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Revision of the diagnostic characters of two morphologically similar snook species, *Centropomus viridis* and *C. nigrescens* (Carangiformes: Centropomidae)

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Abstract

Historically, the taxonomic identification of the two snook species, *Centropomus viridis* and *C. nigrescens*, has been challenging due to their morphological similarity and the inconsistency of the characters used for diagnosis. Therefore, this study aimed to evaluate the usefulness of the morphologic, meristic, and morphometric characters currently being used to identify *C. viridis* and *C. nigrescens*, based on molecular data. The results showed that the gas-bladder shape (i.e., *C. viridis* with diverticula and *C. nigrescens* without diverticula) was the only morphological character univocally related to genetic identification. Likewise, geometric morphometrics separated two groups; each corresponds to only one of two genetically (and gas bladder shape) identified species. Of all the meristic characters examined, only the second dorsal fin ray count (nine for *C. viridis* and ten for *C. nigrescens*) was related to the gas bladder shape and genetic identity; therefore, it is the only external character with a diagnostic utility to separate each species.

Keywords: white snook, black snook, taxonomy, geometric morphometrics, identification key

Introduction

The genus *Centropomus* Lacepède (Carangiformes: Centropomidae *sensu* Girard *et al.* 2020) is a monophyletic group that includes 13 valid species (Carvalho-Filho *et al.* 2019). Commonly known as robalos or snooks, the genus members are diadromous species of amphi-American distribution and tropical-subtropical affinity (Castro-Aguirre *et al.* 1999; Nelson *et al.* 2016). Among the tropical eastern Pacific species, *C. viridis* and *C. nigrescens* reach the largest sizes and command the highest market prices (Alvarez-Lajonchère & Tsuzuki 2008). Because of their high commercial value, wild populations of both species have been overexploited (Arreguín-Sánchez & Arcos-Huitrón 2011; Puentes *et al.* 2014). For this reason, there has been an increasing interest in developing intensive cultivation technology for *C. nigrescens* and *C. viridis* in a closed-cycle system (Resley *et al.* 2014; Ibarra-Castro *et al.* 2017).

The taxonomic validity of *C. nigrescens* and *C. viridis* has been tested using morphological, meristic, and genetic characters (Rivas 1986; Tringali *et al*. 1999a; Fricke *et al*. 2020). However, distinguishing between these morphologically similar species remains problematic (Castro-Aguirre *et al*. 1999; Tringali *et al*. 1999a). The binomen *Centropomus nigrescens* was used for the first time by Risso (1810) to designate a Moronidae species from the Mediterranean (Fricke *et al*. 2020). After, it was reclassified as *Perca nigrescens* by the same author (Bauchot & Desoutter 1987). Later, Günther (1864) used the same binomen to describe a *Centropomus* species from the tropical eastern Pacific. Since then, *C. nigrescens* has maintained its taxonomic validity (e.g., Evermann & Jenkins 1891; Jordan & Evermann 1896; Gilbert & Starks 1904; Alvarez del Villar 1970; Castro-Aguirre 1978; Rivas 1986; Castro-Aguirre *et al*. 1999; Miller *et al*. 2005).

In contrast, *C. viridis* has been synonymized several times with *C. undecimalis* (Evermann & Jenkins 1891; Boulenger 1895; Gilbert & Starks 1904; Castro-Aguirre 1978) and *C. nigrescens* (Meek & Hildebrand 1925). In a broad review of the genus *Centropomus*, Rivas (1986) proposed to differentiate both species by the relative lengths of the third and fourth spines of the first dorsal fin: with the third spine longer in *C. viridis* (giving triangular-shape fin) and equal in *C. nigrescens* (giving blunt-shape fin). The rest of the diagnostic features (i.e., meristic, morphometric, and coloring pattern) remain subordinated to comparing the relative lengths of third dorsal spines between species. Following Rivas (1986), several authors have used this diagnostic characteristic to separate *C. nigrescens* and *C. viridis* (Allen & Roberson 1994; Bussing 1995; van der Heiden *et al*. 1998; Castro-Aguirre *et al*. 1999; Miller *et al*. 2005). However, as noted by Rivas (1986) and Tringali *et al*. (1999a), the coloration and morphological characteristics of young specimens (< 100 mm standard length, SL) of *Centropomus* may be intraspecifically variable and are not reliable for taxonomic diagnosis. Moreover, in *C. viridis*, the shape of the first dorsal fin is highly variable even in late juveniles and young adults. For example, approximately 40% of the juveniles (100–200 mm SL; n = 100) produced in captivity from broodstock phenotypically identified as *C. viridis* and 50% of wild males (300–500 mm SL; n = 50) also identified as *C. viridis*, based on the ray count of the second dorsal fin, presented a blunt-shape first dorsal fin similar to *C. nigrescens* (J. M. Martínez-Brown, personal obsev.). So this feature is of questionable diagnostic value.

Likewise, the presence of anterior diverticula in the gas bladder of *C. viridis* and its absence in *C. nigrescens* has been proposed as a diagnostic character to differentiate the species (Jordan & Evermann 1896; van der Heiden *et al*. 1998). Conversely, Rivas (1986) underestimated the diagnostic value of this character, based on the intraspecific variation of the presence or absence of the diverticula indicated by Meek & Hildebrand (1925). However, gas bladder shape has been used as a reliable taxonomic character in other *Centropomus* species (Chávez 1961) and other teleost species (Chao 1978; Birindelli *et al*. 2009). These discrepancies mentioned above highlight the need to reexamine the role of the gas bladder in differentiating *C. viridis* from *C. nigrescens*.

