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Morphological comparison of *Anisotremus interruptus* (Gill, 1862) (Perciformes: Haemulidae) populations along the Tropical Eastern Pacific.

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Morphological comparison of *Anisotremus interruptus* (Gill, 1862) (Perciformes: Haemulidae) populations along the Tropical Eastern Pacific.

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Abstract

The family Haemulidae is a marine fish group of ample distribution and of fishery importance that has been object of many studies through the Tropical Eastern Pacific (TEP). Phylogenetic studies on the species *Anisotremus interruptus* have revealed a high genetic diversity as well as divergence among its populations into the TEP, suggesting that its taxonomical diversity could be underestimated. *Anisotremus interruptus* possesses a wide distribution ranging from central Baja California Sur and Gulf of Mexico to northern Peru, including all oceanic islands except Clipperton. Recent genetic studies on this taxon indicate the presence of a species complex of at least three distinctive lineages. Under this premise, we performed a comparative study of the population-level meristic and morphometric variation of *A. interruptus* into the TEP, in order to evaluate and identify possible morphological features in support to the recent recognition of the genetically different populations. Our results provide evidence on the presence of two new species for the Revillagigedo and Galapagos archipelagos, respectively.

Key words: taxonomy, morphometry, meristics, analysis, new species.

Resumen

La familia Haemulidae es un grupo de peces marinos de amplia distribución e importancia pesquera que ha sido objeto de diversos estudios en el Pacífico oriental tropical (POT). Los estudios genéticos sobre la especie *Anisotremus interruptus* han revelado una importante diversidad genética así como una divergencia importantes entre sus poblaciones a lo largo del POT, lo que sugiere que su diversidad taxonómica podría estar subestimada. *Anisotremus interruptus* posee una amplia distribución que va desde la zona central de Baja California Sur, el Golfo de California hasta el norte de Perú, incluyendo todas las islas oceánicas, excepto Clipperton. Estudios genéticos recientes sobre este taxón indican la presencia de un complejo de al menos tres linajes distintivos. Bajo esta premisa, realizamos un estudio comparativo de la variación merística y morfométrica a nivel poblacional de *A. interruptus* en el TEP, con el fin de evaluar e identificar posibles rasgos morfológicos que apoyen el reciente reconocimiento de las poblaciones genéticamente diferentes. Nuestros resultados proporcionan pruebas sobre la presencia de dos nuevas especies para los archipiélagos de Revillagigedo y Galápagos, respectivamente.

Palabras clave: taxonomía, morfometria, merística, análisis, nuevas especies.

1. Introduction

The Tropical Eastern Pacific (TEP) has a great diversity of fish with a high number of endemics, which is attributed to the complexity of oceanographic and geological factors that led to the presence of contrasting marine habitats (Robertson & Allen 2015). The Haemulidae family, known as grunts, is represented in the TEP by 10 genera and 35 species. Within these, the genus *Anisotremus* is represented by four species.

Anisotremus interruptus (Gill, 1862), commonly known as burrito grunt, is endemic to the TEP, ranging in distribution from Central Baja California to Peru, including all the oceanic Islands, except Clipperton. This species with a maximum length of 90 cm is found in coastal rocky reefs at maximum depths of 30 m, and it has economic importance for the local fisheries (Cruz-Romero *et al,* 1993; Gonzales-Orozco, 2000; Ruiz-Ramírez *et al,* 2012 y Flores-Ortega *et al,* 2014). *Anisotremus interruptus* have its closest relative in the Atlantic, *A. surinamensis* (Bloch, 1791). The evolution of both species has been attributed to an allopatry process linked to the Panama isthmus formation (Tavera *et al*, 2012; Palmerin-Serrano *et al.,* 2020). *Anisotremus interruptus* was originally described as *Genytremus interruptus* by Gill, 1862, but latter on change for the same author to its actual genus *Anisotremus*.

Recently, a multilocus phylogeny of the Haemulidae evidenced that *Anisotremus interruptus* from Galapagos and continental populations show a substantial genetic isolation (Tavera et al., 2019). Complementarily, phylogeographic study of the species revealed the existence of three highly divergent genetic groups within *Anisotremus interruptus*, a first group distributed in the Galapagos Archipelago–Cocos Island, a second group confined to the Revillagigedo Archipelago and a third group with wide distribution in mainland TEP (Palmerin-Serrano et al., 2020). In this context, other authors recommend a comprehensive study, including morphometric and meristic analyses, in order to elucidate if the genetically divergent populations of *A. interruptus* deserve a new taxonomic status.

Under this premise, we performed a comparative study of the population-level meristic and morphometric variation of this species into the TEP, in order to evaluate possible morphological features in support to the recent recognition of the genetically different populations, and in case of congruence in both approaches, proceeding here with the formal description of these new evolutionary units.

2. Material and methods

The collected specimens were spearing with multi-pronged pole spears and fixed in 10% formalin. Then were preserved in 70% ethanol and incorporated into the Ichthyological collection of the Universidad Michoacana de San Nicolas de Hidalgo. We analyzed twenty-nine individuals in 11 locations on the mainland, 26 in five locations for the Galapagos Islands, two in two locations of Coco Island and 15 in seven locations of the Revillagigedo Archipelago, all of them covering the entire distribution range of the species (Fig. 1 and Table 1).

Meristic data were obtained with the support of AmScope stereomicroscope, the 13 characters explored were: the numbers in parentheses are related to those shown in figure 2. Scales of the lateral line SLL (1); scales of the origin of the dorsal fin to the lateral line SDLL (2); scales around the caudal peduncle SACP (3); caudal peduncle upper scales CPUS (4); scales between the upper beginning of the opercle through the back of the head SBOBH (5); pectoral to pelvic fin scales PecPelS (6); scales from the origin of the dorsal fin to the origin of the anal fin SDA (7); scales of the origin of the dorsal fin at the beginning of the head SODBH (8); scales of the origin of the dorsal rays to the anal fin SODRA (9); rays in the dorsal fin RD, rays in the anal fin RA, rays in the pectoral fin RP and rays in the caudal fin RC. All these measures described above have been widely used in the taxonomic analyzes of fish (Lyons et al. 2004; Dominguez-Dominguez et al., 2009).

Morphometric distances of the examined specimens were obtained from X-ray radiographies taken from the left side of each specimen, using an Ecotron brand portable x-ray device. We obtain 21 linear distances from 16 established landmarks using the ImageJ program (Fig. 3). The measurements were: head length HL (landmark 1 to 3); pre-orbital distance ProD (1-4); eye diameter ED (4-5); postorbital distance PooD (5-3); high of the head HH (6-7); standard length SL (1-2); minimum height of the body MHB (15-13); distance of the anterior insertion of the dorsal fin to the anterior insertion of the ventral fin DAIDAIV (8-9); distance from the posterior insertion of the dorsal fin to the posterior insertion of the anal fin DPDPA (10-12); distance of the origin of the anal fin to the origin of the dorsal fin DOAOD (8-11); dorsal fin base length LD (8-10); distance from the end of the dorsal fin to the upper part of the caudal peduncle DEDUCP (10-15); distance from the lower part of the caudal peduncle to the end of the anal fin DLCPEA (12-13); anal fin base length AL (11-12); distance of the anterior insertion of the anal fin to the insertion of the ventral fin DAIAIV (9-11); distance from the middle part of the caudal peduncle to the anterior insertion of the anal fin DMCPAIA (11-14); distance from the snout to the origin of the anal fin DSOA (1-11); distance from the snout to the ventral fin DSV (1-9); distance from the snout to the origin of the dorsal fin DSOD (1-8); length of the dorsal spine LDS (3-14) and length of the origin to the end of the dorsal rays LOEDR (16-10). To eliminate the effect of the allometry with the size, age and sex of individuals, the measurements were transformed using a ratio of standard length for body measurements and cephalic length for cephalic measurements, this was achieved using the statistical program PAST v. 3.23. This measurements were used in all the analyses (Edison-Zamudio *et al*, 2015) (Table 2 y Figure 3).

To explore the data and obtain the variables that most contribute to group formation, we conducted a principal components analysis (PCA), using the correlation option for the morphometric data and the variance-covariance option for the meristic data. After the exploratory analysis, seven of the original 13 meristic variables and nine of the 21 morphometric variables were maintained to conduct a MANOVA-CVA analysis, and a pairwise Hotelling test to explore statistical differences between groups. We also perform a discriminant function analyses (DFA) to evaluate the correct classification of individuals in the assigned groups, as well as to know which variables discriminate them, using STATISTICA 8.0 software. Box and whiskers plots were made with the most informative variables, as well as a univariate significance test for each variable between the groups.

