pronotum with very fine but dense punctuation. Dark metallic green elytra. Southwest Mexico *C. cyanellus violetae* n. subsp. Posterior edge of pronotum without antescutelar impression. Pronotum with slight punctuation, perceptible only on both sides and posterior margin. The anterior edge of the elytra, elytral suture line, and elytral epipleura are bright green, the elytral disc is orange-reddish; this coloring manifestly predominates. Central and South America *C. cyanellus sallei* Harold.

agriculture, and livestock management. This knowledge will make it possible to elucidate if some populations might be susceptible to ecological changes (e.g., Sierra Madre Oriental and in Tapachula, Chiapas).

The current review is part of an effort to understand the evolutionary history of Scarabaeinae beetles in the Mexican Transition Zone. To sort out the cryptic diversity present in this beetle complex represents an important step in understanding its evolutionary interrelationships. New lines of research must be opened to test hypotheses on the evolution of the genitalia, as



forms that had been considered synonyms of *C. cyanellus* to a new species and a subspecies. Some of these chromatic forms have their correspondence with the clades (e.g., GF, NGM) inferred by Nolasco-Soto et al. (2017, 2020). Phylogeography, combined with traditional morphological taxonomy, provides information on species boundaries, populations, and other intraspecific groups; thus, a better understanding of the speciation processes (Table 2).

4. Pronotum with a dark triangular spot whose tip reaches the base of the elytra, lateral pronotum spots, separate or absent *C. cyanellus sallei* var. *triangulatus* Schmidt. Upper and lower surface dark green; pronotum with four light spots, one at each angle; pygidium usually dark *C. cyanellus sallei* var. *gutticollis* Schmidt.

On the other hand, Nunes (2019) carried out the most recent taxonomic review for the *C. cyanellus* group. Nunes (2019) assigned a new (but not yet named) subgenus to the group “cyanellus” based on the diagnostic character of the clypeal teeth of the anterior margin of the head. In addition, the morphology of the middle peripheral endophallic sclerite allowed him to separate and give preliminary validity to five of the chromatic

1. *Canthon cyanellus* LeConte, 1859 *sensu stricto* is the chromatic form used to describe the species. Nunes (2019) designed this as the subspecies, *C. c. cyanellus*. It corresponds to the clade NGM (Nolasco-Soto et al., 2017). A very dark blue with a rough dorsal surface characterizes it. It is distributed at the northern area of Veracruz in Tuxpan, Papantla, and Tancoco. This species may reach its distribution as far as Texas (Robinson, 1948). However, the genealogical relationship among the populations from Texas and Mexico is unknown.
2. *Canthon speciosus* Harold, 1868. This species was considered a synonym of *C. cyanellus*, and it was assigned to the subspecies *C. c. cyanellus* by Halffter (1961). Harold described it as having a bright green and bluish-green body color, with a smooth dorsal surface. This corresponds to the SGM clade *sensu* Nolasco-Soto et al. (2017), which includes populations from the southern area of Veracruz. Nunes (2019) assigned it as a synonym of *C. spinosus* and delimited its distribution in the eastern coastal zone of Mexico from Veracruz, Yucatan, Quintana Roo, Campeche, Chiapas to the border with Guatemala at the Petén area. Some specimens from Los Chimalapas in Oaxaca, all from Nueva Betania in Chiapas, and some from Calakmul in Campeche sequenced by Nolasco-Soto et al. (2020) were

