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Occurrence of *Sphoeroides rosenblatti* Bussing, 1996 (Teleostei: Tetraodontidae) along the coast of Guayas Province, Ecuador, and a comparison with sympatric *Sphoeroides annulatus* (Jenyns, 1842)

Ocurrencia de *Sphoeroides rosenblatti* Bussing, 1996 (Teleostei: Tetraodontidae) en la costa de la provincia de Guayas, Ecuador, y una comparación con *Sphoeroides annulatus* (Jenyns, 1842)

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Resumen.- Se recolectaron trece ejemplares juveniles de *Sphoeroides rosenblatti* Bussing, 1996 en un bosque pequeño y fuertemente impactado de mangle situado en Palmar, provincia de Guayas, Ecuador. Esta especie era conocida previamente solo de aguas costeras de Costa Rica y Panamá. Se contrastó la ocurrencia y morfología de *S. rosenblatti* con ejemplares de *Sphoeroides annulatus* (Jenyns, 1842) recolectados en la misma área. La presencia de *S. rosenblatti* no estuvo significativamente asociada con la temperatura del agua, salinidad, o morfología del río, mas hubo evidencia de segregación espacial entre *S. rosenblatti* y *S. annulatus*. También encontramos pequeñas pero significativas diferencias entre las características morfométricas de estas especies, que estuvieron en acuerdo con la descripción original basada en especimenes centroamericanos. Utilizando todas las características morfométricas, se realizó un análisis de función discriminante que produjo un vector capaz de clasificar correctamente a todos los ejemplares. Se reporta una ampliación importante de la distribución conocida de *S. rosenblatti* y se agrega una especie nueva a la ictiofauna de la costa continental del Ecuador.

Palabras clave: tamboril, manglar, morfometría, Pacífico tropical este, registro nuevo

Introduction

Sphoeroides rosenblatti Bussing, 1996 is a pufferfish species recently described from shallow brackish water estuaries amongst mangroves or at the mouths of rivers in Costa Rica and Panama (Walker & Bussing 1996). It is generally similar to *Sphoeroides annulatus* (Jenyns, 1842), a common and widespread pufferfish in the tropical eastern Pacific, but differs from it in color pattern (Fig. 1) and subtle morphometric characters. Very little has been published on *S. rosenblatti* since its original description (e.g., Duncan & Szelistowski 1998).

Thirteen juvenile specimens of *S. rosenblatti* were collected in late 2003 during a study of the fish community in a small, highly disturbed mangrove forest located near the town of Palmar (2º00.993'S, 80º44.392'W) in Guayas Province, Ecuador. This collection represents an important expansion of the known range of *S. rosenblatti*. In this paper, we describe the environmental conditions associated with the collection and compare them to those associated with the occurrence of juvenile *S. annulatus* collected in the same area. We also evaluate morphometric and meristic differences between these two species in the mangrove forest of Palmar.