Other characters that are used for the identification of *Centropomus* species (Rivas 1986; Allen & Roberson 1994; Bussing 1995; van der Heiden *et al*. 1998; Castro-Aguirre *et al*. 1999; Miller *et al*. 2005), such as the count of scales on the lateral line, the number of fin rays, gill rakers and scales around the caudal peduncle and the relative length of a character with respect to the standard length may overlap widely between both species. These morphological and anatomical ambiguities lead to misidentifications, particularly in juveniles. For this reason, identification keys based on genetic analysis (i.e., allozymes and mtDNA 16S rRNA gene sequences) were proposed (Tringali *et al*. 1999a). However, genetic identification is still an impractical method for the taxonomic identification of species in the field, particularly when numerous juvenile or young adults are examined. Such as in ecological monitoring or fishery management programs, and the selection of wild specimens captured as broodstock in aquaculture applications. Thus, it is convenient for species identification to have easy-to-recognize, reliable diagnostic characters that do not require procedures that jeopardize the integrity and well-being of the specimens examined (e.g., dissections and excessive manipulation). Therefore, the objective of this study is two-fold: 1) to determine the correlative relationship between morphological (i.e., presence of diverticula in the gas bladder, the shape of the first dorsal fin), meristic, morphometric measures (i.e., shape patterns), and genetic characters (i.e., 16S rRNA gene sequences); and 2) to propose a method for identifying both species from the most reliable and practical diagnostic characters.

Materials and methods

Origin of specimens and collection of samples. Specimens believed to be *C. viridis* or *C. nigrescens* were collected from 3 localities of the Mexican Pacific (Fig. 1): in the northwest, at Estero del Yugo (23°18'11" N, 106°29'00" W),

a small coastal lagoon located in Mazatlan, Sinaloa; in the center, at Laguna de Coyutlan (19°01'47" N, 104°19'14" W), a coastal lagoon located in Manzanillo, Colima; and in the south, at Tonala, Chiapas. In the first locality, the specimens were collected with a cast net (*n* = 23, 280–600 mm SL) and in the second locality with a hand line (*n* = 21, 320–570 mm SL). In the third location, specimens were acquired at a local fish market (*n* = 20, 310–450 mm SL). Additionally, 90 specimens 73–540 mm SL produced in captivity of wild broodstock from Estero del Yugo were used (Ibarra-Castro *et al*. 2017). The specimens set consisted of juveniles, immature adults of both sexes, and mature males. Each specimen was weighed (total weight, TW), and measured (standard length, SL). Samples of the caudal fin or muscle from 15 specimens from Mazatlan and 15 from Tonala were taken, preserved in 95% ethanol, and refrigerated for genetic analysis.
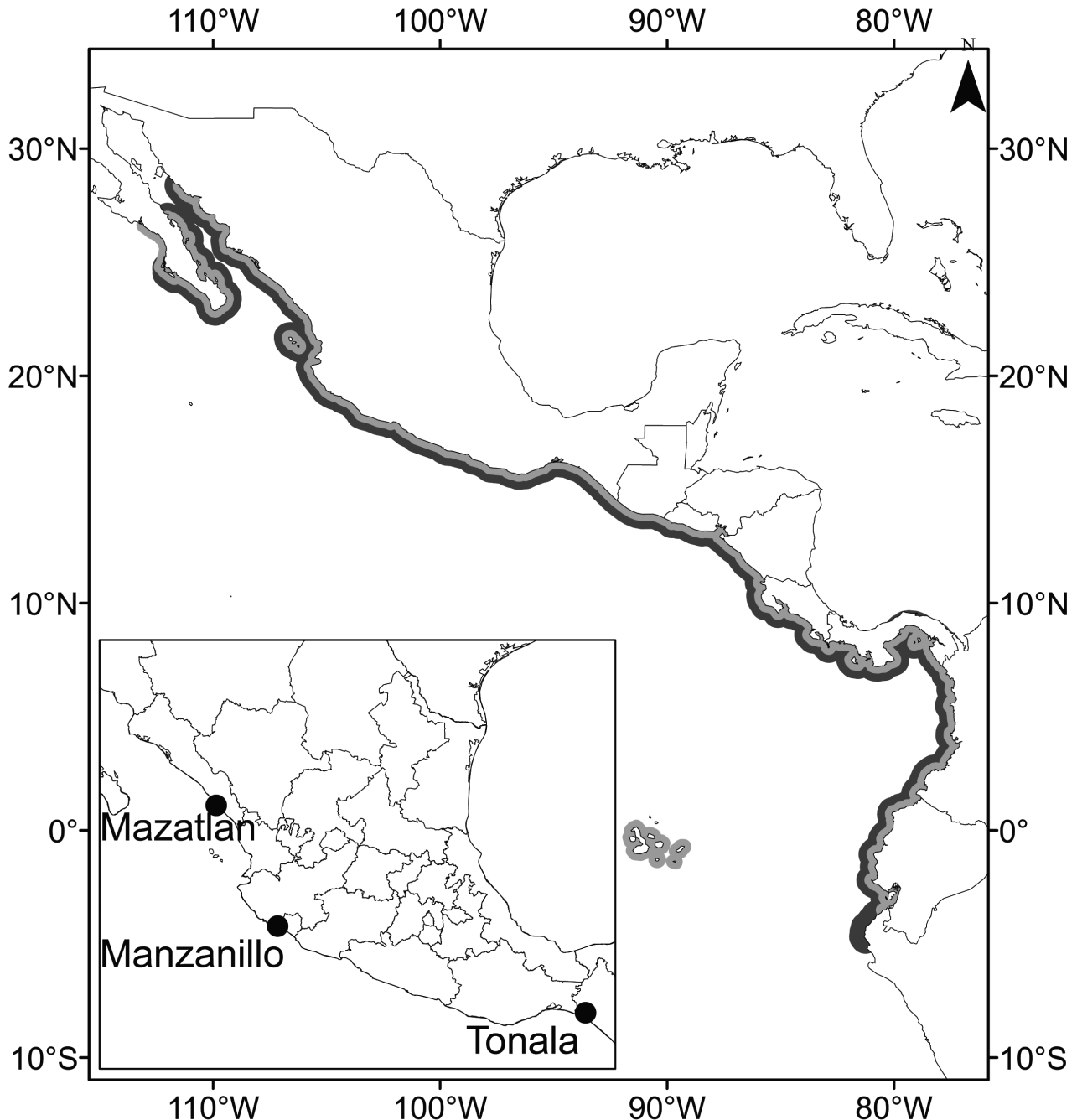

FIGURE 1. The Tropical Eastern Pacific map shows the geographic distribution of *Centropomus nigrescens* (black line) and *C. viridis* (gray line). Sampling localities are shown on the insert. The figure was made based on Robertson & Allen (2015).