3. Results

3.1 Meristic Analysis. The PCA for the meristic variables showed the formation of two groups, one of which corresponding to continental samples, and other to the Galapagos and Revillagigedo Archipelago samples, where an evident overlap is observed (Fig. 4A). PC I and II explained 72.89% of the cumulative variance, with SDLL, SACP, CPUS, SBOBH, SDA, SODBH and SODRA being the variables that most contributed to the variance (Table 3). We found the formation of three statistical significant differentiated groups in the MANOVA-CVA. One corresponds to samples from continental coasts, which do not show overlap with samples from Galapagos and Revillagigedo Archipelagos, the last two groups (Revillagigedo and Galapagos-Cocos) of samples showed a moderate overlap between them (Fig. 5A). The CV 1 account for 90.6% of the variance and 9.34% for CV 2, with a cumulative variance for both of 99.94%. Wilk's lambda value was 0.137, which is indicative of low overlap among groups. Hoteling's pairwise test shows significant differences between Galapagos-Cocos, Continent and Revillagigedo samples ($P < 0.05$), (Table 4). Discriminant function analyses yielded six significantly discriminant variables ($P < 0.05$): SDLL, SACP, CPUS, SDA, SODBH and SODRA. The continental samples show a 100% of correct classification, followed by Galapagos with 81% and Revillagigedo with 60% (Table 5). These results are congruent with the meristic counts, since the variables SDLL, SACP, CPUS, SDA, SODBH and SODRA were significantly different among the three compared populations (Table 5). In all variables, the individuals from Galapagos-Cocos and Revillagigedo Archipelagos had the highest values of meristic characters, while the individuals from the continent had the lowest values. In the univariate significance test the variables such as SDLL, SBOBH, SDA, SODBH and SODRA presented significant differences between the specimens from the Continent *vs* Revillagigedo and Galapagos-Cocos, whereas the variables SACP and CPUS showed significant differences among the three compared groups (Fig. 6).

3.2 Morphometric analysis. The PC I, II and III explained an accumulative variance of 58.59%, being nine characters (DAIDAIV, DOAOD, LD, DLCPEA, DMCPIA, DSOA, DSV, LOEDR and ProD) contributing more to explain the observed variance (Table 3). The samples from the three studied populations showed overlapping (Fig. 4B). The MANOVA-CVA show three statistical significant differentiated groups. One corresponds to samples from continental coasts, other from Galapagos-Cocos and the other from Revillagigedo Archipelago, with little overlap between them (Fig. 5B).The CV 1 explains 90.92% of the variation and 9.07% for CV 2 with a cumulative variance of 99.99%. Wilk's lambda value was 0.147, which is low and indicates that there is little overlap between groups. The Hotelling test shows significant differences between the three groups, Galapagos-Cocos, Continent and Revillagigedo ($P < 0.05$), (Table 4). The DFA show two significant discriminant variable ($P > 0.05$): DSOA and DSV (Table 5). The Continent samples present 95% of correct classification, followed by Galapagos-Cocos with 86% and Revillagigedo with 71%. In the ANOVA the variables DOAOD, LD, DSOA, DSV and LOEDR show significant differences between continent *vs* Revillagigedo and Galapagos-Cocos, the variable DAIDAIV present differences between the three groups Continent-Revillagigedo-Galapagos and the variable DLCPEA presents differences between the continent and the Galapagos specimens (Fig. 6).

4. Description of new species

Anisotremus sp1 new species

(Figure 7a)

Type material: Holotype. CPUM-9858, Tissue number-34922, 30.41 cm SL. Las Pirámides, Isla Clarión, Archipiélago de Revillagigedo, Manzanillo, Colima, México; Geographic coordinates; latitude 18°22´2.03”N, longitude 114°45´27.46”W, 25 July 2015. Col. Edgar Adrián Acevedo-Álvarez, **Paratypes:** CPUM-9845-11194, Bahía del muelle, Isla Clarión, Archipiélago de Revillagigedo, Manzanillo, Colima, México; Geographic coordinates; latitude 18°20´40.44”N, longitude 114°43´54.25”W Col. Edgar Adrián Acevedo-Álvarez, 3 specimens. CPUM-12696-13086, Playa blanca, Isla Socorro Archipiélago de Revillagigedo, Manzanillo, Colima, México; Geographic coordinates; latitude 18°48´51.76”N, longitude 114°2´25.89”W, Col. Carlos Leví Pérez-Hernández, Francisco Martínez-Servín, David Tafolla-Venegas and Edgar Adrián Acevedo-Álvarez, 3 specimens. CPUM-12697 Cabo Paerce, Isla Socorro Archipiélago de Revillagigedo, Manzanillo, Colima, México; Geographic coordinates; latitude 18°46'36.49"N, longitude 110°54'24.72"W, Col. Carlos Leví Pérez-Hernández, Francisco Martínez-Servín, Rosa Gabriela Beltrán-López, David Tafolla-Venegas, Ana Berenice García-Andrade, Moisés Emanuel Bernal-Hernández, 2 specimens. CPUM-13044, La Pared, Isla Clarión, Archipiélago de Revillagigedo, Manzanillo, Colima, México; Geographic coordinates; latitude 18°20'52.49"N, longitude 114°42'10.2"W, Col. Omar Valencia-Méndez and Salvador Romero-Gallardo, 3 specimens. CPUM-13045, Frente al Muelle, Isla Clarión, Archipiélago de Revillagigedo, Manzanillo, Colima, México; Geographic coordinates; latitude 18°20'37.28"N, longitude 114°43'50.11"W, Col. Omar Valencia-Méndez, David Tafolla-Venegas, Yareli López-Arroyo and Salvador Romero-Gallardo, 1 specimens. CPUM-13048, Barbas de Ben Laden, Isla Clarión, Archipiélago de Revillagigedo, Manzanillo, Colima, México; Geographic coordinates; latitude 18°20'23.61"N, 114°45'16.43"W longitude, Col. Omar Valencia-Méndez, David Tafolla-Venegas, Yareli López-Arroyo and Salvador Romero-Gallardo, 2 specimens. Additionally for comparative purposes, we analyzed 13 specimens of *A. surinamensis*, the sister species of *A. interruptus* distributed in the western Atlantic ocean.

Diagnosis. *Anisotremus* sp1 can be distinguish from the rest of the species by the combination of the following meristic characters expressed by their mode value followed by range in parenthesis: 8 (7-10) scales of the origin of the dorsal fin to the lateral line *vs* 7 (6-8) in *A. interruptus* and 7 (7-9) in *A. surinamensis*; 24 (22-27) scales around the caudal peduncle *vs* 22 (21-25) in *A. interruptus* and 27 (23-28) in *A.* sp2; 21 (19-23) caudal peduncle upper scales *vs* 17 (15-20) in *A. interruptus* and 17 (16-20) in *A. surinamensis*; 20 (18-24) scales between the upper origine of the operculum through the back of the head *vs* 17 (15-20) in *A. interruptus* and 19 (18-20) in *A. surinamensis*; 10 (9-12) scales from the origin of the dorsal fin at the beginning of the head *vs* 9 (9-10) in *A. interruptus*; 27 (22-29) scales of the origin of the anal fin to the origin of the dorsal fin *vs* 23 (21-25) in *A. interruptus* and 26 (24-28) in *A. surinamensis* and 25 (22-26) scales from the origin of the dorsal rays to the anal fin *vs* 22 (20-23) in *A. interruptus*, 22 (20-25) in *A. surinamensis* and 27 (21-26) in *A.* sp2 (Table 2). Morphometric characters presented as ratio to the SL are expressed by their average value followed in parentheses by their range: distance from the anterior insertion of the dorsal fin to the anterior insertion of the ventral fin 2.32 (2.19-2.48) *vs* 2.27 (2.22-2.40) in A. sp2 and 2.20 (2.0-2.34) in *A. interruptus*; distance from the origin of the anal fin to the origin of the dorsal fin 1.75 (1.64-1.81) vs 1.75 (1.68-1.88) in *A.* sp2 and 1.64 (1.54-1.71) in *A. interruptus*; dorsal fin length 1.72 (1.64-1.80) *vs* 1.75 (1.61-1.84) in *A.* sp2 and 1.65 (1.56-1.70) in *A. interruptus*; distance from the anterior insertion of the anal fin to the insertion of the ventral fin 3.06 (2.85-3.31) *vs* 3.18 (2.91-3.62) in *A.* sp2 and 2.92 (2.77-3.21) in *A. interruptus*; distance from the snout to the origin of the anal fin 1.38 (1.33-1.44) *vs* 1.39 (1.32-1.44) in *A.* sp2 and 1.35 (1.30-1.39) in *A. interruptus*; distance from the snout to the origin of the dorsal fin 2.30 (2.24-2.53) *vs* 2.19 (2.07-2.35) in *A.* sp2 and 2.23 (2.04-2.36) in *A. interruptus* and length of the origin to the end of the dorsal rays 4.22 (4.01-4.65) *vs* 4.37 (4.08-4.92) in *A.* sp2 and 3.92 (3.72-4.24) in *A. interruptus*. The dorsal fin is supported by 26 pterygiophors inserts in 18 neural spines starting in vertebra number two *vs* 26 pterygiophors inserted in 17 neural spines in *A.* sp2 and 28 pterygiophors inserted in 18 neural spines in *A. interruptus*; the anal fin is supported by 11 pterygiophors inserted in nine hems spines *vs* 11 pterygiophors inserted in eight hems spines in *A.* sp2.