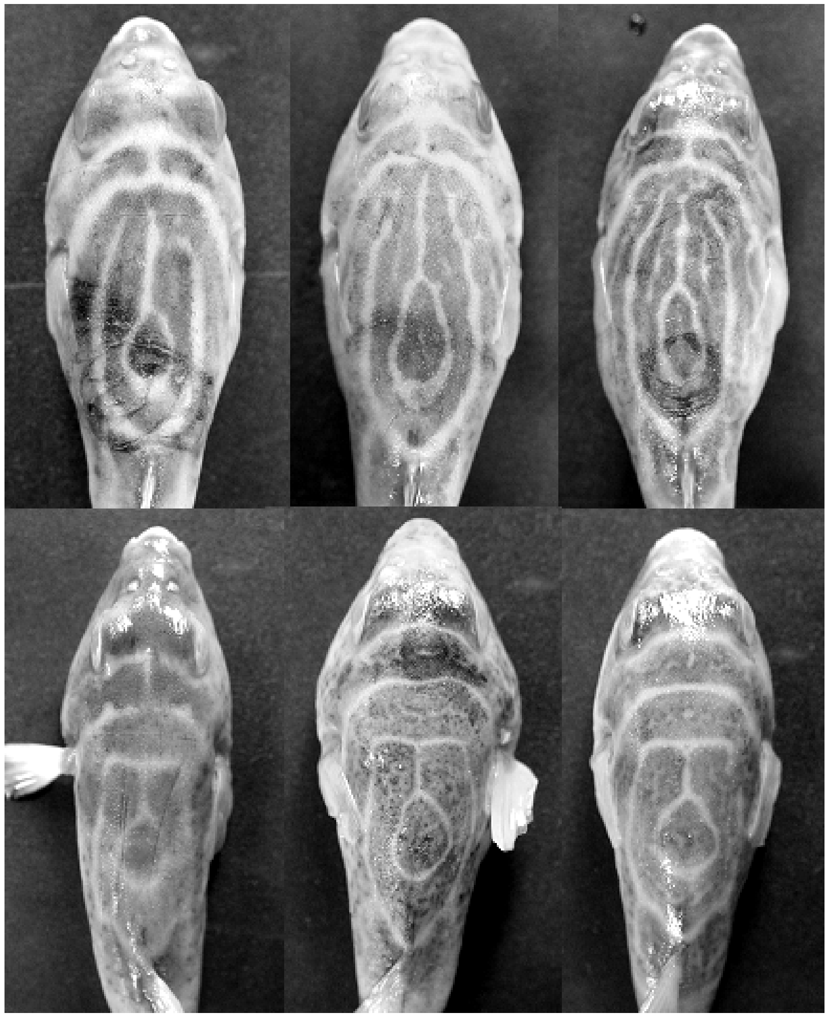

Figure 1

Variation of dorsal color patterns between *Sphoeroides* spp. included in this study. Top: *S. rosenblatti*, bottom: *S. annulatus*

Variación en el patrón dorsal de pigmentación entre *Sphoeroides* spp. incluidas en este estudio. Arriba: *S. rosenblatti*, abajo: *S. annulatus*

Methods

Palmar is a small coastal town located between Salinas and Manglaralto that has approximately 4300 inhabitants, a large proportion of whom are dedicated to fishing (Solís-Coello & Mendívez 1999). The Palmar mangrove forest is a small, isolated patch of mangroves, approximately 33.5 ha in size. The forest was much larger historically, and as recently as 25 years ago it may have been larger by an order of magnitude. Most of the original mangroves within the wetland have been cut for the construction of shrimp farms. The aquatic habitat consists of a main channel connecting the ocean to two sub-channels that flank the major stand of mangrove and shrimp farms (Fig. 2a). We sampled the main channel and the two sub-channels, henceforth identified as channels 1 and 2. We also sampled the Javita River (2º02.088'S, 80º44.255'W), a small coastal river lacking mangroves located approximately 2 km from the Palmar mangrove forest, as a contrasting habitat. Javita River is a shallow, turbid river under strong tidal influence, with a sandy/muddy bottom (Fig. 2b). Each of the primary channels of the mangrove forest and the Javita River was sampled at three different sites located within a few hundred meters of one another. Sampling was carried out over a two-week period in late October to early November, 2003 (henceforth referred to as Nov/03) and late March to early April, 2004 (henceforth referred to as Apr/04). Fish were collected with a 6 m beach seine that had 6 mm mesh size, fixed in 10% formalin, and stored in 50% isopropyl alcohol.

Several environmental variables were measured, including water temperature, salinity and stream morphology. Measures of stream width were taken at three points (beginning, middle, end) along the stream segment that was seined. Depth was taken at three points at each place that width was measured. Thus we had three measures of width and nine measures of depth for each sampling site. These measures were averaged to obtain a single measure per site for statistical analyses. Logistic regression (Floyd 2001) was employed to determine if the occurrence of *S. annulatus* and/or *S. rosenblatti* was associated with any of the environmental variables measured. Occurrence of each species was coded as 1 for presence and 0 for absence and regressed on temperature, salinity, average width and average depth. Odds ratios (reflecting the increase of the odds of collection per unit of the environmental variables measured) are presented for variables that were significant or marginally non-significant.

