Body Shape Variation and Population Genetic Structure of *Rhoadsia altipinna* (Characidae: Rhoadsiinae) in Southwestern Ecuador

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The genus *Rhoadsia* is endemic to western Ecuador and northern Peru and includes two described species that differ in body form, size, and the elevations at which they occur. Unfortunately, there is uncertainty about the number of species that should be recognized in the genus and the causes of the morphological variation documented within and between species. We take advantage of a survey of the fish fauna of the Santa Rosa River in southwestern Ecuador that yielded large numbers of *Rhoadsia altipinna*, to expand knowledge of the ecological, morphological, and genetic variation of this species. Specimens were collected at five sites at elevations between 31 and 613 m above sea level, and each site was sampled in December 2012 and July 2013. *Rhoadsia altipinna* was the second most abundant species in the Santa Rosa River, was one of only three species collected at all elevations, and was more common in pool mesohabitats than riffle mesohabitats. Geometric morphometric analysis of body shape variation indicated strong sexual dimorphism and allometry, with body depth increasing substantially with size. More interestingly, body depth declined with elevation in the Santa Rosa River. This intraspecific pattern of variation mirrored the interspecific divergence reported between the two recognized species. Lower elevation *R. altipinna* are known to be deeper bodied than high elevation *R. minor*. However, specimens of *R. minor* from the paratype series measured for comparison were still more streamlined than all Santa Rosa *R. altipinna* examined, including those collected at the highest elevations and juveniles. Although body shape differed significantly between Santa Rosa River *R. altipinna* and other populations from southwestern Ecuador, the geographic differences appeared small relative to variation attributable to sexual dimorphism and allometry. Finally, sequencing of a fragment of the cytochrome oxidase I gene for samples from the Santa Rosa River and two samples from the neighboring Guayas River drainage failed to recover a monophyletic Santa Rosa lineage. Although haplotype frequencies differed significantly between the Santa Rosa and Guayas River samples, the lack of monophyly and similarity among the haplotypes make the genetic data more consistent with divergence of geographically isolated populations within a single species than with interspecific divergence. Further analysis of morphological and genetic variation throughout the range of the genus will help elucidate its ecological and evolutionary dynamics.

El género *Rhoadsia* es endémico del oeste del Ecuador y noroeste del Perú e incluye a dos especies reconocidas, que se diferencian en la forma y tamaño del cuerpo y su distribución altitudinal. Existe incertidumbre sobre el número de especies que deben ser reconocidas como parte de este género y sobre las causas de la variación morfológica entre poblaciones de una misma especie y entre las dos especies. Durante un estudio sobre la composición de especies de peces del río Santa Rosa, en el suroeste del Ecuador, colectamos un gran número de especímenes de *Rhoadsia altipinna* en cinco sitios localizados entre los 31 y 613 m de altitud, lo cual aprovechamos para ampliar el conocimiento sobre la variación ecológica, morfológica y genética de esta especie. El trabajo de campo fue realizado en diciembre de 2012 y julio de 2013. En el río Santa Rosa, *Rhoadsia altipinna* fue la segunda especie más abundante y una de las tres especies colectadas en las cinco estaciones muestreadas en diferentes altitudes. Fue más común en hábitats de aguas quietas que en los de aguas rápidas. El análisis de morfometría geométrica de la variación de la forma del cuerpo indica un fuerte dimorfismo sexual y alometría con relación a la profundidad del cuerpo, siendo mucho mayor en los adultos. Se destaca el hecho de que la profundidad del cuerpo se redujo a medida que se incrementaba la altitud del sitio de muestreo. Este patrón de variación intraespecifica es similar al patrón de divergencia interespecifica conocida para las dos especies del género *Rhoadsia*. *Rhoadsia altipinna*, que normalmente ha sido reportada para sitios de baja altitud, tiene un cuerpo más profundo que *R. minor*, una especie reportada en sitios de mayor altitud. Sin embargo, los especímenes de *R. altipinna* colectados en los sitios de mayor altitud en el río Santa Rosa, incluyendo los juveniles, presentaron cuerpos con mayor profundidad que una muestra de la serie de paratipos de *R. minor* examinada. También se comparó la forma del cuerpo de la población de *R. altipinna* del río Santa Rosa con la de otras poblaciones de ríos del suroeste del Ecuador y las diferencias son pequeñas con relación a la variación atribuible al dimorfismo sexual y a la alometría. Finalmente, la secuenciación de un fragmento del gen citrocromo oxidasa I de una muestra del río Santa Rosa y de dos muestras de la cercana cuenca del río Guayas, no evidenció un linaje monofilético para el río Santa Rosa. Aunque las frecuencias de haplotipos difirieron significativamente entre las muestras, los datos genéticos fueron más consistentes con la divergencia entre poblaciones geográficamente aisladas dentro de la misma especie que con la divergencia interespecifica por la falta de linajes monofileticos y la similitud entre haplotipos. Un análisis más detallado de la variación morfológica y genética en toda el área de distribución del género ayudaría a aclarar su dinámica ecológica y evolutiva.