Description. *Anisotremus* sp1 possesses 17 to 18 dorsal rays, usually 17; from 9 to 11 anal rays (mode= 10) and 17 to 19 pectoral rays (mode= 18); caudal rays from18 to 19,usually 19; seven to 10 scales from the origin of the dorsal fin to the lateral line, usually 8; , scales on the lateral line ranging from 53 to 55, usually 54; 22 to 27 scales around the caudal peduncle, usually 24; scales from the upper origin of the operculum through the back of the head 18 to 24 (mode= 20); ten to 12 scales between the pectoral to pelvic fins, usually 12; scales from the origin of the dorsal fin to the origin the anal fin from 22 to 29 but usually 27; nine to 12 scales of the origin of the dorsal fin at the beginning of the head, usually 10; caudal peduncle upper scales 17 to 23 (mode= 21) and 22 to 26 scales of the origin of the dorsal rays to the anal fin (usually 25). High and laterally compressed body, its maximum height is 2.32 times the SL and its minimum height is 8.43 times the SL. Head high and robust, its length is 3.53 times in the SL, eye diameter 3.17 times in the head length, thick and fleshy lips, finely serrated preopercle, tip of the long pectoral fin reaching the origin of the anal fin. Forked caudal fin, slightly large scales, present in all the body and head except chin, snout and lips. (Table. 2). Twenty vertebrae (10 precaudal plus 15 caudal), the caudal region supports 19 branched rays with two upper and one lower hipural plates, two upper epural plates and one lower parhipural plate.

Pigmentation. Dorsum yellow with silvery tones near to the head and towards the anal region with silvery tones. Edges of the scales slightly yellow. Posterior upper region of the body with soft yellow tonalities, slightly dark head. Pectoral, caudal, dorsal, ventral and anal fins with black color.

Sexual dimorphism. Not present evident sexual dimorphism.

Etymology. To be defined based on toponymy.

Habitat and Distribution: Endemic to Revillagigedo Archipelago, Manzanillo, Colima Mexico. Collections were performed in the Clarion and Socorro Islands, at an average depth of 4 meters. All individuals were found in shoals, always associated with rocky reef, so this is considered its main habitat.

Conservation. As this species seems to be restricted to the islands of Revillagigedo Archipelago, more studies are needed to accurately state the conservation status of the populations. The samplings conducted showed a low occurrence of the species, with few organisms and not easy to find, however these are empirical data. The Revillagigedo Archipelago was declared a Natural Protected Area since 1994, a biosphere reserve since 2008 and in 2017 was declared a natural heritage of humanity by UNESCO as well as a national park, becoming the largest in North America, so the commercial extraction of marine fauna is prohibited. Accordingly, this species seems to be protected. However, it is necessary to pay special attention to the conservation of the habitats of shallow rocky reefs close to the coast, any impact on shallow coastal rocky reefs can have a negative impact on the populations of the species.

Anisotremus sp2 new species

(Figure 7b)

Type material: Holotype. CPUM-13493, tissue number-46746, 28.37 cm LE. Cerro Gallina, Isla Santa Cruz, Galapagos Archipelago, Ecuador, Geographic coordinates: latitude 00°43´40.2"N longitude 90°39´54.32"O, 13February 2017. Col. Rolando Quetzalcoatl Torres-García, Juan Carlos Quevedo-Machado y Paola Nallely Palmerín-Serrano. **Paratypes:** CPUM-13493, same data as holotype, 13 specimens. CPUM-13494, Islote Espejo, Isla Marchena, Galapagos Archipelago, Ecuador. Geographic coordinates: latitude 00°18´46.18"N longitude 90°24´4.64"W, Col. Dahiana Arcila, Adrián Jaramillo-López, Rosa Gabriela Beltrán-López, 3 specimens. CPUM-13496, Poza Lobos, Isla Marchena, Galapagos Archipelago, Ecuador. Geographic coordinates: latitude 00°19´6.29"N, longitude

90°28´38.34”W, Col. Dahiana Arcila, Adrián Jaramillo-López, Rosa Gabriela Beltrán-López, 1 specimens. CPUM-13495, Bahía Darwin, Isla Genovesa, Galapagos Archipelago, Ecuador. Geographic coordinates: latitude 00°18´58.38”N, longitude 89°57´15.71”W, Col. Yareli López-Arroyo, Omar Domínguez-Domínguez, Francisco Martínez-Servín, Carmen del Rocío Pedraza-Marrón, Eduardo Espinoza, Emanuell Duarte-Ribeiro, 7 specimens. CPUM-6073, Punta Pitt, Isla San Cristóbal, Galapagos Archipelago, Ecuador. Geographic coordinates: latitude 00°42´44.08”N, longitude -89°14´51.91”W, Col. Salvador Romero-Gallardo, Eloísa Torres-Hernández, Oscar Lasso-Alcalá, Julio César Orantes-Ávalos, 1 Specimens. CPUM-Num.-Tejido-58719, Frente a Ulloa, Isla del Coco, Costa Rica. Geographic coordinates: latitude 5°33´5.04”N longitude 87°2´11.77”W, Col. Yareli López-Arroyo and Francisco Martínez-Servín, 1 specimens. CPUM-Num.-Tejido-58729, Manuelita Profundo, Isla del Coco, Costa Rica. Geographic coordinates: latitude 5°33´40.08”N longitude 87°2´54.29”W, Col. Yareli López-Arroyo, Francisco Martínez-Servín, 1 specimens.

Diagnosis. *Anisotremus* sp2 differs from the rest of the studies species within the Pacific as well as its sister species of the Atlantic *A. surinamensis* by the combination of the following meristic characters, expressed by their mode followed in parentheses by their range: 8 (7-9) scales of the origin of the dorsal fin to the lateral line *vs* 7 (6-8) in *A. interruptus* and 7 (7-9) in *A. surinamensis*; 27 (23-28) scales around the caudal peduncle *vs* 22 (21-25) in *A. interruptus* and 24 (22-27) in *A.* sp1; 21 (19-24); caudal peduncle upper scales *vs* 17 (15-20) in *A. interruptus* and 17 (16-20) in *A. surinamensis*; 20 (18-24); scales between the upper origin of the operculum through the back of the head *vs* 17 (15-20) in *A. interruptus* and 19 (18-20) in *A. surinamensis*, 10 (9-12); scales from the origin of the dorsal fin at the beginning of the head *vs* 9 (9-10) in *A. interruptus*, 27 (24-29); scales of the origin of the anal fin to the origin of the dorsal fin *vs* 23 (21-25) in *A. interruptus* and 26 (24-28) in *A. surinamensis* and 27 (21-26) and scales from the origin of the dorsal rays to the anal fin *vs* 22 (20-23) in *A. interruptus*, 22 (20-25) in *A. surinamensis* and 25 (22-26) in *A.* sp1 (Table 2). Morphometric characters presented in proportion of the SL expressed by their average followed in parentheses by their range: distance from the anterior insertion of the dorsal fin to the anterior insertion of the ventral fin 2.27 (2.22-2.40) *vs* 2.32 (2.19-2.48) in *A.* sp1 and 2.20 (2.0-2.34) in *A. interruptus*; distance from the origin of the anal fin at the origin of the dorsal fin 1.75 (1.68-1.88) *vs* 1.64 (1.54-1.71) in *A. interruptus*; dorsal fin length 1.75 (1.61-1.84) *vs* 1.72 (1.64-1.80) in *A.* sp1 and 1.65 (1.56-1.70) in *A. interruptus*; distance from the anterior insertion of the anal fin to the insertion of the ventral fin 3.18 (2.91-3.62) *vs* 3.06 (2.85-3.31) in *A.* sp1 and 2.92 (2.77-3.21) in *A. interruptus*; distance from the snout to the origin of the anal fin 1.39 (1.32-1.44) *vs* 1.38 (1.33-1.44) in *A.* sp1 and 1.35 (1.30-1.39) in *A. interruptus*; distance from the snout to the origin of the dorsal fin 2.19 (2.07-2.35) *vs* 2.30 (2.24-2.53) in *A.* sp1 and 2.23 (2.04-2.36) in *A. interruptus* and length of the origin to the end of the dorsal rays 4.37 (4.08-4.92) *vs*. 4.22 (4.01-4.65) in *A.* sp1 and 3.92 (3.72-4.24) in *A. interruptus*. The dorsal fin is supported by 26 pterygiophors inserted in 17 neural spines, starting in vertebrate two *vs* 26 pterygiophors inserted in 18 neural spines in *A.* sp1 and 28 pterygiophors inserted in 18 neural spines in *A. interruptus;* the anal fin is supported by 11 pterygiophors inserted in eight hems spines *vs* 11 pterygiophors inserted in nine hems spines in *A.* sp1.