Twelve linear measures (standard length [SL], head length [HL], head width [HW], fleshy orbit diameter [EYE], snout length [SN], postorbital length [POL], dorsal-fin length [DL], anal-fin length [AL], pectoral-fin length [PL], caudal-fin length [CL], predorsal length [PD], and preanal length [PA]) and three counts (dorsal, anal, and pectoral fin rays) were taken from all *S. rosenblatti* collected and 27 specimens of *S. annulatus* of comparable size. The traits measured are those described in Walker & Bussing (1996) minus the bony interorbital, which was not included because of the large error we found associated with measuring this trait in small specimens. We collected many more *S. annulatus* (133) than *S. rosenblatti* (13), but most *S. annulatus* were much smaller than the smallest *S. rosenblatti*, and thus not included in the morphometric analysis. These small *S. annulatus* were identified exclusively based on their color pattern. Measurements were taken to hundredths of a millimeter under a dissecting microscope (with the exception of SL and HL) with Fowler Ultra-Cal Mark III digital calipers. All measures were $\log_{10}$ transformed. Statistical analyses were carried out with SPSS 11.0 (SPSS, Inc. 2001) and BIOMstat 3.30 (Applied Biostatistics, Inc. 2002).

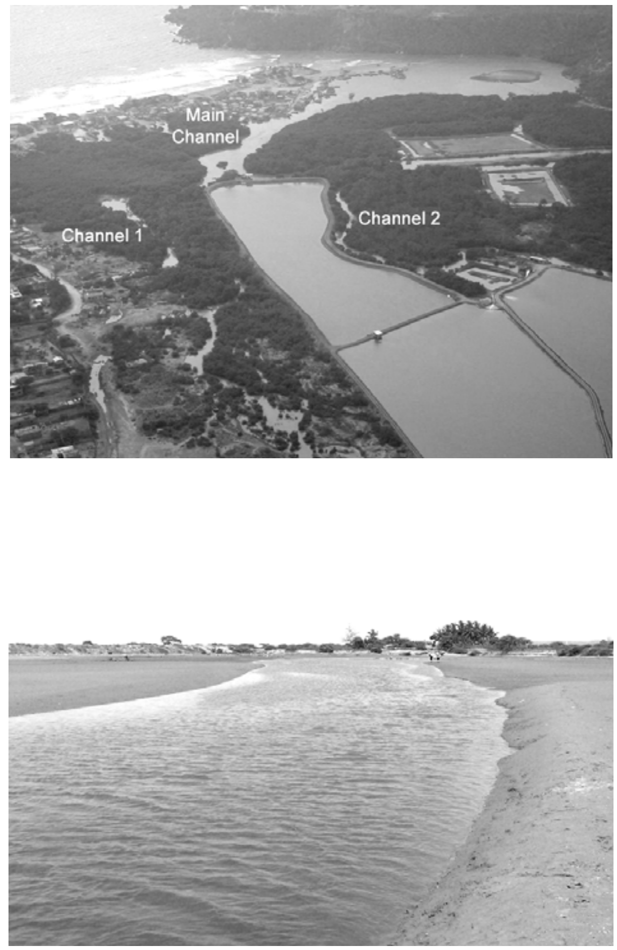

Figure 2

a. Aerial view of the Palmar mangrove forest. Part of the town of Palmar is visible in the background, as is the Pacific Ocean. The rectangular ponds are artificial shrimp ponds. b. The Javita River

a. Vista aérea del bosque de manglar de Palmar. Parte del pueblo de Palmar está visible en el fondo, como también lo está el Océano Pacífico. Las pozas rectangulares son piscinas camaroneras. b. El río Javita

Results

Both the Palmar mangrove forest and the Javita River had generally hypersaline, warm water (Table 1). Mean salinities did not differ significantly between seasons (ANOVA, df = 1.6, F= 0.351, *P*=0.575). The salinity in both areas typically ranged from full strength ocean salinity (35 psu) to hypersaline, with two of the localities registering average salinities over 50 psu in Apr/04. The average water temperatures for the mangrove forest and the Javita River were 27.3°C and 25.4ºC respectively in Nov/03. In Apr/04, the average water temperature was significantly greater, rising to 30.8°C and 31.2°C for the mangrove forest and Javita River respectively (ANOVA, df = 1,6, F= 24.16, *P*=0.003). This is in accordance with seasonal changes in the temperature of the ocean along the continental coast of Ecuador due to shifts in the warm El Niño and cold Humboldt ocean currents. Both average channel width and depth were greater at most sites in Apr/04 than in Nov/03, which may be related to increases in precipitation along the coast of Ecuador during that time.