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THE Rhoadsiinae are a small subfamily within the Characidae (Teleostei: Characiformes) that includes six species in three genera, *Rhoadsia*, *Carlana*, and *Parastrema,* occurring in Central America and the Pacific coast of Colombia, Ecuador, and Peru (Cardoso, 2003). The geographically isolated genus *Nematocharax* in Brazil has also been reported as possibly related (Weitzman et al., 1986) and is sometimes included in the subfamily (e.g., Froese and Pauly, 2014). Fishes in this group are small, reaching a maximum size of 170 mm standard length (Jiménez Prado et al., 2015), have rhomboidal bodies, and often exhibit extreme sexual dimorphism (Fig. 1A). The morphological distinctiveness and low species diversity of the subfamily makes it a group of interest from evolutionary and conservation perspectives. Moreover, many areas within the range of the subfamily are under threat from anthropomorphic pressures. One such area is western Ecuador, which harbors the genus *Rhoadsia*. Isolated for millions of years from the major South American river systems by the rise of the Andes (Hoorn et al., 2010), the region forms part of one of the most important biodiversity hotspots on Earth (Myers et al., 2000) and harbors a unique fish fauna with high levels of endemism (Eigenmann, 1921; Albert et al., 2011; Barriga, 2012). Unfortunately, the region is experiencing elevated rates of habitat loss and degradation related to human actions (Aguirre et al., 2013).

Two species of *Rhoadsia* occur in western Ecuador. *Rhoadsia altipinna* is found at low elevations in southwestern Ecuador (Barriga, 2012) and northern Peru (Ortega et al., 2011), and *Rhoadsia minor* occurs at high elevations in river systems in northwestern Ecuador (Barriga, 2012). The two species are similar morphologically and can be distinguished by differences in size, body depth, and other minor features, with *R. altipinna* being larger and deeper bodied than *R. minor* (Eigenmann and Henn, 1914). Although the two species are presently considered valid, there appears to be substantial morphological overlap between them including in diagnostic characters (Böhlke, 1958), such that the status of *R. minor* as a valid species has been questioned (Géry, 1977). Additionally, in the most recent list of freshwater fish species for Ecuador, Barriga (2012) lists *R. altipinna* as restricted to the Guayas River drainage and lists an undescribed species of *Rhoadsia* as occurring in the Catamayo drainage system in southwestern Ecuador. Thus, there is significant taxonomic uncertainty, and it is presently unclear how many species of *Rhoadsia* exist and what their distribution is. Furthermore, very little has been published on *Rhoadsia* spp., beyond their occurrence in different rivers and distinguishing morphological characteristics (e.g., Eigenmann and Henn, 1914; Böhlke, 1958; Glodek, 1978; but see Loh et al., 2014).

In this paper, we take advantage of a survey of the fish fauna of the Santa Rosa River in southwestern Ecuador conducted in 2012–2013 that yielded large numbers of specimens of *Rhoadsia*, to expand knowledge of the morphological, ecological, and genetic variation that this species exhibits in the region and gain insight into the causes of this variation. Specifically, we address the following issues: 1) We examine the abundance of *Rhoadsia* in the Santa Rosa River along an altitudinal gradient between 31 and 613 m in pool and riffle mesohabitats to gain a better understanding of the ecological distribution of the species in streams of southwestern Ecuador. 2) We use geometric morphometric methods to quantify body shape variation among *Rhoadsia* based on size, sex, and collection site. Because the two described species of *Rhoadsia* differ primarily in body shape and have been described from different elevations, we specifically examine body shape variation of *Rhoadsia* collected at different altitudes in the Santa Rosa River to examine whether intraspecific variation associated with elevation is concordant with the interspecific divergence documented between *R. altipinna* and *R. minor.* If so, we would expect body depth to decrease with elevation in the Santa Rosa River *Rhoadsia*. We also compare body shape variation of Santa Rosa River *Rhoadsia* with museum samples from southwestern Ecuador to examine the extent of morphological divergence between these drainages, and to specimens of the paratype series of *R. minor* from the Esmeraldas River drainage in northwestern Ecuador to put intraspecific variation in body shape of *Rhoadsia altipinna* in the context of the difference between species. 3) Finally, we sequence a fragment of the DNA barcoding gene cytochrome oxidase I (COI) to examine the magnitude of genetic divergence between Santa Rosa River *Rhoadsia* and two samples from the Guayas River drainage. This provides another metric of divergence between Santa Rosa River *Rhoadsia* and other populations from southwestern Ecuador to help gauge whether the two forms represent two distinct species or geographically isolated (and perhaps morphologically divergent) forms within the same species.

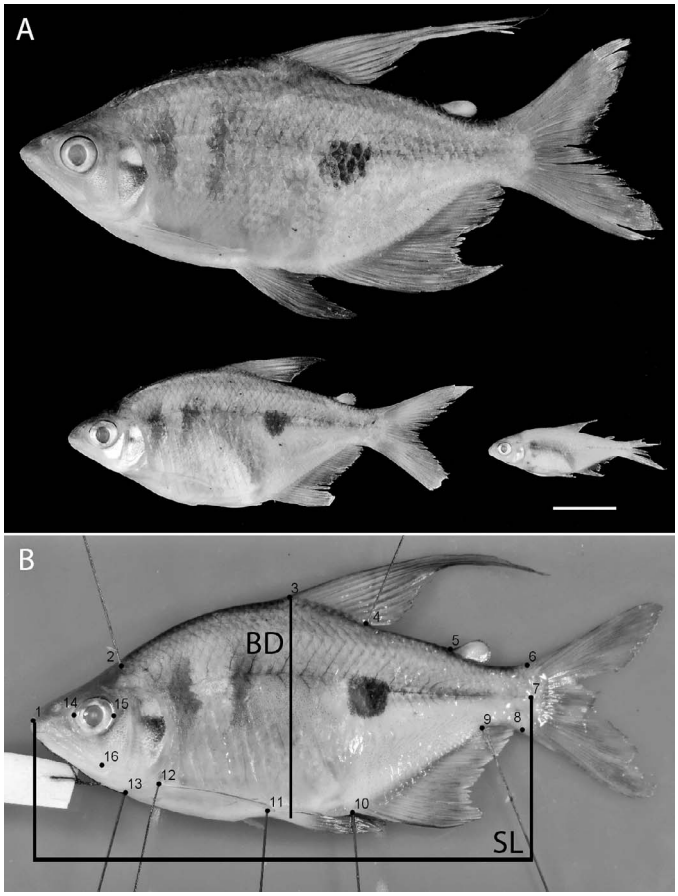

Fig. 1. (A) Preserved adult male (top), female (lower left), and juvenile (lower right) *Rhoadsia altipinna* from the Santa Rosa River (31 m site). Scale bar is 10 mm. (B) Digital image of male specimen of *Rhoadsia* showing the 16 landmarks and two linear measures used in the study. Insect pins were used to mark some landmarks.