Description. *Anisotremus* sp2 has 16 to 18 dorsal rays (mode= 17), nine to 10 anal rays (mode= 10), 17 to 19 pectoral rays (mode=18), and 17 to 19 caudal rays (mode= 18); number of scales ranges from seven to nine from the dorsal fin origin to the lateral line (mode= 80 ; fifty-one to 56 scales on the lateral line (mode= 54); scales around the caudal peduncle from 23 to 28, being frequently 27; eighteen to 24 scales between the upper origin of the opercule to the back of the head (mode= 20); number of scales between pectoral and pelvic fins ranging from 10 to 13 (mode= 10); twenty-four to 29 scales from the origin of the dorsal fin to the origin the anal fin, being usually 27; nine to 12 scales from the origin of the dorsal fin to the beginning of the head, being usually 10; caudal peduncle upper scales from 19 to 24, usually numbering 21; and 21 to 26 scales from the origin of the dorsal rays to the anal fin, having usually 24. Body high and laterally compressed, its maximum height contains 2.74 times within the SL and its minimum height 8.42 times in the SL. Head high and robust with a slightly pronounced nape, its length is 3.41 times in the SL, eye diameter 2.95 times in the head length; lips are thick and fleshy; serrated pre-opercule, long pectoral fin its tip reaching the origin of the anal fin; forked caudal fin; body covered with slightly large scales throughout the body and head except chin, front of snout and lips (Table 2). Twenty-five vertebrae (10 precaudal plus 15 caudal), the caudal region supporting 19 branched rays with two upper and one lower hipural plates, two upper epural plates and one lower parhipural plate.

Pigmentation. Body is silvery through the body, having the center of the scales and the caudal peduncle dark. Pectoral, caudal, dorsal, ventral and anal fins of yellowish color.

Sexual dimorphism. Without external evident sexual dimorphism.

Etymology. Could be based on toponymy.

Distribution and habitat: Endemic to Galapagos Archipelago and Coco Island. Collections were made at an average depth of 4 to 8 maind all individuals were found associated with rocky reefs, assuming that this biotope could be the main habitat of the species, even some individuals were observed in underwater caves, the collections were made during the day. Individuals were observed in medium and large shoals.

Conservation. Individuals of this new species are common in the study locations, forming small and large schools. This species is not caught commercially in the area due that the Galapagos Archipelago and Coco Island are National Parks and both are under strict protection and management, so the populations until now seems to be protected.

5. Identification key for the three species in the *Anisotremus interruptus* species group from the tropical Eastern Pacific.

1a. Number of scales in an oblique line from the dorsal fin origin to the lateral line equal to or less than six; number of scales in the upper region of the caudal peduncle equal to or less than 17; number of scales in an oblique series from the dorsal fin origin to the region of the anal fin origin equal to or less than 23 (fig.2)……………………………………………………………………...….***Anisotremus interruptus***

1b. Number of scales in an oblique line from the dorsal fin origin to the lateral line greater than six; number of scales in the upper region of the caudal peduncle greater than 17; number of scales in an oblique series from the dorsal fin origin to the region of the origin of the anal fin greater than 23………….....................2

2a. 24 scales around the caudal peduncle; 26 dorsal pterygiophors supported by 18 neural spines; 11 anal pterygiophors supported by nine hems spines; ventral, anal and caudal fins dark……………………….……………………....……….….………...……….....***Anisotremus sp1***

2b. 27 scales around the caudal peduncle; 26 dorsal pterygiophors supported by 17 neural spines; 11 anal pterygiophors supported by eight hems spine; dorsal, ventral, anal and caudal fins bright yellow ……………………….………………………...………………………….......***Anisotremus sp2***

6. Discussion

The results presented herein, using morphologic and meristic data, give substance to the existence of three well-differentiated morphologically groups within *Anisotremus interruptus* complex, supporting the finding of three independent genetic groups previously identified by Palmerin-Serrano et al., (2020). The significant morphologic and meristic differences determined in the studied populations allow to distinguish the existence of three morphotypes that are congruent with the genetic groups reported by Palmerin-Serrano et al. (2020) in these same locations, one distributed in the Continental coast of the Tropical Eastern Pacific, other distributed in the Galapagos Archipelago and Cocos Island and other distributed in the Revillagigedo Archipelago. The description of the two new species pointed out the underestimation of the fish diversity in the TEP, as have been shown in other groups as Gobiidae (Sandoval-Huerta et al., 2019) and Gobiesocidae (Torres-Hernández et al., 2020), even in well studied areas as Galapagos Archipelago, or in species as *Anisotremus interruptus* (Cruz-Romero et al, 1993; Gonzales-Orozco, 2000; Bernardi & Lape 2005; Pereira-Dias 2007; Ruiz-Ramírez et al, 2011; Tavera et al. 2011; Tavera et al. 2012; Tavera et al., 2018; Palmerin-Serrano et al., 2020).

It is interesting to note that, although in the phylogeographic study of Palmerin-Serrano et al., (2020), the populations of *Anisotremus interruptus* from the Continental TEP and Revillagigedo Archipelago were the closet related, the Galapagos-Cocos population is revealed in all the analyses presented herein as the most genetically differentiated, open new questions about the role of the habitat on the modeling of the morphology of fish species.

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8. FIGURES

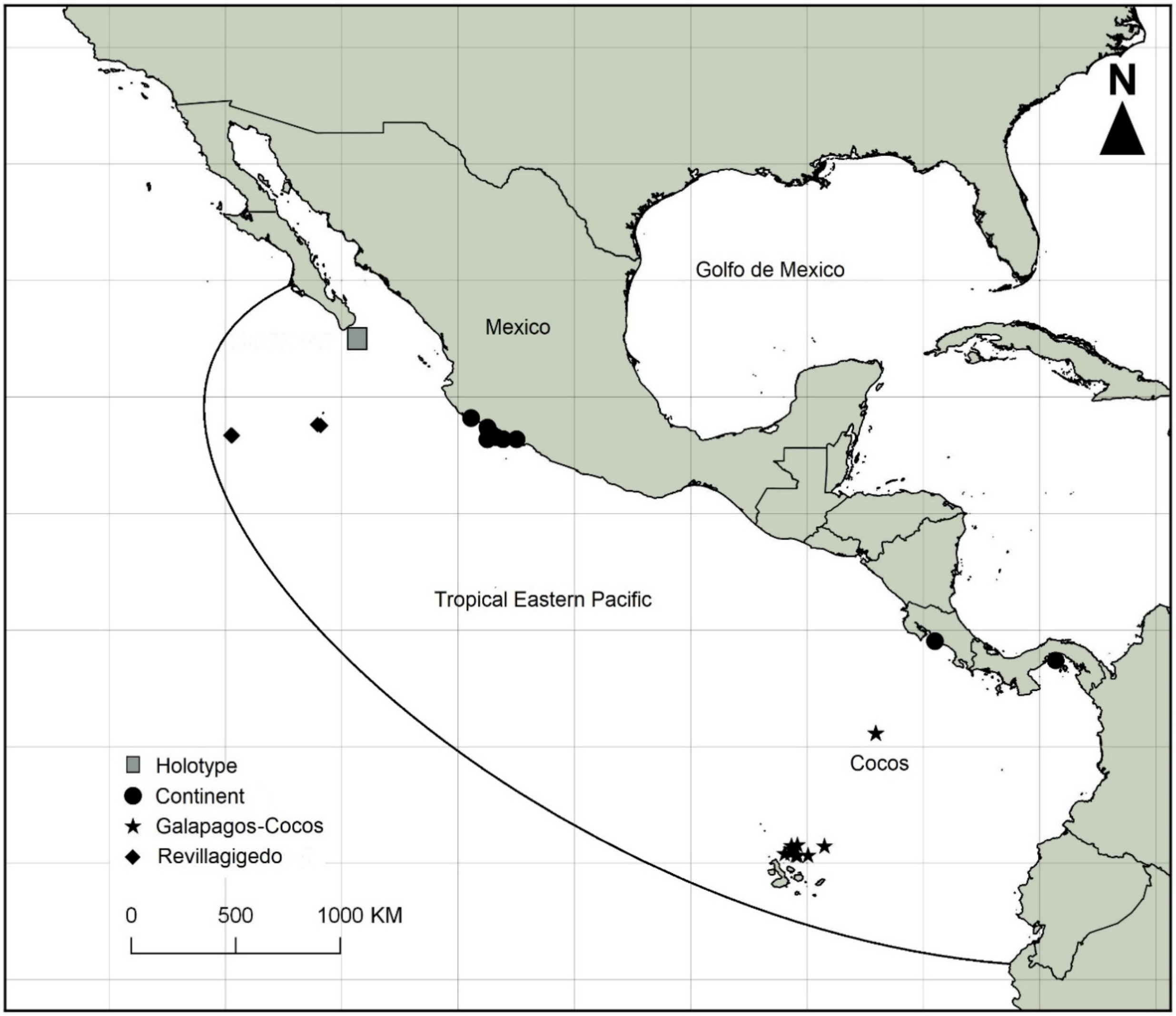


FIGURE 1. Sampled locations along the Tropical Eastern Pacific (TEP).