The occurrence and size distribution of pufferfish in Palmar exhibited relatively strong seasonal patterns. The mean SL (± standard error) of the *S. rosenblatti* collected was 56.30 ± 4.81 mm and the range was 26.30 to 91.19 mm, whereas the mean SL of *S. annulatus* was 22.43 ± 1.04 mm and ranged from 9.32 to 61.66 mm (Fig. 3). For specimens of *S. annulatus* included in the morphometric analysis (n=27), the mean SL was 41.09 ± 2.10 mm and the range was 25.94 to 61.66 mm. *Sphoeroides rosenblatti* exhibited a limited spatial and temporal distribution in the mangrove forest, with all 13 specimens collected during Nov/03 in the mangrove forest (Table 1). All specimens of *S. annulatus* over 30 mm SL were also collected in Nov/03. Smaller specimens were present during this period and the size range of the specimens collected was 9.3 to 63.7 mm SL. *Sphoeroides annulatus* was collected from all areas sampled with the largest numbers of specimens (n=16) collected in Javita River and the smallest (n=2) in channel two of the mangrove forest (Table 1). This is in stark contrast to the collection of *S. rosenblatti,* in which most specimens were collected in channel two of the mangrove forest and none were collected in the Javita River. In Apr/04, 92 small *S. annulatus* ranging in size from 11.9 to 28.1 mm SL were collected primarily in the Main Channel of the mangrove forest and the Javita River.

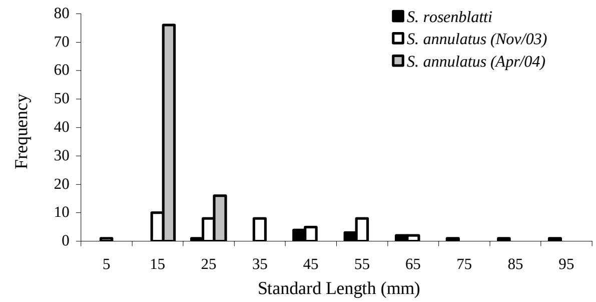

Figure 3

Size (SL) distribution of *S. rosenblatti* and *S. annulatus*. Specimens of *S. annulatus* collected in Nov/03 and Apr/04 are plotted separately

Distribución de tamaño (SL) de *S. rosenblatti* y *S. annulatus.* Especímenes de *S. annulatus* recolectados en nov/03 y abr/04 están graficados por separado

The association between the occurrences of *S. rosenblatti* and *S. annulatus*, and the environmental variables was weak. None of the environmental variables were significantly associated with the occurrence of *S. rosenblatti* when data for both Nov/03 and Apr/04 were included (logistic regression, *P*>0.206 for all variables). Limiting the analysis to Nov/03 (the season in which *S. rosenblatti* were collected) resulted in marginal probability values for water temperature (logistic regression, odds ratio=1.445, *P*=0.088) and channel depth (logistic regression, odds ratio=1.074, *P*=0.0847) with the probability of presence tending to increase as both water temperature and channel depth increased. The weighted averages (± standard error) of the temperature and salinity (with the site data weighted by the number of specimens collected per site) at which we collected *S. rosenblatti* were 28.16 ± 0.93ºC (range=25.0-35.5ºC) and 39.82 ± 1.14 psu (range=33.4-45.3 psu) respectively. Channel width was significantly associated with the occurrence of *S. annulatus* when data for both Nov/03 and Apr/04 and all specimens collected were included (logistic regression, odds ratio=1.124, *P*=0.0282), with the odds of *S. annulatus* being present significantly increasing as channel width increased. None of the other variables had a significant effect (*P*>0.262). Restricting the analysis to Nov/03 and including only specimens > 26 mm SL to minimize discrepancies with *S. rosenblatti* (the smallest *S. rosenblatti* collected was 26.30 mm SL), resulted in channel width becoming marginally non-significant (logistic regression, odds ratio=1.215, *P*=0.108).