MATERIALS AND METHODS

Study area.—The Santa Rosa River is a small high gradient river that runs between the Andes and Pacific Ocean in the province of El Oro, southwestern Ecuador (Aguirre et al., 2014). It originates at approximately 2,200 m of elevation in an extension of the Andes Mountains and runs 41 km before merging with the Buena Vista River and draining into the Gulf of Guayaquil. The entire Santa Rosa River drainage basin (including the Buena Vista River and other tributaries) covers an area of approximately 756 km^2. The mean temperature and precipitation at lower elevations (130 m) are approximately 24.7°C and 773 mm/year and at higher elevations (1,150 m) are 21.7°C and 1,029 mm/year, respectively. At 89 m of elevation (Station 2 in this study), mean water flow is approximately 1.55 m^3/s (Conchas Egas, 2009). The drainage basin is heavily impacted by human activities and harbors many human settlements including the city of Santa Rosa (population size: ~49,000), which is located close to the mouth of the river and uses it as its primary source of freshwater. Farming, cattle, and gold mining are important contributors to habitat transformation and degradation in the area. As of 2005, approximately 1,143 ha of forest were reported to remain above 600 m of elevation in the basin (Conchas Egas, 2009).

Field collections.—Fishes were collected at five sites in the Santa Rosa River at elevations of 31 (3°30′06.0″S, 29°57′25″W), 86 (3°33′31.4″S, 79°56′45.5″W), 189 (3°34′53.0″S, 79°54′44.8″W), 382 (3°35′23.9″S, 79°50′33.1″W), and 613 m (3°35′9.3″S, 79°48′32.6″W) above sea level (Figs. 2, 3). To take temporal variability into consideration, each site was sampled twice, once on 7–9 December 2012, immediately preceding the onset of the rainy season in western Ecuador, and once on 28–30 July 2013, a few months after its conclusion. To account for habitat variability within the river, riffle mesohabitats that form where the river constricts or large rocks and boulders have accumulated and pool mesohabitats that form where the river width expands or at the sides of the river, were sampled at each site (Fig. 3). Water velocity was measured using a Globalwater flowmeter. Although water velocity varied among stations within mesohabitat types, it was always greater at riffle than in pool stations at a particular site. The average water velocity was 0.75 meters per second (m/s) at riffle sites (range: 0.40 to 1.66) and 0.20 m/s at pool sites (range: 0.10 to 0.41 m/s). The sites were selected after visual inspection of potential areas close to elevations of 0, 100, 200, 400, and 600 m above sea level and were chosen because they had adjacent pool and riffle mesohabitat and were accessible for sampling. The maximum elevation of ~600 m was used because sites higher in the river were inaccessible from roads and the river is not navigable, making continuous sampling along an altitudinal gradient very difficult. Fish diversity is also likely low at high elevations in this system because of the high gradient that the river exhibits.

Specimens were collected with a Smith-Root LR-24 electrofishing backpack using a standard pulse, voltage varying between 200 and 450 volts, frequency varying between 30 and 60 hz, and duty cycle varying between 25 and 45%. Settings were altered as needed so that catch efficiency was relatively homogeneous given differences in conductivity and water flow among sites. Stunned specimens were collected with seines and dip nets and sampling continued until representative fish samples were obtained and new species stopped appearing. Sampling time was recorded to standardize catch by sampling effort.

Morphometric analysis.—Geometric morphometric methods were used to quantify body shape variation and generate visual depictions of body shape differences (Zelditch et al., 2012). Specimens were straightened (if necessary) using insect pins and photographed on their left side with a 10.3 megapixel Nikon Coolpix P100 digital camera. Two dimensional coordinates were collected for 16 landmarks (Fig. 1B) digitized on each specimen using TPSDIG, version 2.17 (Rohlf, 2013a). Some of the landmarks that were difficult to see in lateral pictures were marked with insect pins. The landmark data were aligned using Procrustes superimposition implemented in Relative warps, version 1.53 (Rohlf, 2013b) to eliminate variation related to rotation, translation, and size. Thin-plate spline version 1.20 (Rohlf, 2004) was used to compare consensus configurations of samples and create deformation grids depicting differences among sample means.

Standard length (SL) and body depth (BD) were also measured from the digital images using tpsDig 2.17 (Fig. 1B), because differences in body shape between *R. altipinna* and *R. minor* have previously been described using linear measures, especially the ratio of standard length to maximum body depth (SL/BD), which is also known as the fineness ratio (e.g., Li and Li, 2006). Standard length was measured from the tip of the snout to the end of the caudal peduncle. Body depth was measured from the origin of the dorsal fin down to the ventral edge of the abdomen along a line perpendicular to SL, which was typically the point of maximum body depth. The fineness ratio was used as a measure of body depth with larger values indicating more streamlined bodies. Sex was determined through inspection of the gonads.