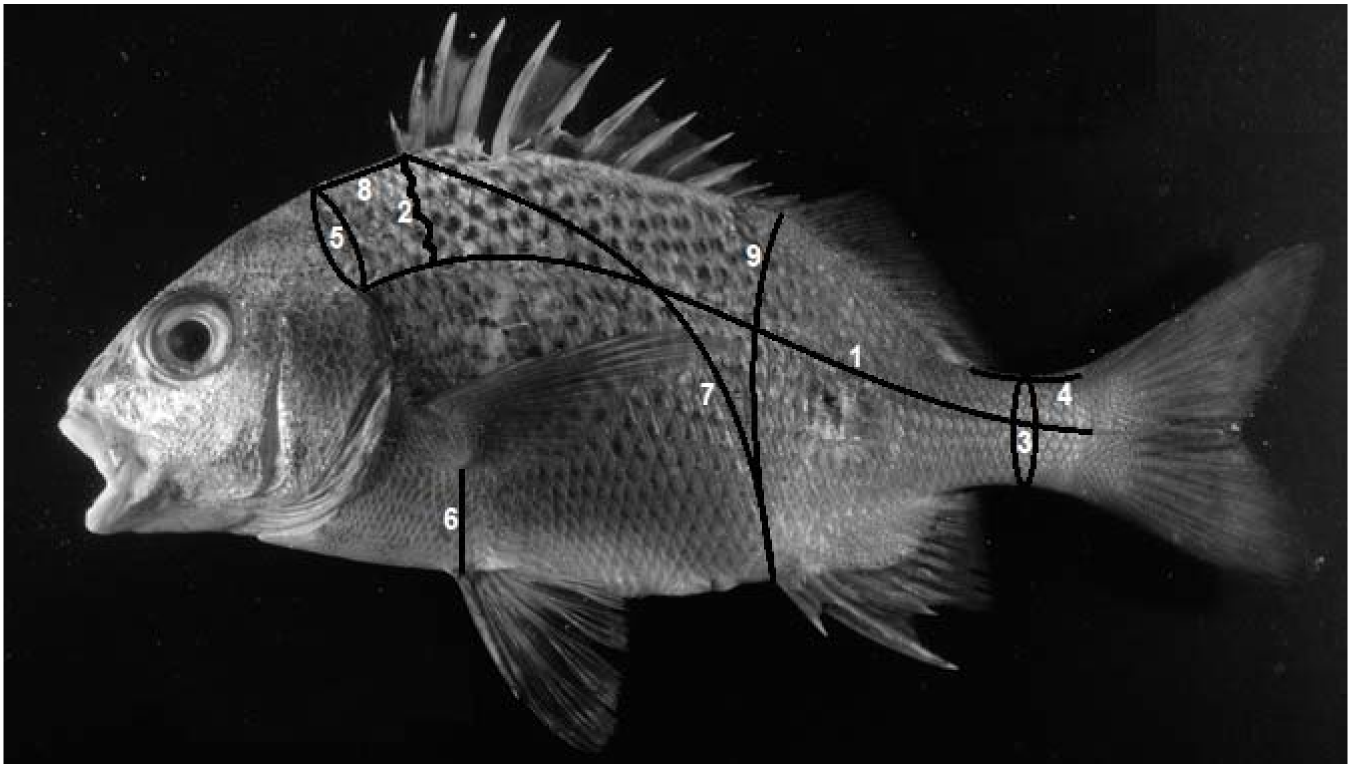


Figure 2. Meristic variables used. The numbers are associated with the text and correspond only to the meristic variables involving scale counts

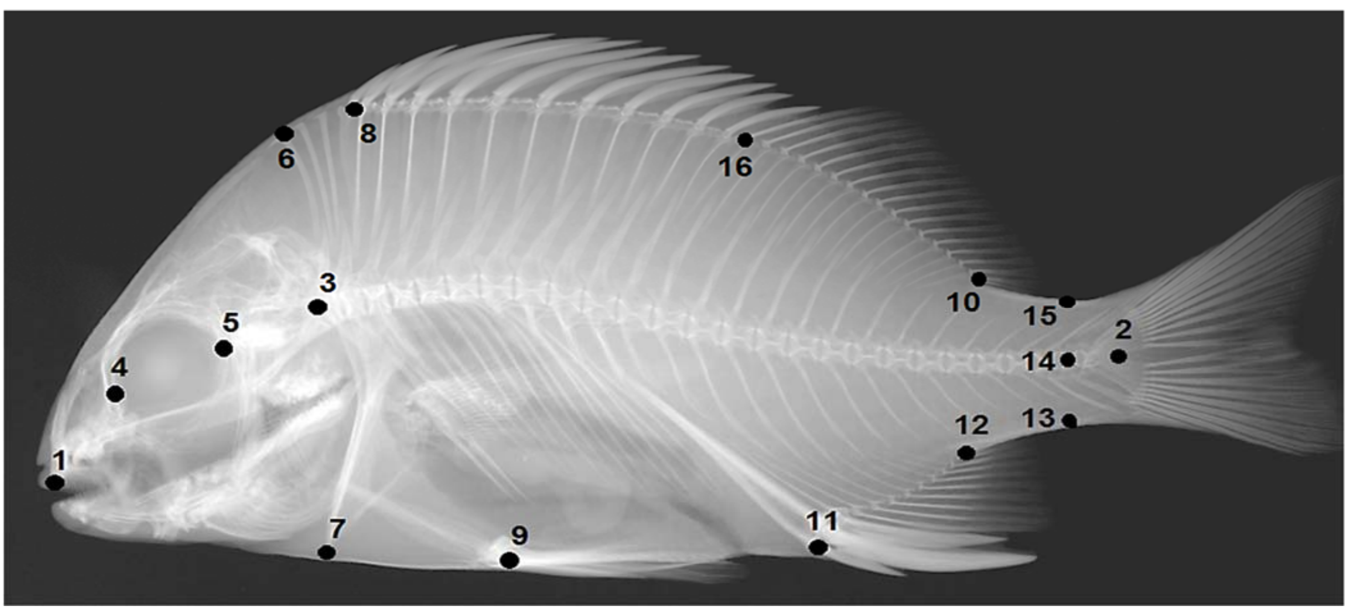
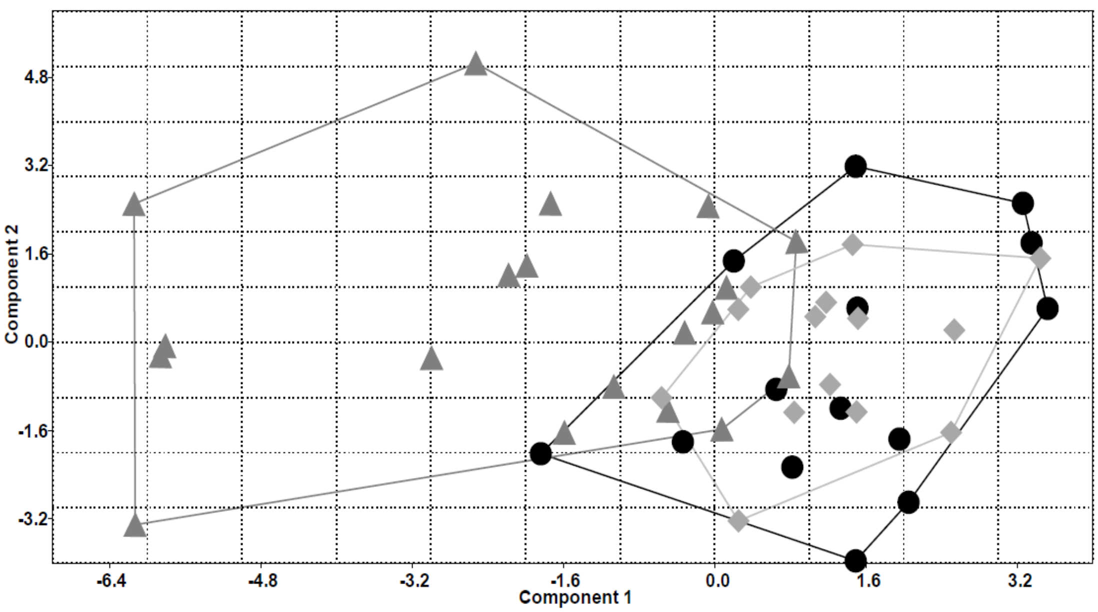


FIGURE 3. Landmarks used to obtain morphometric measurements.