Table 1

Averages (± standard error) of the environmental and stream morphology variables for channels sampled and the number of *S. rosenblatti* and *S. annulatus* collected per location

Promedios (± error estándar) de las variables ambientales y morfológicas para los canales muestreados, con el número de *S. rosenblatti* y *S. annulatus* recolectados por sitio

Location	Temp (ºC)	Salinity (psu)	Width (m)	Depth (cm)	*S. rosenblatti*	*S. annulatus*
Oct/Nov 2003						
Channel 1	27.20±1.16	43.73±1.94	11.79±2.48	48.58±10.66	0	11
Channel 2	27.50±1.32	41.27±2.11	10.25±2.98	53.15±7.97	11	2
Main Channel	27.18±2.79	37.93±2.42	14.74±3.38	45.50±3.15	2	13
Javita River	25.35±0.74	42.35±0.54	20.72±6.15	33.81±4.43	0	16
Mar/Apr 2004						
Channel 1	28.92±0.20	40.18±2.91	15.20±3.50	68.74±18.48	0	0
Channel 2	31.85±0.47	50.03±0.88	12.08±1.67	50.61±7.56	0	1
Main Channel	31.60±0.12	33.37±2.46	27.40±5.77	51.66±4.96	0	58
Javita River	31.23±0.33	52.67±1.45	29.26±2.79	51.37±1.65	0	32
Total N					13	133

Values of the three meristic traits counted for *S. rosenblatti* collected in Palmar were similar to those reported by Walker & Bussing (1996) for Central American specimens. Unfortunately, the counts were not useful for distinguishing between species occurring sympatrically in the mangrove forest of Palmar. The number of dorsal fin rays was always eight, the number of anal fin rays was seven except in a single specimen of *S. annulatus,* in which it was eight, and the number of pectoral fin rays was 16 with the exception of one specimen of *S. rosenblatti* that had 15 pectoral fin rays and four specimens of *S. annulatus*, three with 15 rays and one with 17 rays.

Of the twelve morphometric measurements, only snout length (Fig. 4a) and head length differed significantly (ANCOVA, covariate=SL, df=1,26, HL: F=17.167, P<0.0002; SN: F=82.166, P=6.218X10^{-11}) between species after a sequential Dunn-Sidak correction for multiple tests was performed (Sokal & Rohlf 1995). The difference in HL is probably driven by the difference in SN. Snout length was one of the key traits described by Walker & Bussing (1996) as differentiating these two species. Walker & Bussing (1996) also indicated that POL differed, but it did not differ between species in our samples (ANCOVA, covariate=SL, df=1.26, F=0.717, P=0.403). This may be due to regional differences in this trait in one or both species. Alternatively, the difference may result from divergent allometric growth trajectories between species since most of the specimens collected in this study are much smaller than those described in Walker & Bussing (1996). We evaluated all linear traits for growth allometry using SL as a proxy for size and considered growth significantly allometric if the confidence intervals of the slope of the log-transformed trait regressed on log SL did not overlap with one. Postorbital length grew isometrically in both species over the size range examined, thus the growth trajectory for this trait must shift in larger specimens than those examined here, in one or both species, if the discrepancy between our results and those of Walker & Bussing (1996) is due to size. Five of the other traits measured exhibited significant negative allometric growth in *S. rosenblatti* (HL, EYE, PL, CL, PD) and four of these also exhibited negative allometric growth in *S. annulatus* (Table 2). The difference was PL, which was negatively allometric in *S. rosenblatti* but isometric in *S. annulatus*. Interestingly, SN, which differed significantly between species, was the only trait that exhibited positive allometric growth. It did so in both species and the values of the allometric coefficients were quite similar (1.133 ± 0.052 and 1.163 ± 0.028 in *S. rosenblatti* and *S. annulatus* respectively).

A discriminant function analysis including all the morphometric variables ($\log_{10}$ transformed) yielded a single function that correctly assigned all specimens (Fig. 4b):

D = -58.74 + 96.85(SL) - 46.24(HL) + 11.39(HW) - 1.10(EYE) - 44.10(SN) + 14.62(POL) - 6.42(DL) - 5.43(AL) -5.03(PL) - 6.23(CL) + 16.57(PD) - 22.33(PA)

The posterior probabilities of correct assignment were generally high, averaging 0.987. Only two specimens, one *S. rosenblatti* (0.751) and one *S. annulatus* (0.780) had posterior assignment probabilities of less than 0.98. Therefore, morphometric differences between these two species appear substantial even at small sizes. Given the allometry documented, however, the discriminant function is probably only valid over the size range examined here.