Collections at the 31 m site carried out in July 2013 yielded a good mix of juveniles and adult males and females occurring in the same area at the same time. This allowed the opportunity to examine static allometry and sexual dimorphism for the species independent of confounding factors like elevation of collection and habitat differences. Twenty or more juveniles, adult males, and adult females were separated for analysis of allometry of body shape using geometric morphometrics and of body depth using the fineness ratio. The sample of 20 juvenile individuals examined measured between 18.4 and 24.5 mm SL (mean = 21.6), the sample of 23 males measured between 38.7 and 84.9 mm SL (mean = 71.5), and the sample of 21 females measured between 38.8 and 64.6 mm SL (mean = 46.9). Samples of 21 specimens from 86 m, 39 specimens from 189 m, 20 specimens from 382 m, and 1 specimen from 613 m (the only specimen collected) were also analyzed to examine divergence in body shape among sites at different elevations. One additional male and seven females collected in December 2012 collected at the 31 m site were also included in this analysis. See the Material Examined section for more details on the size ranges of these samples.

MANOVA as implemented in tpsRegr 1.40 (Rohlf, 2011) was used to examine the contribution of allometry, elevation (sampling site), sex, and the interaction of elevation by sex to the variation of body shape among Santa Rosa *R. altipinna*. The significance of each factor was evaluated by running the complete model with and without the factor of interest and testing the significance of the difference in the residual sums of squares matrices. To estimate the relative importance of

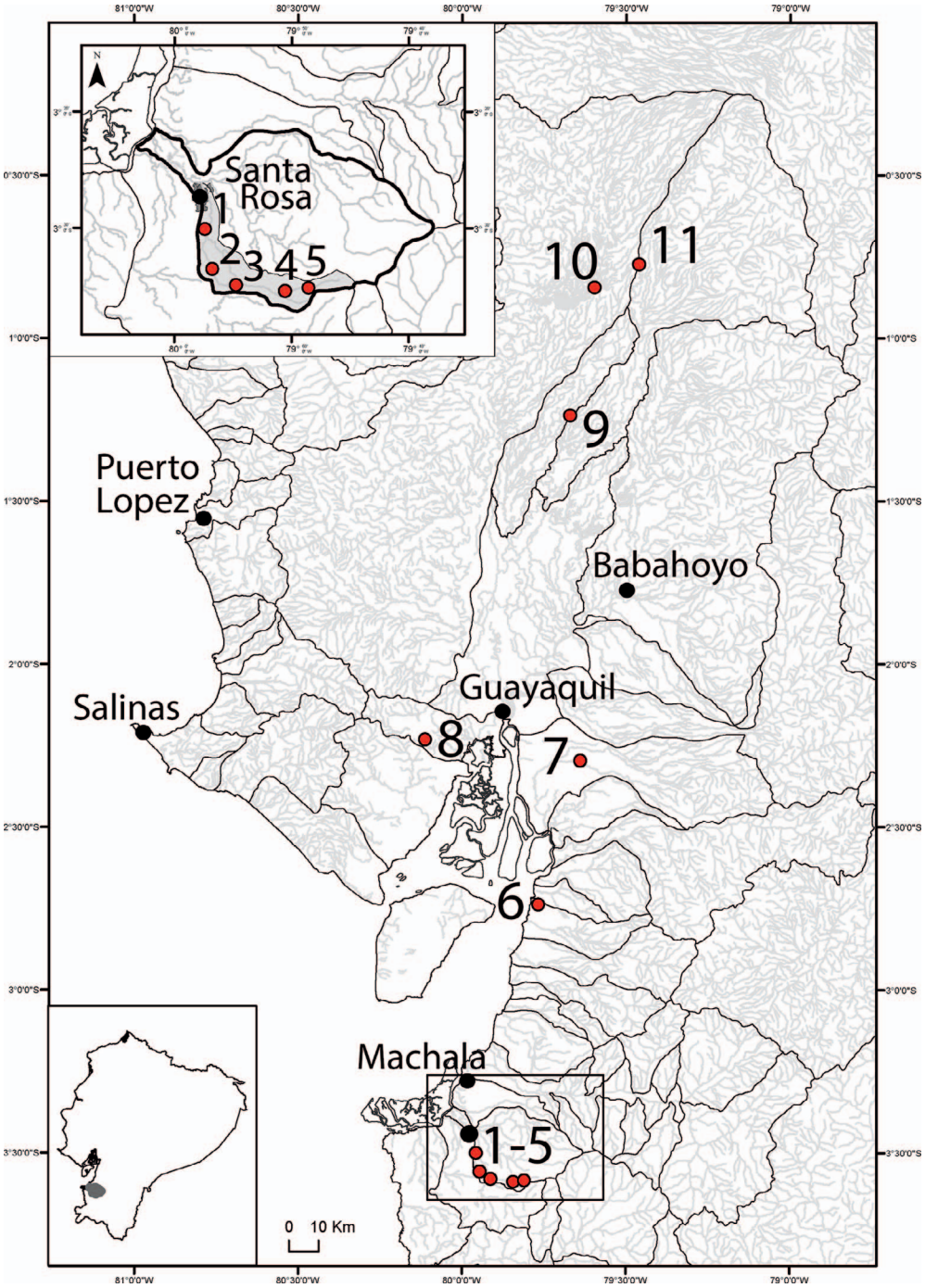

Fig. 2. Map of sampling sites (1–11) with some major cities indicated for reference. 1–5 Santa Rosa River sampling sites, 6 Balao Chico, 7 Río Culebras, 8 Chongón, 9 Jauneche, 10 Salapi Chico, 11 Estación Científica Río Palenque. Inset on the bottom left shows the position of the Santa Rosa River drainage in western Ecuador. Inset on the top left shows a close up of the Santa Rosa River drainage.

each of these factors to body shape variation of Santa Rosa *R. altipinna*, Wilks' η^2 was calculated following Langerhans and DeWitt (2004). Wilks' η^2 is a measure of the strength of the effect of a particular factor that takes into account differences in the number of groups for different variables. It is calculated as Wilks' $\eta^2 = 1-\lambda^{1/s}$, where λ is Wilks' λ, a common multivariate test statistic, and s is the smaller of the number of dependent variables or the df_{effect}, the degrees of freedom for the particular factor of interest. Larger numbers imply greater importance of a particular factor.