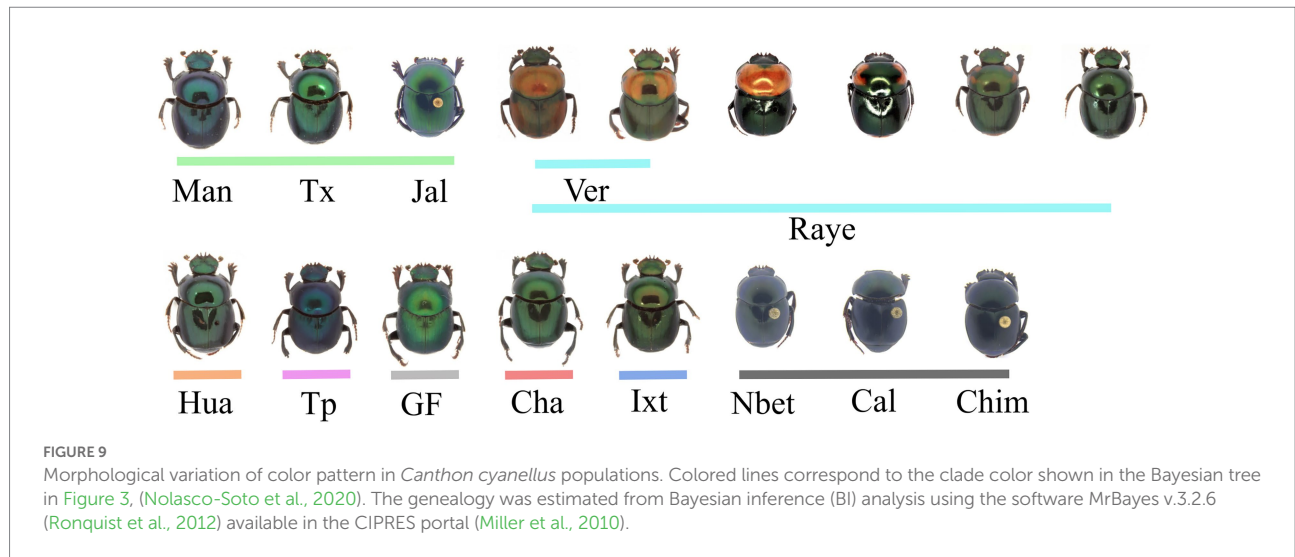


FIGURE 9 Morphological variation of color pattern in *Canthon cyanellus* populations. Colored lines correspond to the clade color shown in the Bayesian tree in Figure 3, (Nolasco-Soto et al., 2020). The genealogy was estimated from Bayesian inference (BI) analysis using the software MrBayes v.3.2.6 (Ronquist et al., 2012) available in the CIPRES portal (Miller et al., 2010).

TABLE 2 Taxonomic division of *Canthon cyanellus* carried out by Nunes (2019) compared to the clades inferred by Nolasco-Soto et al. (2017, 2020)

Nunes (2019)	Nolasco-Soto et al. (2017, 2020)
<i>Canthon cyanellus cyanellus</i> LeConte, 1859	NGM
<i>Canthon cyanellus</i> (undescribed subspecies) Nunes & Vaz-de-Mello	GF
<i>Canthon sallei</i> Harold, 1863 status reviewed	Col
<i>Canthon speciosus</i> Harold, 1868 status reviewed	SGM
<i>Canthon triangulatus</i> Schmidt, 1920 novo status	Col
<i>Canthon violeatae</i> Halffter, 1961 novo status	SPS
<i>Canthon</i> (undescribed species) Nunes y Vaz-de-Mello	Ixt
Specimens under review	Hua
Specimens under review	ChaCal

Clade code as in Table 1.

recovered within the SEM clade, therefore, *C. speciosus* would be a polyphyletic group. The SEM clade seems to correspond to the material reviewed by Harold referring to *C. spinosus* from southern Mexico. To resolve this problem material from the localities examined by Nunes should be sequenced, as well as a detailed review of the endophallic sclerites of the SEM clade individuals must be performed.