Genetic analysis. The genetic analysis was based on 15 specimens from Mazatlan and 15 specimens from Tonala, the most distant sampling localities. Since this study is approached in a taxonomic context and not in popu-

lation genetics, the number of specimens examined were considered sufficient to support the congruence of the genetic and morphological differences between the two species (e.g. Tringali *et al.* 1999a, b).

The DNA was extracted from 20 mg of fin or muscle from each organism, using The KingFisher™ Duo Prime system (Thermo Fisher Scientific, Vernon Hills, IL) and the KingFisher Cell and Tissue DNA kit (Thermo Fisher Scientific, Vernon Hills, IL, catalog no. 97030196). The quality, concentration, and integrity of the extracted DNA were evaluated by spectrophotometry with NanoDrop 2000 (Thermo Fisher Scientific, Inc., Wilmington, DE, USA) and by electrophoresis in 0.8% agarose gels stained with GelRed 30X (Biotium). Gels were visualized in a photo-documentation system (BioRad). The amplification of a segment of the 16S rRNA gene was carried out through a polymerase chain reaction (PCR), using GoTaq® Green Master Mix (Promega, Madison, Wisconsin, USA, catalog no. M7121), 0.32 µM of each of the 16SAR and 16SBR primers (Geller et al. 1993; Palumbi 1996) and 50–100 ng of total DNA. The thermocycler conditions were as follows: 94 °C for 1 min; 28 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min; and final elongation at 72 °C for 8 min.

PCR products were electrophoresed on a 1.5% agarose gel stained with GelRed 30X (Biotium) and visualized in a photo-documentation system (BioRad). Subsequently, each amplified product was purified with the kit QIA-quick PCR Purification (Qiagen, Valencia, California, USA, catalog no. 28106). The purified products were sent to SeqXcel, San Diego, California, for sequencing in both directions, using an ABI Prism® sequencer Capillary Electrophoresis Genetic Analyzer and BigDye® Terminator chemistry. Sequences were reviewed and arranged with the CLC-Genomics Workbench 10 (CLC-Bio) software and were aligned in MEGA X (Kumar *et al.* 2018), using the CLUSTAL W algorithm (Thompson *et al.* 1994; Higgins *et al.* 1996). These sequences were blasted in GenBank. From the 60 *Centropomus* 16S sequences found in the GenBank (March 2020), only those sequences that overlap with the sequences from this study were selected to construct a phylogenetic tree using MEGA version X software (Kumar *et al.* 2018; Stecher *et al.* 2020). The best-fitted model found was used to estimate the tree was the Tamura 3-parameter with a gamma distribution (shape parameter = 1). The reliability of the nodes was assessed with 1000 bootstrap iterations.

Available GenBank 16S sequence of *Lates niloticus* (accession number LNU85007) was used as outgroup. The new sequencing data for *C. viridis* were deposited in GenBank databases under accession numbers (GAN) MN196668 and MN196669.

Morphological and meristic examinations. All specimens (*n* = 154) were examined for morphological and meristic characters. The morphological examination consisted of 1) determining the presence or absence of diverticula in the gas bladder (gas bladder shape). 2) determining the shape of the unfolded first dorsal fin by comparing the length of the third spine in relation to the fourth (Rivas 1986). When the third spine was longer than the fourth, giving a triangular-shape fin, specimens were designated as a *C. viridis* type. When the third and fourth spines were equal in length, giving a blunt-shape fin, specimens were designated as being a *C. nigrescens* type. 3) in some specimens, the relative length of the second anal fin spine and the last radius of the folded anal fin was compared (van der Heiden *et al.* 1998). If the second anal spine was longer, specimens were assigned as *C. viridis* type; otherwise, they were assigned *C. nigrescens* type.

For meristic examination, the following counts were determined for each specimen according to Rivas (1986): the spines and rays of the dorsal fins, anal fin, and left pectoral fin and counts of the gill rakers and rudiments (lower and upper) of the first branchial arch. In addition, the lateral line scales were counted, including the entire caudal portion.

Geometric morphometric analysis. The left side of 34 *Centropomus* specimens (15 genetically identified specimens from Mazatlan; 15 genetically identified specimens and four genetically unidentified specimens from Tonala) was photographed using a digital SLR camera (Canon EOS Rebel T5, ES-F lens 18-55 mm). A ruler was placed next to each specimen to obtain scaling information. A template was constructed from the digital image using the MakeFan8 module (Sheets 2014a) to provide equal angular spacing guidelines and identify one point along the body curve (semi-landmark) between the snout and dorsal fin. Thus, digitized configurations were obtained using ten landmarks and one semi-landmark (Fig. 2) with TpsDig2 Ver 2.31 software (Rohlf 2017).