To put the body shape variation of *Rhoadsia* in the Santa Rosa River in context of body shape variation at broader geographic scales, several museum samples from collections made in the Guayas River and other drainages in southwestern Ecuador were examined (Fig. 2; Material Examined). A sample of specimens for the paratype series of *R. minor* was also included in many of the morphological analyses to put intraspecific variation in context of differences between the two species recognized in the region. The specimens of *R. minor* were smaller than most of the *R. altipinna* examined but not smaller than the juveniles of *R. altipinna* sampled from the Santa Rosa River 31 m site in July 2013, allowing comparisons between specimens of similar sizes. Sex determination, which requires making an incision to examine gonads, was not conducted for the museum specimens. Principal component analysis (PCA) as implemented in relative warps was used to examine body shape variation. Because these museum samples were fixed in formalin, they were not used in the genetic analyses.

DNA sequencing and analysis of COI.—To examine the extent of genetic divergence of Santa Rosa River *Rhoadsia* from those

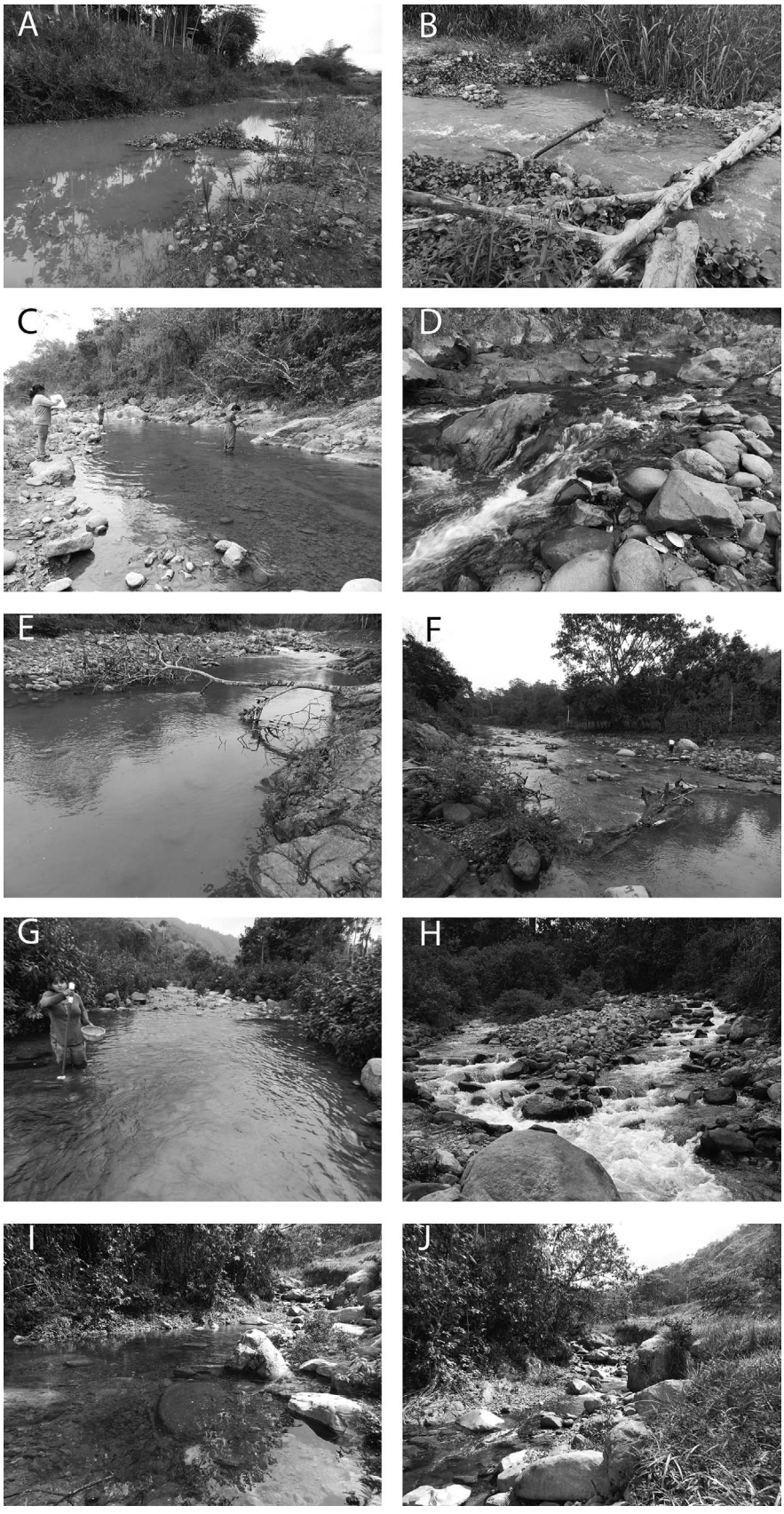

Fig. 3. Collection sites in the Santa Rosa River illustrating pool (left column) and riffle (right column) mesohabitats sampled. (A) 31 m pool site, (B) 31 m riffle site, (C) 86 m pool site, (D) 86 m riffle site, (E) 189 m pool site, (F) 189 m riffle site, (G) 382 m pool site, (H) 382 m riffle site, (I) 613 m pool site, (J) 613 m riffle site. Pictures from December 2012, except for (H) taken July 2013.