A



B

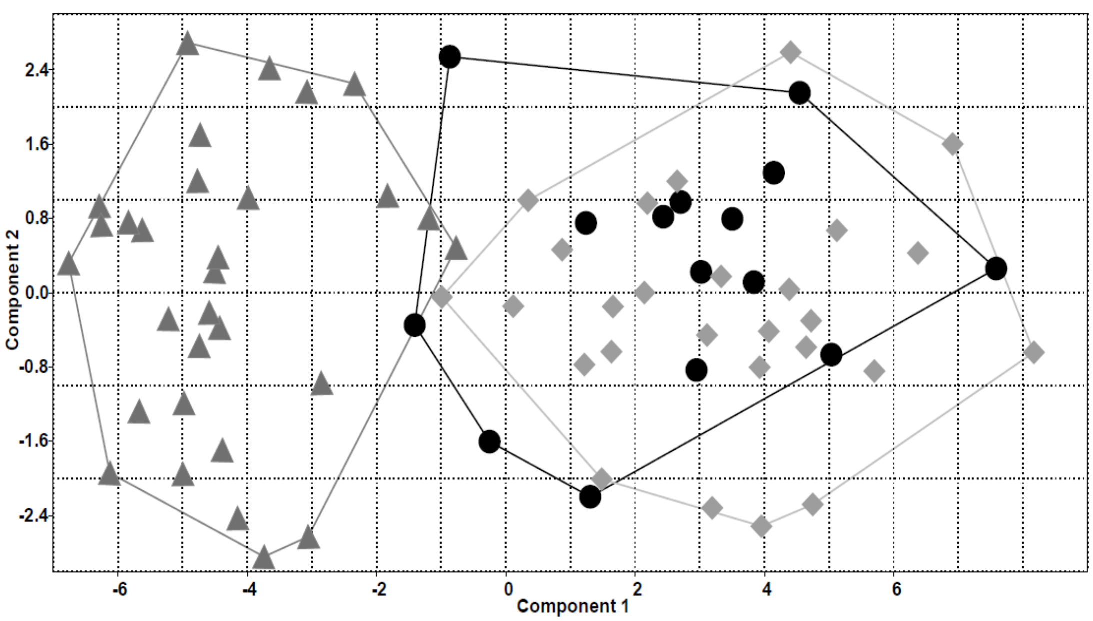
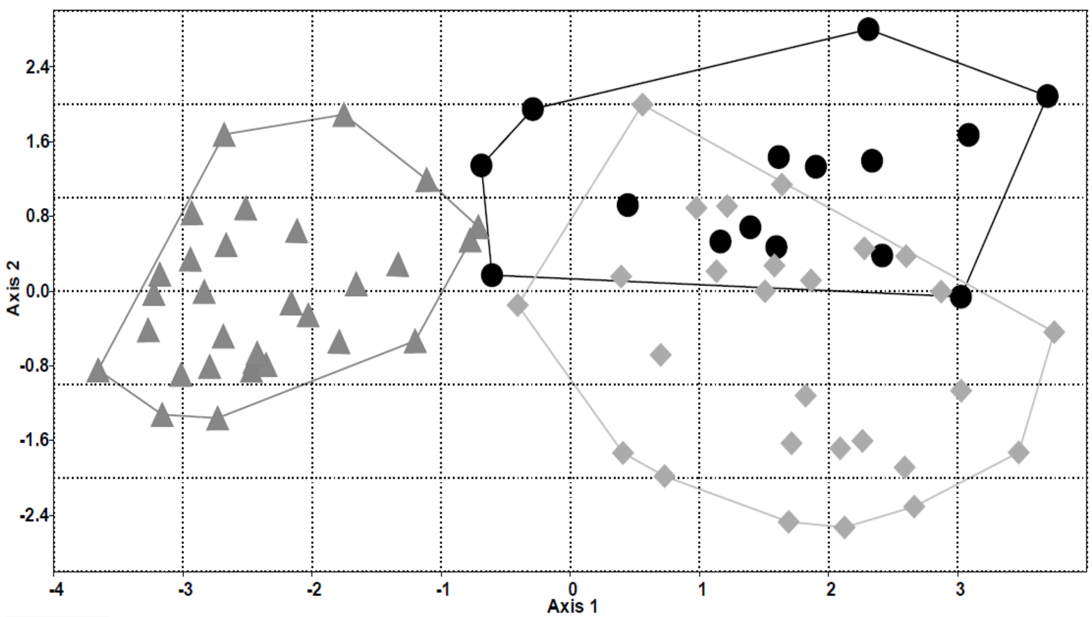


FIGURE 4. Analysis of principal components of meristic (A) and morphometric (B) characters. Continent (triangles), Revillagigedo (circles) and Galapagos (rhombuses).

A



B

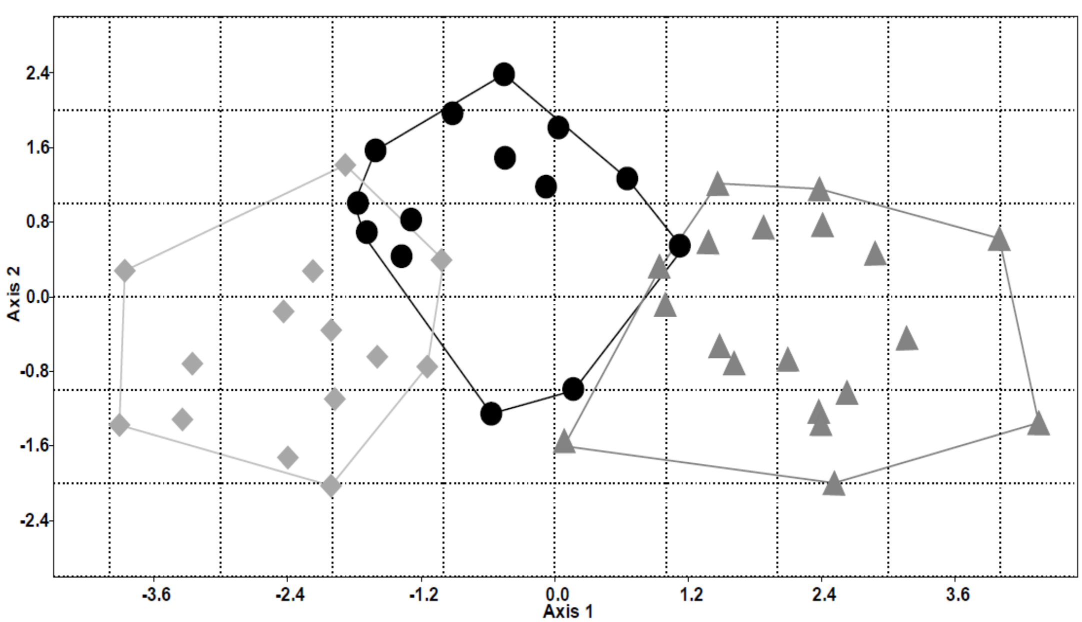


FIGURE 5. MANOVA-CVA for the three groups taking only the variables that contribute more weight in the PCA for meristic (A) and morphometric (B) characters. Continent (triangles), Revillagigedo (circles) and Galapagos (diamonds).