Once the specimens were digitized, all the information that was unrelated to shape, such as differences attributed to location, scale, and rotation, was removed using the generalized Procrustes analysis with the CoordGen8 module (Sheets 2014b), and the alignment of the semi□landmark was performed with SemiLand8 modules (Sheets 2014c). Partial warp scores, which were obtained from the thin-plate spline interpolation function (Bookstein 1989), were used in a principal component analysis (PCA) to explore the grouping of the data in multivariate space by

PCAGen8 software (Sheets 2014d). To estimate the morphometric differences between the species, we calculated the partial Procrustes distance (PPD) between the mean shapes. We estimated its significance using an F-test and 900 bootstraps to determine whether the observed F-value could have been produced by chance, considering the bootstrapped F-values distribution. This analysis was carried out using the TwoGroup8 module (Sheets 2014e). Finally, a canonical variate analysis (CVA) was performed using the CVAGen8 module (Sheets 2014f) based on partial warp scores. The Mahalanobis distances between a specimen and the mean of a group obtained from the CVA scores were used to estimate *a posteriori* classification matrix.
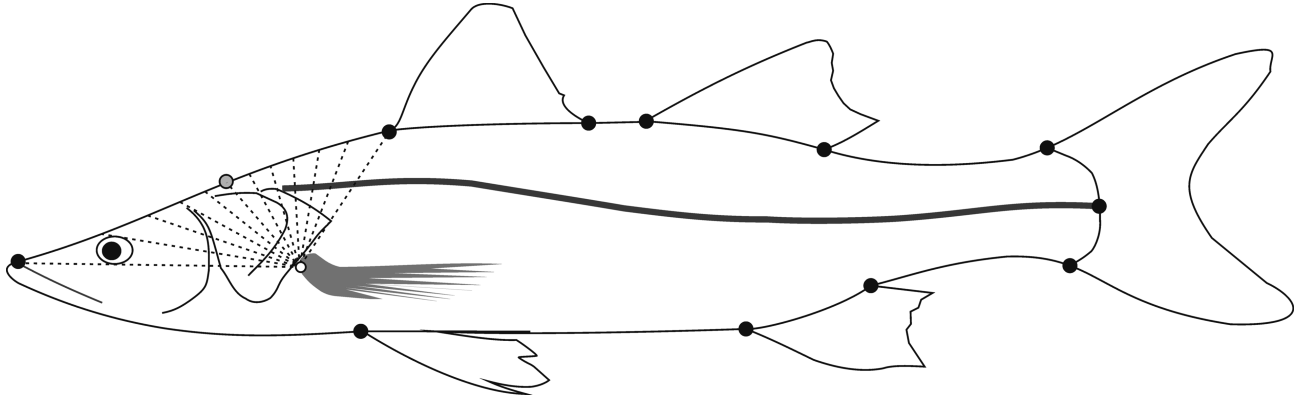

FIGURE 2. Representation of the generalized morphology of *Centropomus,* indicating ten landmarks (black circles) and one semi-landmark (gray circle) on the position of the anatomical structures compared in this study.

TABLE 1. Mode, frequency, and range for morphologic and meristic characters of genetically identified specimens of *Centropomus viridis* and *C. nigrescens* from Mazatlan, and Tonala, Mexico. n = number of specimens. [a]1 = with diverticula, 0 = without diverticula. [b]1 = triangular-shape fin, 2 = blunt-shape fin. [c]1 = second spine is shorter than the last ray, 2 = second spine equal to the last ray.

Character	C. viridis			C. nigrescens		
	n	Mode (Frequency)	Range	n	Mode (Frequency)	Range
Anatomic character						
Swim bladder shape[a]	16	1 (100%)		14	0 (100%)	
Morphologic characters						
First dorsal fin shape[b]	16	1 (50 %)	1–2	14	2 (86%)	1–2
Anal fin shape[c]	11	1 (81%)	1–2	11	1 (100%)	
Meristic characters						
Spines of the first dorsal fin	16	8 (87%)	7–8	14	8 (100%)	
Rays of the second dorsal fin	16	9 (94%)	8–9	14	10 (100%)	
Rays of pectoral fin	16	15 (87%)	14–16	11	15 (100%)	
Spines of anal fin	16	3 (100%)		14	3 (100%)	
Rays of anal fin	16	6 (100%)		14	6 (100%)	
Scales on the lateral line	16	96 (19%)	84–99	14	84 (28%)	80–97
Gill raker count	16	20 (37%)	17–21	14	12 (50%)	11–13
Upper limb	16	2 (62%)	2–3	14	1 (42%)	1–3
Lower limb	16	5 (37%)	2–6	14	3 (100%)	

Results

Genetic characterization. The sequences of all 15 specimens from Mazatlan produced three haplotypes. Haplotype 1 was identical to the sequence of *Centropomus viridis* (GAN DQ307689). There was only one difference between haplotypes: at positions 260 (haplotype 2, one specimen) and 274 (haplotype 3, one specimen) with respect to hap-

lotype 1. Thus, it was considered that all these three haplotypes belong to this species. Of the 15 specimens from Tonala, only one specimen had a sequence equal to haplotype 1 (*C. viridis*). The remaining 14 sequences (haplotype 4) were identical to *C. nigrescens* (GAN CNU85015). Thus, the phylogenetic three supported that the three haplotypes from Mazatlan belong to the same clade as *C. viridis* (GAN DQ307689), with maximum bootstrap support (Fig. 3). In contrast, the haplotype found only in Tonala grouped with *C. nigrescens*, with maximum bootstrap support (Fig. 3). *Centropomus viridis* had 40, 44, and 57 nucleotide differences with the closest species: *C. poeyi* (GAN CPU85014), *C. undecimalis* (GAN AF247436), and *C. nigrescens* (GAN CNU85015), respectively. The genetic distance between *C. viridis* and *C. poeyi* or *C. undecimalis* was 0.058. The genetic distance between *C. viridis* and *C. nigrescens* was 0.100. These findings support the taxonomic identity of each of the specimens that were genetically examined.