3. *Canthon violeatae* Halffter, 1961. It is characterized by dark-green metallic elytra, as well as reddish-orange pronotum, and pygidium disc, except the lateral-posterior margins. The ventral part of the body is blackish-green and with some abdominal segments reddish-orange. It is endemic

to the center and southwest area of the state of Chiapas. This is the SPS clade *sensu* Nolasco-Soto et al. (2017, 2020) that includes individuals from localities as El Vergel (also reviewed by Nunes, 2019), and Raymundo Enríquez, which are genetically differentiated from other populations in Chiapas. At the Raymundo Enríquez locality, there are individuals with completely green or reddish-orange cuticular colors (or a mixed pattern of these colors in different parts of the body; i.e., head, pronotum, abdomen, and pygidium). These color forms have been reported by Solís and Kohlmann (2002) in Costa Rica. This may suggest a possible hybridization zone or a species in which color variation is controlled by several codominant genes.

4. *Canthon sallei* Harold, 1863. Orange color at the cuticula, body, and elytra with black outline, may have a triangular-shaped spot in the center of the pronotum, sometimes it appears as a longitudinal line (Nunes, 2019). Nunes (2019) assigned *Canthon dentiger* Harold, 1968 as a synonym of *C. sallei*. This species is distributed from northwestern Colombia to southern Mexico and may occur in sympatry with *C. triangulatus* at its southern limit and with *C. violeatae* at its northern limit (Nunes, 2019). The material reviewed by Nunes came from Nicaragua, Costa Rica, Colombia, and Panama. He also reviewed beetles from El Vergel (Chiapas, Mexico), which corresponds to the SPS clade *sensu* Nolasco-Soto et al. (2017). This clade contains large part of the color variation reported for the “cyanellus” group (e.g., metallic green, orange, and combinations of these colors in different parts of the body). Further molecular analyses of specimens from the localities reviewed by Nunes (2019) should be included, as well as a detailed review of the middle peripheral sclerite morphology must be carried out to determine if all the observed variation represents a polymorphic population or if this is a species complex.

5. *Canthon triangulatus* Schmidt, 1920. Synonyms: *C. sallei guticollis* Schmidt, 1920 and *C. cyanellus havranekae* Martínez, 1988 (Nunes, 2019). The species is distinguished by an opaque dorsal surface, pronotum, and elytra of orange color and black outline. On the pronotum, it has a longitudinal central spot. The pygidium is orange (Nunes, 2019). According to Nolasco-Soto et al. (2020), it corresponds to the Colombian clade. The phenotype corresponded to the one described for the variety that Schmidt called *C. sallei triangulatus*, which Halffter (1961) synonymized with *C. cyanellus sallei*. It is possible that populations from the Natagaima region in Tolima have their own identity and evolutionary history (Nolasco-Soto et al., 2020). These populations are enclosed between the central and eastern Andes Mountains in Colombia.
6. *Canthon cyanellus* (undescribed subspecies) Nunes & Vaz-de-Mello. It is characterized by a bright green color with a rough dorsal surface. According to Nunes (2019), it is an endemic subspecies in the region of “El Cielo” Biosphere Reserve in Tamaulipas (Mexico). This taxon corresponds to the GF clade *sensu* Nolasco-Soto et al. (2017).
7. *Canthon* (undescribed species) Nunes & Vaz-de-Mello. It has bright dark green color. This species corresponds to the clade Ixt located in Ixtapa, Zihuatanejo in the Pacific (Nolasco-Soto et al., 2020). However, it is easily confused with specimens recovered in the SGM, SEM, Hua, and ChaCal clades (Nolasco-Soto et al., 2020). Based on the material reviewed by Nunes (2019), which included specimens from Ixtapa, Zihuatanejo in Guerrero, El Aguacero, and El Vergel in Chiapas, the author concluded that this new species of *Canthon* might be in sympatry with *C. violetae* y *C. sallei*.
8. Other cryptic species. The localities of Huatulco in Oaxaca (Clade Hua) and Chamela Biosphere Reserve (Clade Cha)

in Jalisco are monophyletic clades that may represent distinct and new species (Nolasco-Soto et al., 2017).

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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