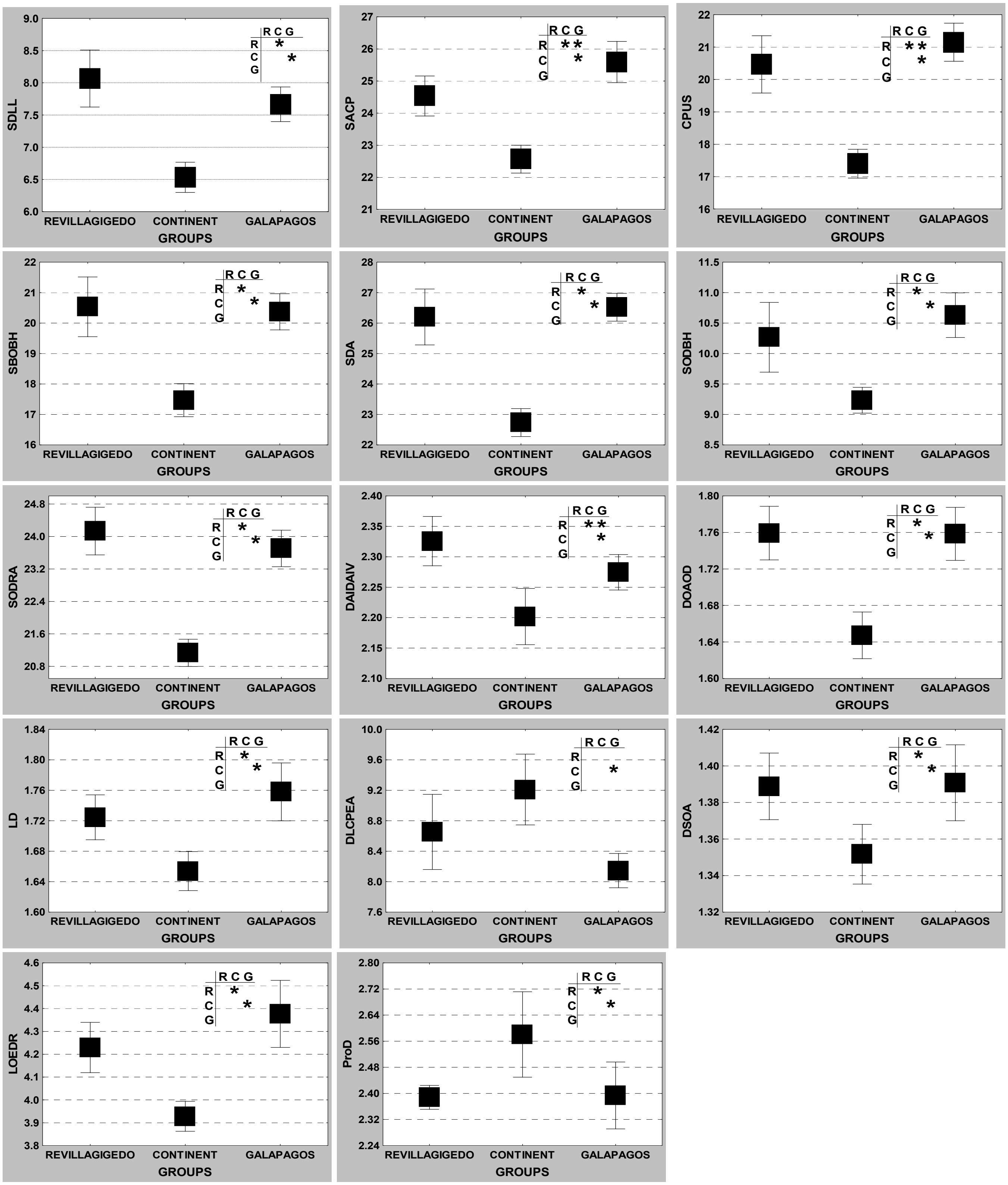


FIGURE 6. Box and whiskers diagrams for seven meristic (SDLL, SACP, CPUS, SBOBH, SDA, SODBH and SODRA) and nine morphometric variables (DAIDAIV, DOAOD, LD, DLCPEA, DMCPAIA, DSOA, DSV, LOEDR and ProD) that resulted to be discriminant for populations of

Anisotremus interruptus from Tropical Eastern Pacific. In the table are the paired comparisons of Hotelling, only the significantly different ones are represented. R = Revillagigedo, C = Continent, G = Galapagos. * p<0.05.

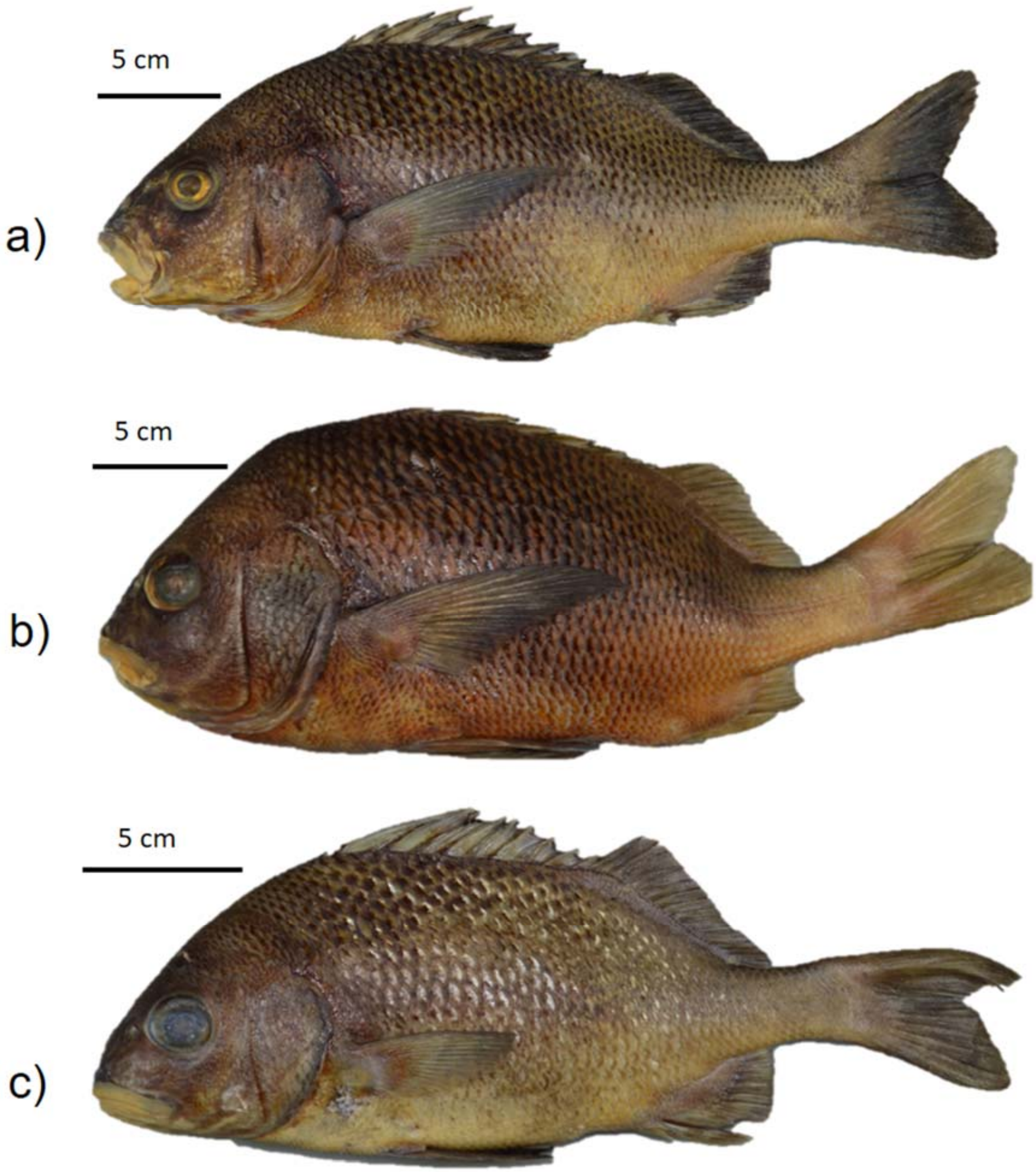


FIGURE 7. a) *Anisotremus* sp1, Holotype CPUM-9858 Las Pirámides, Isla Clarión, Archipelago de Revillagigedo, Manzanillo, Colima, México. b) *Anisotremus* sp2, Holotype CPUM-1349 Cerro Gallina, Isla Santa Cruz, Archipiélago de las Galápagos, Ecuador. c) *Anisotremus interruptus*, CPUM-9924, Playa La Privada, San Juan de Alima, Michoacán, México.

9. TABLES

TABLE 1. Sampled locations, number of specimens analyzed by location and geographic coordinates.

Location	Specimens	Coordinates	
Continent	**29**		
Carrizitos, México	1	18°16'52.48"N	103°22'42.41"W
Chucutitán, México	1	18°01'12.5"N	102°30'01.4"W
Pichilinguillo, México	1	18°11'26.3"N	103°3'54.91"W
Frente a Boca de Apiza, México	1	18°41'5.77"N	103°44'29.16"W
El Morro Chino, México	1	18°11'12.7"N	103°03'23.1"W
Playa La Privada, México	9	18°36'13.7"N	103°42'27.1"W
Lázaro Cárdenas, México	6	17°57'17.1"N	102°11'18.4"W
Boca de Apiza, México.	2	18°41'11.0"N	103°44'8.2"W
Pescadores Esterillo del Oeste, Costa Rica.	1	9°31'26.24"N	84°30'1.49"W
Bahía Panamá, Panamá	4	8°56'02.0"N	79°32'51.0"W
Isla San José, Panamá	2	8°15'05.0"N	79°06'10.8"W
Revillagigedo Archipelago, Mexico	**15**		
Bahía del muelle, Isla Clarión.	3	18°20'40.44"N	114°43'54.25"W
La Pared, Isla Clarión.	3	18°20'52.49"N	114°42'10.2"W
Frente al Muelle, Isla Clarión.	1	18°20'37.28"N	114°43'50.11"W
Barbas de Ben Laden, Isla Clarión.	2	18°20'23.61"N	114°45'16.43"W
Las Pirámides, Isla Clarión.	1	18°22´2.03"N	114°45´27.46"W
Cabo Paerce, Isla Socorro.	2	18°46'36.49"N	110°54'24.72"W
Playa blanca, Isla Socorro.	3	18°48'51.76"N	111°2'25.89"W
Galápagos-Cocos Islands, Ecuador	**28**		
Cerro Gallina, Isla Santa Cruz	14	0°43'40.2"S	90°39'54.32"W
Islote espejo, Isla Marchena	3	0°18'46.18"S	90°24'4.64"W
Poza Lobos, Isla Marchena	1	0°19'6.29"S	90°28'38.34"W
Bahía Darwin, Isla Genovesa	7	0°18'58.38"S	89°57'15.71"W
Punta Pitt, Isla San Cristóbal	1	0°42'44.08"S	89°14'51.91"W
Frente a Ulloa, Islas del Coco	1	5°33'5.04"S	87°2'11.77"W
Manuelita profundo, Islas del Coco	1	5°33'40.8"S	87°2'54.29"W

TABLE 2. Descriptive statistics of meristic and morphometric characters of the examined populations of *Anisotremus interruptus* from the Tropical Eastern Pacific. Abbreviation of the measurements are given in the section of method. SL and HL in millimeters. The values of the holotypes of the new species are also depicted.