Table 2

Allometric coefficients (± standard error), 95% confidence intervals and coefficients of determination (r^2) for morphometric traits measured (see text for trait definitions). The "Allom." column indicates the type of growth allometry exhibited, with "-" indicating negative allometry, "+" indicating positive allometry, and "iso" indicating isometry

Coeficientes alométricos (± error estándar), intervalos de confianza de 95%, y coeficientes de determinación (r^2) para los caracteres morfométricos medidos (vea el texto para definiciones). La columna "Allom." indica el tipo de crecimiento alométrico, con "-" indicando alometría negativa, "+" indicando alometría positiva, e "iso" indicando isometría

	Sphoeroides rosenblatti				*Sphoeroides annulatus*			
Traits	Allom. Coeff.	95% CI	r^2	Allom.	Allom. Coeff.	95% CI	r^2	Allom.
HL	0.891±0.019	0.849-0.934	0.995	-	0.900±0.014	0.871-0.929	0.994	-
HW	0.989±0.037	0.908-1.069	0.985	iso	0.912±0.043	0.824-1.000	0.948	iso
EYE	0.570±0.048	0.463-0.676	0.927	-	0.721±0.051	0.617-0.825	0.891	-
SNT	1.133±0.052	1.018-1.248	0.977	+	1.163±0.028	1.106-1.220	0.986	+
POL	1.031±0.027	0.972-1.090	0.993	iso	1.055±0.043	0.967-1.143	0.961	iso
DL	1.002±0.036	0.923-1.081	0.986	iso	1.048±0.065	0.914-1.183	0.912	iso
AL	1.001±0.034	0.925-1.077	0.987	iso	0.965±0.069	0.824-1.107	0.887	iso
PCTL	0.880±0.047	0.777-0.984	0.970	-	0.934±0.047	0.837-1.032	0.939	iso
CFL	0.858±0.035	0.781-0.935	0.982	-	0.929±0.034	0.859-0.998	0.969	-
PDL	0.959±0.014	0.928-0.990	0.998	-	0.948±0.020	0.908-0.988	0.989	-
PAL	0.994±0.013	0.966-1.022	0.998	iso	0.980±0.015	0.949-1.011	0.994	iso

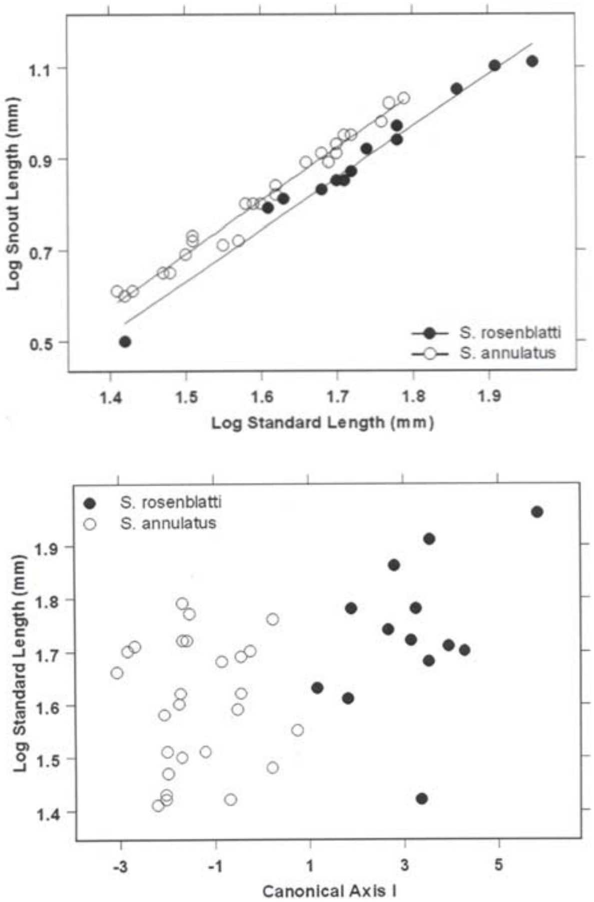

Figure 4

a. Log_{10} snout length (SN) plotted against log_{10} SL for *S. rosenblatti* and *S. annulatus*. b. Discriminant function scores (Canonical Axis I) for both species plotted against log_{10} SL

a. Log_{10} longitud del hocico (SN) graficado contra log_{10} SL (longitud estándar) para *S. rosenblatti* y *S. annulatus*. Valores de la función discriminante (eje canónico I) para ambas especies graficados contra log_{10} SL