in the Guayas River drainage, samples collected in 2008 from the Santa Rosa River at the 86 m site ($n = 16$) and the Guayas River drainage at sites in Jauneche ($n = 15$, 01°14′17.6″S, 79°40′21.3″W) and from the Estación Científica Río Palenque ($n = 16$, 00°34′26.3″S, 79°21′43.5″W) were sequenced. The fin clips were preserved in 95% ethanol in the field and stored at –80°C until DNA extraction. In the lab, pectoral-fin clips were placed in a mixture of tissue digestion buffer (10 mM Tris, pH 8.0, 100 mM NaCl, 10 mM EDTA, 0.5% SDS) and proteinase K (20 mg/ml), and incubated at 55°C

overnight. The next day, DNA was isolated using 25:24:1 phenol:chloroform:isoamyl alcohol. The mixture was centrifuged (13,750 g for 10 min) to separate the DNA, which was then washed in 100% and 70% ethanol. DNA was resuspended in 100 ml of TE and diluted to a 1:10 working stock in water.

The mitochondrial cytochrome oxidase I gene was chosen because it is the DNA barcoding gene and has been commonly used to identify species and examine phylogenetic relationships (Hebert et al., 2003). The primers used to amplify a fragment approximately 670 bp long were: FISH-BCL: 5′–TCAACYAATCAYAAAGATATYGGCAC–3′ and FISH-BCH: 5′–TAAACTTCAGGGTGACCAAAAAATCA–3′ (Baldwin et al., 2009). PCR reactions were carried out in 30 μl volumes consisting of 1X PCR buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl), 2 mM $MgCl_2$, 0.25 mM dNTP (Invitrogen), 0.35 μM primers, 1 unit of Taq DNA polymerase (Invitrogen), and approximately 50 ng of template DNA. PCR conditions consisted of one cycle at 95°C for 1 min 45 s, 50°C for 45 s, and 72°C for 45 s; followed by four cycles of 94°C for 45 s, 50°C for 45 s, and 72°C for 45 s; then 30 cycles of 92°C for 30 s, 50°C for 45 s, and 72°C for 45 s; and a final extension of 72°C for 7 min. PCR products were purified using ExoSAP-IT® (USB Corporation). Forward and reverse strands were sequenced on an Applied Biosystems 3730 DNA Analyzer at the University of Arizona Genetics Core. Forward and reverse sequences were manually checked for errors, and contigs were constructed with Chromas Lite (Technelysium, Pty Ltd., South Brisbane, Queensland, Australia; http://www.technelysium.com.au/chromas_lite.html). Haplotype sequences were deposited in GenBank under the accession numbers KU252601–KU252647.

Sequences were aligned with ClustalW as implemented in BioEdit 7.2.3 (Hall, 1999), and alignments were manually inspected for errors. A maximum parsimony median joining network depicting relationships among haplotypes was constructed using Network 4.6.1.2 (Fluxus Technology Ltd., Suffolk, England; http://www.fluxus-engineering.com/sharenet.htm). Haplotype richness (H, the number of haplotypes per population) was calculated for each population, as were the number and percentage of private haplotypes (H_p, haplotypes found exclusively in the i^{th} sample) and haplotype diversity (H_d, calculated as $N(1–\Sigma p_i^2)*(N–1)^{–1}$, where p_i is the frequency of the i^{th} allele and N is the number of individuals in the sample). Arlequin 3.5.1.2 (Excoffier et al., 2005) was used to calculate pairwise F_{ST} values between populations and conduct an AMOVA to examine the components of genetic variation associated with divergence between river drainages (Santa Rosa vs. Guayas), divergence among populations within drainages (Guayas River), and divergence among individuals within populations.

RESULTS

Distribution and abundance of* R. altipinna *in the Santa Rosa River.—Fish diversity and community structure in the Santa Rosa River are being treated separately, so here we focus on the major aspects that are relevant for putting the collections of *Rhoadsia* in a broader context.

Species diversity in the Santa Rosa River was relatively low, and only 19 fish species were collected across all sites and both collection seasons (Appendix 1). Species richness also declined significantly with elevation, with a maximum value of 15 species at the 31 m site in July 2013 and only two (December 2012) or four species (July 2013) collected at the 613 m site (Dec/12: r = –0.954, $P = 0.012$, $n = 5$; Jul/13: r = –0.884, $P = 0.046$, $n = 5$). *Rhoadsia altipinna* was the second most abundant species with 548 specimens (20% of fishes) collected, and exhibited a broad altitudinal range, being one of only three species collected at all elevations (Appendix 1). As was the case with fishes in general, however, *R. altipinna* was more abundant at the lower elevation sites. In total, 513 of the 548 specimens collected were caught at 189 m of elevation or less, and only one individual (a single adult female) was caught at the 613 m site across both collection trips. This trend held when catch data were standardized by sampling effort; standardized catch values tended to be greater at lower elevations than at higher elevations (Table 1). *Rhoadsia* were also more common in pool mesohabitats than in the riffles. Within sites, abundance was greater in pool habitats than in riffle habitats at every elevation and in both collection trips, with the exception of the 189 m site in December 2012 for which 66 specimens were collected in riffle habitat but only 23 were collected in pool habitat (Sign test, two-tail $P = 0.039$, $n = 9$). This difference in abundance held at all sites in both seasons (except as described above) when percentage of catch per site was used or always when catch data were standardized by sampling effort. The average difference in abundance between habitats within collection sites was +19.1 specimens in favor of pool habitats for the standardized catch data (+25.1 for the raw abundance data), with *R. altipinna* constituting an average of 33.1% of fishes collected in pool habitats and only 9.1% of fishes collected in riffle habitats.