Morphology and meristic examinations. The anatomical examination revealed a univocal correspondence (100%) between the presence of diverticula in the gas bladder and the genetically identified *C. viridis* group and between the absence of diverticula and the genetically identified *C. nigrescens* group (Table 1). In contrast, the shape of the first dorsal fin was variable in both genetically identified species (Table 1; Fig. 4). In Mazatlan specimens (*C. viridis*), only 50% (8 of 16) of the genetically identified specimens presented a triangular shape of the first dorsal fin. In specimens caught in Tonala (*C. nigrescens*), 86% (12 of 14) of the genetically identified specimens presented a blunt shape of the first dorsal fin. Likewise, if all specimens (genetically identified plus genetically unidentified) are separated using the presence or absence of diverticula in the gas bladder as a taxonomic criterion, then in *C. viridis*, 72% (57 of 79) of the specimens showed a triangular shape of the first dorsal fin. In contrast, in *C. nigrescens*, 84% (16 of 19) showed a blunt shape of the first dorsal fin (Table 2).

TABLE 2. Mode, frequency, and range for morphologic and meristic characters of specimens grouped by the presence (*Centropomus viridis*) or absence (*C. nigrescens*) of diverticula in the gas bladder, from Mazatlan, Manzanillo and Tonala, Mexico. *n* = number of specimens. [a]1 = triangular-shape fin, 2 = blunt-shape fin. [b]1 = second spine is shorter than the last ray, 2 = second spine equal to the last ray.

Character	*C. viridis*			*C. nigrescens*		
	n	Mode (Frequency)	Range	*n*	Mode (Frequency)	Range
Morphologic characters						
First dorsal fin shape[a]	79	1 (72%)	1–2	19	2 (84%)	1–2
Anal fin shape[b]	128	1 (56%)	1–2	15	1 (100%)	
Meristic characters						
Spines of the first dorsal fin	133	8 (68%)	6–9	21	8 (100%)	
Rays of the second dorsal fin	133	9 (97%)	8–10	21	10 (100%)	
Rays of pectoral fin	131	15 (87%)	13–16	15	15 (93%)	14–15
Spines of anal fin	133	3 (100%)		21	3 (100%)	
Rays of anal fin	133	6 (95%)	5–8	21	6 (100%)	
Scales on the lateral line	131	88 (16%)	70–116	21	84 (23%)	74–97
Gill raker count	131	14 (50%)	12–15	21	12 (52%)	11–13
Upper limb	131	3 (75%)	2–7	21	2 (38%)	1–3
Lower limb	131	1 (43%)	1–6	21	3 (90%)	2–3

The majority of the meristic parameters (i.e., spines and rays of the anal fin and the left pectoral fin; gill rakers and rudiments [lower and upper] of the first branchial arch; scales on the lateral line) and morphologic characters (i.e., the relative length among the second spine and the last ray of the folded anal fin) showed a broad overlap between both genetically identified specimens, and between groups formed by the absence or presence of diverticula.

On the other hand, one meristic character showed differentiation between species. In genetically identified specimens (Table 1), the number of rays of the second dorsal fin had minimal intraspecific variation in *C. viridis* (mode nine rays, *n* = 16; one with eight rays), while in *C. nigrescens* the number of rays was constant (mode ten rays, *n* = 14). Likewise, in specimens separated by the presence or absence of diverticula (genetically identified

plus genetically unidentified specimens; Table 2), the same pattern in the number of rays of the second dorsal was obtained: specimens with diverticula (i.e., *C. viridis* specimens) showed a small variation (mode nine rays, $n = 133$; two with eight rays; 1 with ten rays), while in specimens without diverticula (i.e., *C. nigrescens* specimens) the number of rays was constant (mode ten rays, $n = 19$).
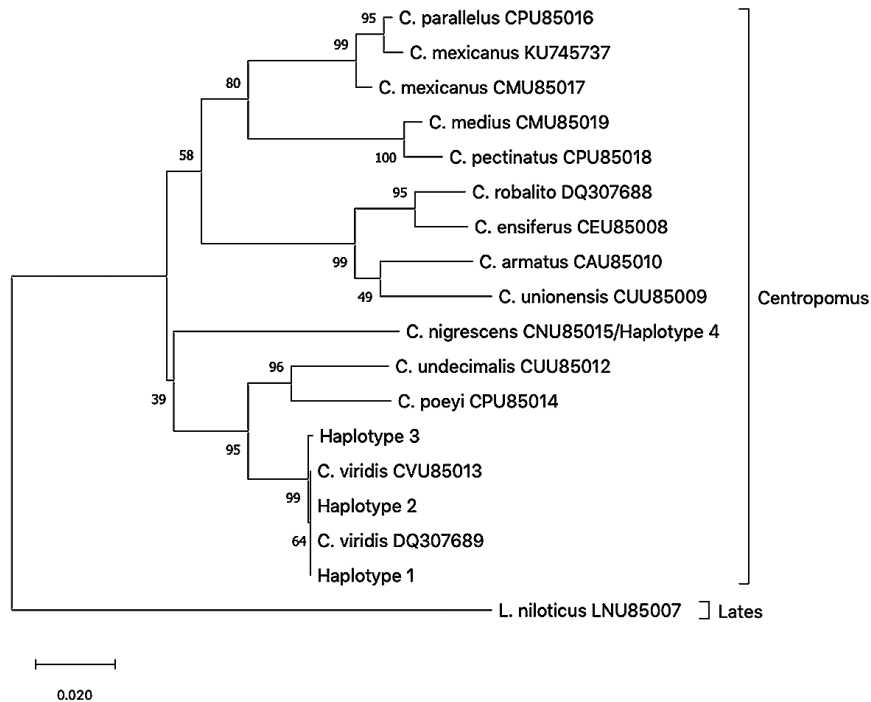