Population	Revillagigedo			Continent		Galapagos		
Variables	Range	Mode	Type	Range	Mode	Range	Mode	Type
Meristic								
SLL	53-55	54	**53**	51-55	53	51-56	54	**53**
SDLL	7-10	8	**8**	6-8	6	7-9	8	**9**
SACP	22-27	24	**25**	21-25	22	23-28	27	**27**
CPUS	19-23	21	**19**	15-20	17	19-24	21	**21**
SBOBH	18-24	20	**18**	15-20	17	18-24	20	**22**
PecPelS	10-12	12	**12**	9-11	11	10-13	10	**11**
SDA	22-29	27	**27**	21-25	23	24-29	27	**28**
SODBH	9-12	10	**10**	9-10	9	9-12	10	**12**
SODRA	22-26	25	**26**	20-23	22	21-26	24	**24**
RD	16-18	17	**17**	18-19	18	16-18	17	**17**
RA	9-10	10	**9**	10-11	10	9-10	10	**10**
RP	17-19	18	**17**	17-19	18	17-19	18	**18**
RC	18-19	19	**19**	18-20	19	17-19	18	**18**
	Revillagigedo			**Continent**		**Galapagos**		

	Range Average SD	Type	Range Average SD	Range Average SD	Type
Morphometric					
HL	59.7-115.7(83.7/13.2)	**85.6**	38.8-83.7(62.6/13.0)	54.1-86.3(74.5/11.2)	**80.7**
ProD	2.29-2.50(2.50/.06)	**2.32**	2.23-3.28(2.58/0.27)	2.0-2.8(2.39/0.17)	**2.31**
ED	2.99-3.47(3.17/0.12)	**3.26**	2.44-3.76(2.87/0.26)	2.67-3.37(2.95/0.21)	**3.10**
PooD	2.82-3.44(3.11/0.16)	**3.22**	2.55-3.41(3.13/0.23)	3.04-3.49(3.3/0.12)	**3.40**
HH	0.67-0.76(0.71/0.02)	**0.72**	0.64-0.72(0.7/0.02)	0.65-0.73(0.7/0.02)	**0.65**
SL	213-383.3(292.9/40)	**304.1**	125.4-304(213/50.5)	184-284.3(254/37.3)	**283.72**
MHB	7.61-8.99(8.43/0.36)	**8.80**	6.06-8.77(8.15/0.59)	8-9.04(8.42/0.31)	**8.49**
DAIDAIV	2.19-2.48(2.32/0.07)	**2.39**	2-2.34(2.20/0.09)	2.20-2.40(2.27/0.05)	**2.25**
DPDPA	5.50-6.78(6.24/0.38)	**6.62**	5.36-6.53(6.03/0.29)	5.57-6.47(6.01/0.24)	**6.20**
DOAOD	1.64-1.81(1.75/0.05)	**1.79**	1.52-1.71(1.64/0.05)	1.68-1.88(1.75/0.05)	**1.70**
LD	1.64-1.80(1.72/0.05)	**1.79**	1.56-1.74(1.65/0.05)	1.61-1.84(1.75/0.06)	**1.78**
DEDUCP	10.5-12.7(11.6/0.75)	**10.70**	9.78-15.2(12.25/1.17)	9.94-12.8(11.22/1.0)	**12.83**
DLCPEA	7.31-10(8.65/0.85)	**8.87**	7.06-10.8(9.20/0.96)	7.43-18.74(8.14/0.39)	**7.80**
AL	6.11-7.58(6.78/0.45)	**6.85**	5.61-7.20(6.49/0.39)	6.33-7.98(6.81/0.46)	**7.09**
DAIAIV	2.84-3.31(3.06/0.14)	**2.96**	2.75-3.21(2.92/0.11)	2.91-3.62(3.18/0.19)	**3.01**
DMCPAIA	3.27-4.04(3.52/0.22)	**3.50**	3.01-4.04(3.54/0.23)	3.29-3.57(3.43/0.08)1	**3.52**
DSOA	1.33-1.44(1.38/0.03)	**1.36**	1.24-1.39(1.35/0.03)	1.32-1.44(1.39/0.03)	**1.33**
DSV	2.31-2.64(2.47/0.10)	**2.42**	2.16-2.62(2.45/0.11)	2.32-2.58(2.41/0.07)	**2.35**
DSOD	2.14-2.53(2.30/0.09)	**2.37**	2.04-2.36(2.23/0.09)	2.05-2.35(2.19/0.07)	**2.18**
LDS	1.37-1.44(1.41/0.02)	**1.43**	1.35-1.45(1.41/0.02)	1.36-1.48(1.41/0.02)	**1.40**
LOEDR	4.01-4.06(4.22/0.19)	**4.59**	3.72-4.24(3.92/0.13)	3.97-4.92(4.37/0.25)	**4.71**

TABLE 3. Eigenvalues and eigenvectors for the first three principal components (PC1, PC2 and PC3) of the 13 meristic and 21 morphometric variables that were included in the analysis for the three compareds populations of *Anisotremus interruptus* from the Tropical Eastern Pacific. Abbreviation of the measurements are given in method section.

	Eigenvalues	% of Variation
PC1	17.4	65.9
PC2	1.84	6.99
PC3	1.65	6.25
Eigenvectors		
SLL	0.55	-0.07
SDLL	**0.70**	**0.14**
SACP	**0.80**	**-0.42**
CPUS	**0.87**	**0.31**
SBOBH	**0.86**	**-0.21**
PecPelS	0.24	0.07
SDA	**0.89**	**-0.27**
SODBH	**0.69**	**0.21**
SODRA	**0.88**	**0.04**
RD	-0.59	0.17
RA	-0.18	0.07
RP	0.04	0.02
RC	-0.45	-0.01

	Eigenvalues	% of Variation
PC1	5.82	29.10
PC2	3.50	17.53
PC3	2.39	11.96
PC4	1.86	9.32
Eigenvectors		
MHB	0.57	0.19
DAIDAIV	**0.87**	**-0.13**
DPDPA	0.56	0.25
DOAOD	**0.90**	**-0.16**
LD	**0.79**	**0.27**
DEDUCP	-0.18	0.38
DLCPEA	**-0.18**	**0.60**
AL	0.51	0.23
DAIAIV	0.35	-0.29
DMCPAIA	**0.06**	**0.80**
DSOA	**0.43**	**-0.76**
DSV	**0.04**	**-0.64**
DSOD	0.42	-0.27
LDS	0.39	0.64
LOEDR	**0.68**	**0.18**
HL	0.56	-0.48
ProD	**-0.80**	**-0.01**
ED	0.20	-0.03

PooD	0.56	0.22

TABLE 4. Pairwise comparison between groups of *Anisotremus interruptus* from the Tropical Eastern Pacific, using the Hotelling test for meristic (above the diagonal) and morphometric characters (below the diagonal).

	REV	CON	GAL
REV	0	**5.98829E-11**	**0.00518538**
CON	**0.00023837**	0	**6.30081E-16**
GAL	**0.0305135**	**2.85981E-17**	0

TABLE 5. Wilks' Lambda values, significance (p) and tolerance for meristic and morphometric variables from the discriminant function analysis for populations of *Anisotremus interruptus* in the Tropical Eastern Pacific. Values of significant discriminant variables in bold,

Var. Meristics	Wilks' Lambda	Partial Lambda	F-remove (2,57)	p-level	Toler.	Toler. (R-Sqr.)
SDLL	0.158810	0.859854	5.134131	**0.008598**	0.779998	0.220002
SACP	0.155935	0.875710	4.470795	**0.015288**	0.742484	0.257516
CPUS	0.164230	0.831480	6.384266	**0.002988**	0.801777	0.198223
SBOBH	0.138791	0.983881	0.516051	0.599374	0.621234	0.378766
SDA	0.159634	0.855418	5.324113	**0.007305**	0.741610	0.258390
SODBH	0.151774	0.899716	3.511053	**0.035836**	0.711117	0.288883
SODRA	0.170170	0.802453	7.754623	**0.000975**	0.631826	0.368174

Var. Morphometrics	Wilks' Lambda	Partial Lambda	F-remove (2,57)	p-level	Toler.	Toler. (R-Sqr.)
DAIDAIV	0.163907	0.894326	2.126899	0.133943	0.225889	0.774111
DOAOD	0.148947	0.984149	0.289910	0.750061	0.156644	0.843356
LD	0.168852	0.868130	2.734212	0.078439	0.168672	0.831328
DLCPEA	0.154825	0.946786	1.011688	0.373708	0.600204	0.399796
DMCPAIA	0.151601	0.966920	0.615814	0.545794	0.479933	0.520068
DSOA	0.206402	0.710197	7.345098	**0.002113**	0.182997	0.817003
DSV	0.185388	0.790698	4.764683	**0.014595**	0.475740	0.524260
LOEDR	0.168622	0.869317	2.705922	0.080390	0.385176	0.614825
ProD	0.148968	0.984009	0.292517	0.748139	0.493766	0.506234