***Allometry and sexual dimorphism of* R. altipinna.**—Collections at the 31 m site conducted in July 2013 yielded good samples of adult male, adult female, and juvenile *Rhoadsia*, allowing examination of shape variation associated with allometry and sexual dimorphism, independent of temporal or spatial factors. Body shape variation between juveniles and adults was substantial, as was variation between adult males and adult females. A PCA conducted on specimens collected at this site yielded significant clustering of specimens by size and sex, with PCs I and II accounting for 60.7 and 14.4% of the variation, respectively (Fig. 4A). Juveniles were grouped by themselves in one portion of the space and had bodies that were more streamlined than adults. Females and small males resembled each other in body shape and were more similar to juveniles than large males but exhibited much deeper bodies than juveniles (Procrustes distance between consensus of adult females and consensus of juveniles = 0.059). The clustering of smaller males with adult females indicates that males go through the portion of body shape space occupied by females as they develop, becoming morphologically distinct as they grow and mature sexually. Larger adult males were approximately 42% more divergent from juveniles than females were (Procrustes distance between consensus of adult males and consensus of juveniles = 0.085). Large males also had deeper and relatively shorter bodies, greatly expanded maxillary bones, and much larger mouths than juveniles and females.

The fineness ratio was used to specifically examine changes in body depth with size. Higher fineness ratios indicate a more streamlined body shape. In *R. altipinna*, body depth increased with size. The fineness ratio decreased significantly with increasing SL for the fish collected at 31 m (Fig. 4B). The sample of 20 juveniles had substantially higher fineness ratios ranging between 2.70 and 3.09 and averaging (±

Table 1. Environmental data and abundance of *Rhoadsia* in the Santa Rosa River. Elev. is the site elevation, Veloc. is the average water velocity measured at the time of collection, Time is the sampling time in minutes, *n* is the number of specimens of *Rhoadsia* collected, Catch/hr is the catch standardized by collection time (the number of *Rhoadsia* collected per hour), and Catch% is the percent of the catch that consisted of *R. altipinna*.

Elev.	Type	Veloc. (m/s)	Temp. (°C)	Time (min)	*n*	Catch/hr	Catch%
December 2012							
31	Pool	0.24	26.0	59	46	46.8	38.0
31	Riffle	0.74	26.0	35	1	1.7	1.8
86	Pool	0.23	25.9	30	4	8.0	29.8
86	Riffle	0.96	26.0	60	0	0.0	0.0
189	Pool	0.31	23.6	60	23	23.0	27.4
189	Riffle	0.62	23.7	80	6	4.5	50.8
382	Pool	0.41	25.0	58	15	15.5	30.0
382	Riffle	0.61	23.3	60	11	11.0	8.5
613	Pool	0.13	21.2	47	0	0.0	0.0
613	Riffle	0.61	21.2	50	0	0.0	0.0
July 2013							
31	Pool	0.10	22.0	65	145	133.8	60.9
31	Riffle	0.51	22.1	30	59	118.0	7.4
86	Pool	0.21	23.7	120	68	34.0	93.2
86	Riffle	0.67	25.0	101	13	7.7	7.8
189	Pool	0.14	21.6	60	68	68.0	38.0
189	Riffle	1.66	20.3	55	10	10.9	13.9
382	Pool	0.11	19.6	39	7	10.8	12.7
382	Riffle	0.70	20.6	60	1	1.0	0.6
613	Pool	0.12	19.1	80	1	0.8	1.4
613	Riffle	0.40	18.9	60	0	0.0	0.0

standard error) 2.84±0.02, indicating that they exhibit more streamlined bodies. The fineness ratio declined to 2.32±0.02 (range: 2.18–2.52) and 2.36±0.02 (2.13–2.56) in adult males and females, respectively, and appeared to stabilize approximately between 50 and 60 mm SL.

Variation in body depth with elevation in the Santa Rosa River.—As was the case for fish collected at the 31 m site, body depth increased with size (SL) across samples in the Santa Rosa River as evidenced by a decrease in the fineness ratio (Fig. 5A). However, there was also a significant change in body depth associated with the elevation of the collection sites (Fig. 5B). The mean fineness ratio was significantly correlated with elevation in females ($r = 0.941$, $P = 0.017$, $n = 5$), with fishes from higher elevations tending to have more streamlined bodies. This is consistent with the difference described between *R. altipinna* and *R. minor* in which the species occurring at higher elevations (*R. minor*) has a more streamlined body. The same trend was observed in males although it was not statistically significant ($r = 0.617$, $P = 0.383$, $n = 4$). This pattern of body depth variation was not due to allometry. According to the allometric relationship documented between SL and the fineness ratio, smaller fish tend to be more streamlined. Although mean SL was associated with elevation in females ($r = 0.903$, $P = 0.035$, $n = 5$), it increased with elevation (mean female size was larger at higher elevation sites), the opposite of what was expected if the increase in elongation with elevation was due to allometry. Standard length was not associated with elevation in males ($r = -0.273$, $P = 0.727$, $n = 4$). Despite the variation in body depth documented for Santa Rosa *R. altipinna*, the paratype series of *R. minor* were more streamlined than all the samples of *R. altipinna* examined, including small specimens of similar size (Fig. 5). The average fineness ratio in the paratype series of *R. minor* was 3.09 and ranged from 2.79 to 3.42. The fineness ratio was also correlated with SL in *R. minor* and decreased with increasing SL (fish become deeper bodied with size), as was the case for the Santa Rosa *R. altipinna* ($r = -0.781$, $P < 0.001$, $n = 19$).