FIGURE 3. Phylogenetic relationships among the *Centropomus* 16S rRNA gene sequences found in the GenBank (March, 2020), only those sequences which overlap with the sequences from this study were selected. Relationships are based on the neighbor-joining method and the Tamura 3-parameter with a gamma distribution (shape parameter = 1). Node value support higher than 60% are shown.
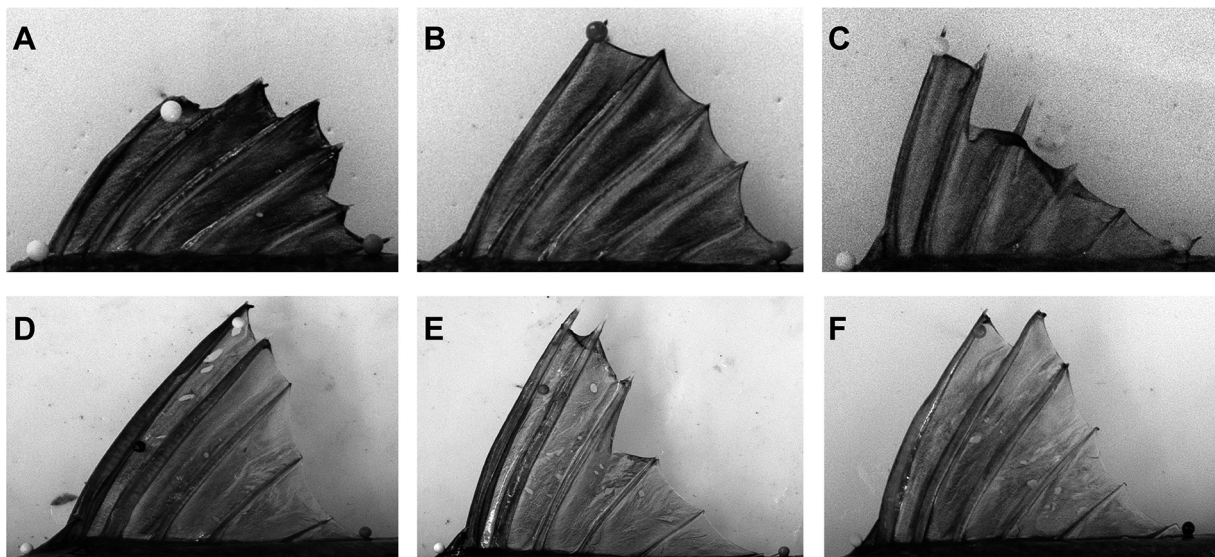

FIGURE 4. Some morphological variants of the first dorsal fin of genetically identified *Centropomus nigrescens* (A-C) and *C. viridis* (D-F) specimens. A and D represent the typical shape accepted for each species (blunt-shaped fin in *C. nigrescens* and triangular-shaped fin in *C. viridis*).

Geometric morphometrics. PCA analyses showed that two principal components contributed to 56% of the variance. These components supported the existence of two taxonomic entities that were morphometrically differentiated, with one corresponding to 16 specimens that were genetically identified as *C. viridis* and the other 18 specimens corresponding to *C. nigrescens* (14 genetically identified and four identified by gas bladder shape; Fig.

5). The F-test indicated significant differences between the mean body shapes of both species based on the partial Procrustes distance (*PPD*) ($F = 9.35$, $P = 0.0011$, $PPD = 0.0295$), showing a deeper body in *C. nigrescens* than in *C. viridis* (Fig. 6). A morphometric difference between the two species was also supported by the CVA, which indicated significant differences between the groups (Wilks' Lamba = 0.0367, $\chi^2 = 72.7$, $P < 0.05$). The percentage of correct assignment based on the Mahalanobis distance of CVA scores was 100% for each species, which indicated a high percentage of morphometric discrimination.
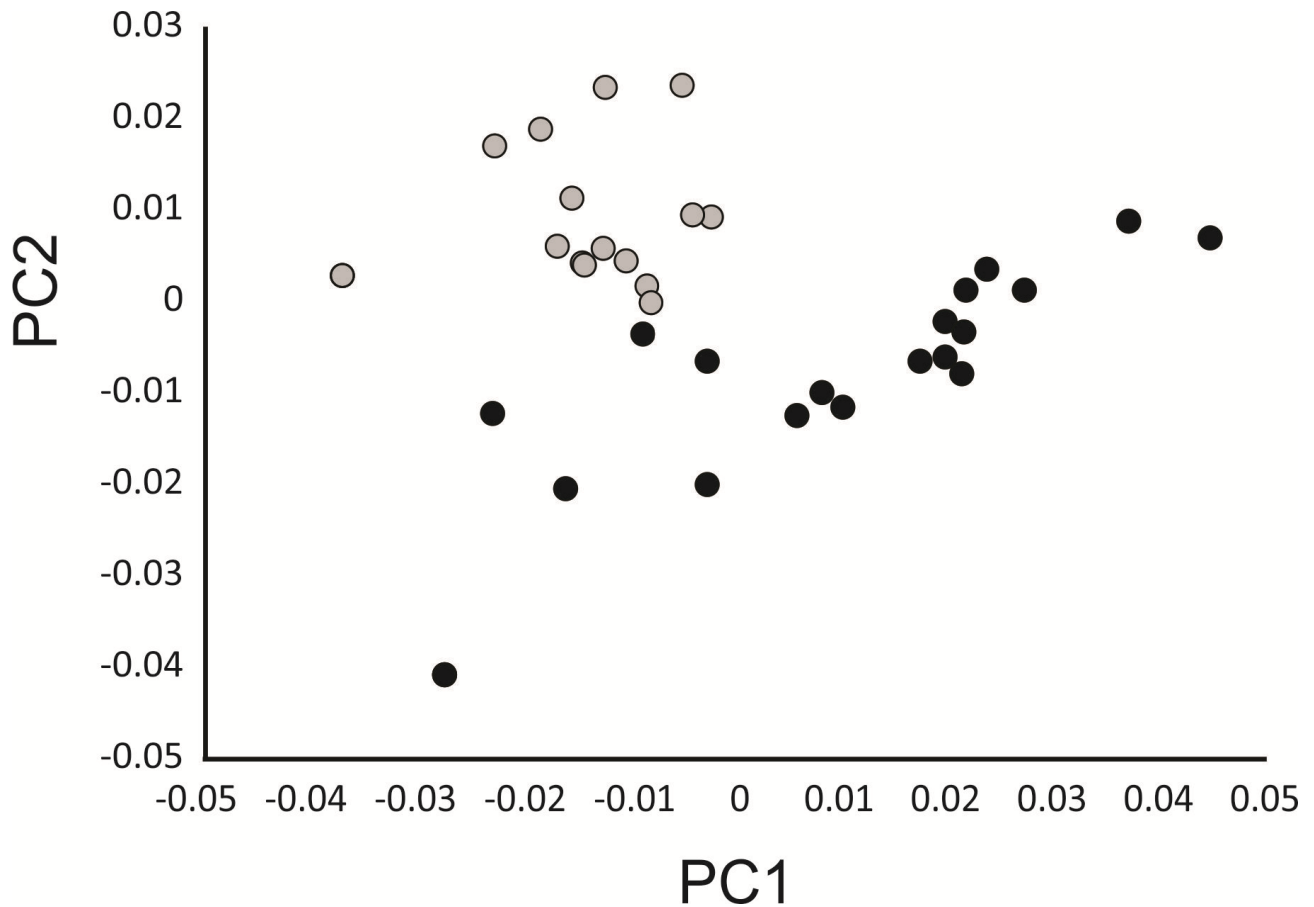