Discussion

The occurrence of *S. rosenblatti* in Ecuador represents an important expansion of its known range. The small size of the mangrove forest in Palmar suggests that *S. rosenblatti* occurs in other parts of Ecuador and the Pacific coast of South America and has not been reported previously because it was only recently described. However, given that all specimens were collected in a relatively restricted area, and that the area is highly disturbed and harbors numerous shrimp farms, we cannot rule out the possibility that the specimens we collected represent a recent accidental introduction.

The number of specimens of *S. rosenblatti* collected was small for the sampling effort and also relative to the number of *S. annulatus* collected, suggesting it may not be very common in the area. In addition, *S. rosenblatti* was only collected in Nov/03 and in a relatively small portion of the mangrove forest, further suggesting that it may occur only in areas exhibiting a restricted set of environmental conditions. Unfortunately, the environmental data available were not intended to document microhabitat use and the number of *S. rosenblatti* collected was small. Consequently, our ability to detect associations between the environmental variables measured and the occurrence of *S. rosenblatti* was limited. The negative results of the logistic regression carried out, therefore, do not indicate that these variables are unimportant for the occurrence of *S. rosenblatti*, but simply that their influence is small enough that we could not detect it with our data. The inverse abundance patterns for *S. rosenblatti* and *S. annulatus* in Nov/03 (when both were present) is suggestive of segregation in habitat use between species. Eleven of the thirteen specimens of *S. rosenblatti* were collected in Channel 2 of the mangrove forest, which on average had the highest water temperature, and was the narrowest, deepest channel. On the other hand, the largest number of *S. annulatus* were collected in Javita River where no *S. rosenblatti* were collected, moderate numbers of *S. annulatus* were collected in Channel 1 and the Main Channel of the mangrove forest, where *S. rosenblatti* was sparse or absent, and the smallest number of *S. annulatus* were collected in Channel 2, where *S. rosenblatti* was most abundant (Table 1). However, research specifically designed to assess how these two species segregate in their use of the mangrove forest is needed to validate the pattern observed here and we strongly recommend that such research be carried out.

Superficially, *S. rosenblatti* and *S. annulatus* were quite similar morphologically at the size at which they occurred in the mangrove forest of Palmar. This similarity was also reflected in their growth trajectories. Of 11 traits in which growth allometry was assessed, 10 exhibited the same type of growth: four exhibited negative allometry, one exhibited positive allometry, and five exhibited isometry. Pectoral fin length exhibited negative allometry in *S. rosenblatti* and isometry in *S. annulatus*. Snout length, the trait that most clearly separated the two species, was the only trait that exhibited positive allometry. Given the small sizes of the specimens measured, the difference in SN between species seems to originate quite early in development. The divergent growth trajectory of SN relative to the other traits and the early divergence observed between species suggests that this trait may be of particular importance from a functional perspective in both species and perhaps subject to divergent selection between species. If adaptive, differences in SN are probably associated with differences in feeding habits, which is generally one of the most important ways in which closely related species segregate ecologically (Schluter 2000). Despite their superficial similarity, the discriminant function analysis including all traits correctly classified all specimens. The high posterior probabilities of the analysis suggest that there were substantial morphometric differences between species despite the small size of the specimens examined.

In summary, we report the occurrence of a new species of pufferfish, *S. rosenblatti*, for the continental coast of Ecuador, which appears closely related to, and occurs, sympatrically with, the common pufferfish species *S. annulatus* in mangrove forests. The two species differ morphologically (although the differences are subtle among juvenile fish), and variation in the most divergent trait that we measured, snout length, is generally associated with divergence in feeding in other groups, hinting at a potential mechanism for ecological segregation. We also found evidence of an inverse abundance pattern for these two species suggesting the potential for spatial segregation as well. More research is needed, however, on both these potential mechanisms of ecological segregation. Another direction for future research is the extent to which these species differ genetically, which would help shed light on their evolutionary history. Finally, more work is also needed to document the actual distribution of *S. rosenblatti* along the Pacific coast of South America.

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