FIGURE 5. Scatter plot showing scores on the first two principal components explaining 56.3% of the total variance. Two taxonomic groups are detected, indicating that shape variables obtained by PCA are significant discriminators. *Centropomus nigrescens* is represented by black circles and *C. viridis* by gray circles.
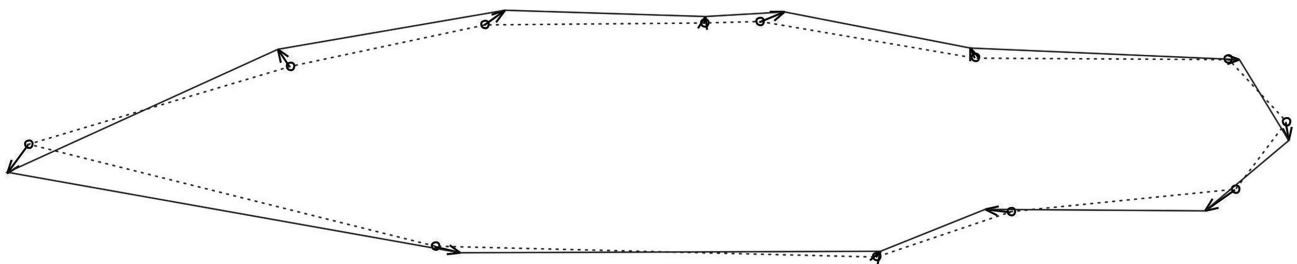

FIGURE 6. Difference between the mean shape of *Centropomus nigrescens* (solid line) and that of *C. viridis* (dashed line) based on partial Procrustes distances. Arrows indicate the direction of the change.

Discussion

The taxonomic identification of our specimens by genetic analysis allowed us to clearly identify two distinct clades of fish from distinct geographic locations. This result is significant because it allows us to determine the diagnostic value of each morphological and meristic character. Of these characters, only the gas bladder shape (presence or absence of diverticula) and the number of rays of the second dorsal fin were useful for distinguishing between both

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species. All the genetically verified specimens of *C. viridis* presented anterior diverticula in the gas bladder. In this species, the smallest specimen examined (73 mm SL) had much shorter diverticula (but recognizable) than larger specimens (from 210 mm SL). Therefore, diverticula may begin to develop in this species around that size. In *C. nigrescens*, anterior diverticula were not found at any size range. These results confirm the usefulness of gas bladder shape as a diagnostic character.

In the original description of *C. nigrescens*, Günther (1864) pointed out that this species does not have diverticula in the gas bladder. On the other hand, in the original description of *C. viridis*, Lockintong (1877) did not mention anything about the gas bladder; however, this author indicates a close relationship between *C. viridis* and *C. appendiculatus* (synonym of *C. undecimalis*). Subsequently, Boulenger (1895) differentiated *C. nigrescens* from *C. viridis* (synonymized in his work with *C. undecimalis*) based on the lack of the diverticula of the gas bladder in *C. nigrescens*. Similarly, Jordan & Evermann (1896) and Regan (1907) pointed out the lack of diverticula of the gas bladder in *C. nigrescens* to distinguish it from *C. viridis*. Conversely, Meek & Hildebrand (1925) did not recognize the diagnostic value of this character to separate both species, arguing that the specimens of *C. nigrescens* (including *C. viridis* as a synonym) can present either gas bladders without diverticula or bladders with different states of development of diverticula. However, they did not indicate the same for other *Centropomus* species. From this last point of view, Rivas (1986) took the variation of such character as a general pattern in *Centropomus* and excluded it as a diagnostic character in his genus review. However, this author acknowledged that Meek & Hildebrand (1925) reported *C. nigrescens* as a mixture of this species with *C. viridis* but did not consider this circumstance to make a critical assessment of the diagnostic value of the presence or absence of diverticula.

The gas bladder shape has diagnostic value in other families of fishes. For example, in a broad review of the gas bladder morphology in Doradidae (thorny catfishes), Birindelli *et al.* (2009) found a lower intraspecific variation that reflects ontogenetic states. Therefore, they pointed out that interspecific and intergeneric variations have a taxonomic and phylogenetic value in that group. Similarly, Chao (1978) used the gas bladder morphology as a taxonomic classification criterion in Sciaenidae (drums). Functionally, the gas bladder participates in buoyancy regulation, but in some fishes, it is involved in producing sound or auditory reception (Fine & Parmentier 2015). Notably, in some species, a functional relationship has been demonstrated between the anterior diverticula of the gas bladder and the inner ear, which plays a role in hearing (Parmentier *et al.* 2011; Schulz-Mirbach *et al.* 2013). In an evolutionary context, if the mechanisms of premating reproductive isolation (which includes behavioral barriers, such as sexual selection) are the first to appear (Futuyma & Kirkpatrick 2017), it can be argued that differences in the gas bladder shapes of close species may reflect differences in the patterns of intraspecific communication that originate from their divergence.

Although the gas bladder shape has taxonomic value in *Centropomus*, it does not have a deep phylogenetic signal because the presence of diverticula is not related to the monophyly of the genus (Fig. 3; Tringali *et al.* 1999b). Of the 13 valid species of *Centropomus*, only five species show diverticula (Table 3). In contrast, recently, Girard *et al.* (2020) found that the presence of diverticula is one of the morphological characters that support the monophyly of the suborder Centropomoidei (Latidae, Centropomidae, Lactariidae, Sphyraenidae), which would indicate that this character is a symplesiomorphy for *Centropomus*. Based on the above, the gas bladder shape in *Centropomus* species could result from a speciation process related to establishing premating reproductive barriers by sexual selection. Future studies are required to test this hypothesis.

In the present work, the number of rays of the second dorsal fin was the only external character that allowed for the separation of *C. viridis* from *C. nigrescens*. This character was used for the first time by Walford (1937) as a unique meristic character for identifying both species. Subsequently, some authors included it in their taxonomic keys (Rivas 1986; Castro-Aguirre *et al.* 1999; Miller *et al.* 2005). Others included it only in the synopsis of each species (Allen & Robertson 1994; Bussing 1995). Similarly, the ray count of the second dorsal fin is enough to distinguish *C. undecimalis* from *C. poeyi* (Rivas 1986). However, Rivas (1986) found that of 41 examined specimens of *C. viridis* (neotype and lectotypes with nine rays), only two had ten rays; of 38 examined specimens of *C. nigrescens* (holotype and lectotypes with ten rays), only two had nine rays. Likewise, van der Heiden *et al.* (1998) found that of the 40 examined *C. viridis*, one had ten rays, and of 14 specimens of *C. nigrescens*, one had nine rays. If the data from the present work and those of previous studies are considered together (212 *C. viridis* and 73 *C. nigrescens*), then the global probability of incorrectly identifying *C. viridis* as *C. nigrescens* using only the count of dorsal fin rays is approximately 0.02 and that for *C. nigrescens* as *C. viridis* is approximately 0.04. Therefore, the gas bladder morphology is the more robust character for the correct taxonomic identification of specimens that can be dissected.

On the other hand, the results obtained from the morphometric analysis indicated significant differences between the two species, which supports that the body shape can be used in the discrimination of these two groups: *C. nigrescens* shows a deeper bodied and more "humpback" than *C. viridis* (Fig. 5). However, the intraspecific variability of this character added to the observer subjectivity limits its practical utility in the field.

TABLE 3. Distribution of the presence (1) or absence (0) of gas bladder diverticula in the *Centropomus* clades, arranged in descending order according to their phylogenetic relationship (Tringali et al. 1999b; Carvalho-Filho et al. 2019).

Clade	Species	Diverticula	Note	Reference
1	*C. undecimalis*	1	Large	Chávez (1961)
	C. irae	?	unknown	Carvalho-Filh et al. (2019)
	C. poeyi	1	Shorts	Chávez (1961)
	C. viridis	1	Large	This study
	C. nigrescens	0		This study
2	*C. parallelus*	0		Meek & Hildebrand (1925)
	*C. mexicanus**	?	unknown	Jordan & Everman (1896)
3	*C. pectinatus*	0		Meek & Hildebrand (1925)
	C. medius	0		J. M. Martínez-Brown, pers. obs.
4	*C. ensiferus*	0		Meek & Hildebrand (1925)
	C. robalito	1	Short	Meek & Hildebrand (1925)
5	*C. unionensis*	1	Short	Meek & Hildebrand (1925)
	C. armatus	0		Meek & Hildebrand (1925)

* *C. constantinus* is currently a synonym of *C. mexicanus*. In the description of *C. constantinus* it is mentioned that it has two short blunt diverticula.

The intraspecific variation of the external morphology of both species that was observed in this work makes it difficult to correctly identify specimens that need to be kept alive. However, based on our results and in agreement with other authors (Walford 1937; Rivas 1986; Allen & Robertson 1994; Bussing 1995; van der Heiden *et al.* 1998; Castro-Aguirre *et al.* 1999; Miller *et al.* 2005), the following taxonomic identification procedure is recommended: first, determine whether the specimen belongs to the *C. viridis–C nigrescens* group. For this, it must be checked whether the tip of the second spine of the anal fin, when folded to the body, does not exceed three quarters the length of the caudal peduncle; otherwise, it belongs to some other species of the Pacific. Second, for identification at the species level, if the second dorsal fin has nine (rarely eight) rays, the specimen corresponds to *C. viridis*; however, if there are ten (rarely 11) rays, the specimen corresponds to *C. nigrescens*. This procedure assumes a low probability of misidentification. Therefore, in doubtful cases, confirmation of the taxonomic identification by genetic analysis will be necessary.

In conclusion, the results presented here show a univocal relationship of the gas bladder shape with each species, which validates its taxonomic value. Likewise, the meristic count of the second dorsal fin is validated as a diagnostic character due to its consistency in each species. Therefore, these characters are proposed to be useful and reliable taxonomic identification criteria for biological, ecological, fishing, or aquaculture studies.

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