Body shape variation among sites in the Santa Rosa River and comparison with other populations.—PCA of body shape variation including all samples from the Santa Rosa River and samples from other drainages in south western Ecuador yielded results that were generally similar to those including only specimens from the Santa Rosa 31 m site. Across sites in the Santa Rosa River, the general pattern of distribution of juveniles in one portion of the space, females and small males towards the middle of the space, and large adult males on the left side of the shape space, generally held (Fig. 6). The distribution of individuals from different sites in the Santa Rosa River in the PCA does suggests some heterogeneity in body shape based on sampling site within sexes, especially among males. However, this appeared small relative to variation associated with size and sex. Centroid size, elevation (sampling site), sex, and the interaction of elevation and sex, all contributed significantly to the variation in body shape among Santa Rosa fishes as inferred from MANOVA (Table 2). Centroid size appeared to have the strongest impact on body shape variation as indicated by the Wilks' η^2 values calculated, highlighting the importance of allometry in this species. Elevation and sex appeared to contribute similarly to body shape variation, with elevation having a slightly higher value of Wilks' η^2 than sex.

Expanding the comparison across different river drainages, there appears to be substantial similarity in body shape between Santa Rosa *R. altipinna* and populations from the other rivers sampled in southwestern Ecuador. The Santa Rosa specimens spanned the space occupied by other samples of *R. altipinna* with little obvious segregation from samples from the other rivers (Fig. 6). Although adult Santa Rosa specimens differed significantly in body shape from

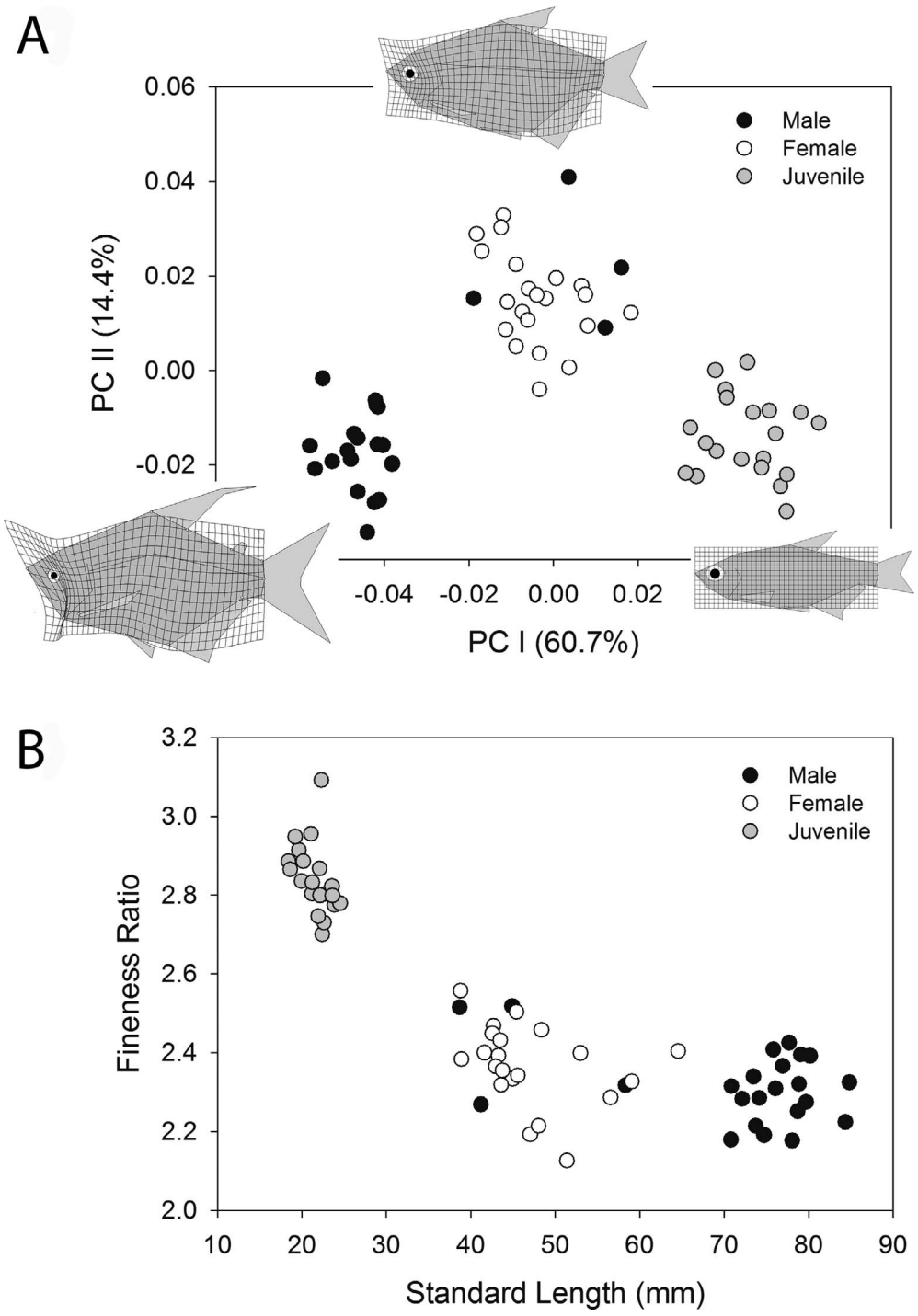

Fig. 4. (A) Principal Components Analysis (PCA) depicting body shape variation of juvenile, adult male, and adult female samples of *Rhoadsia* collected at the 31 m site in July 2013. Percentages next to the axes labels indicate the percent of body shape variation accounted for by each PC. Deformation grids are consensus configurations for juveniles, males, and females, as deformations of the juvenile consensus. (B) Change in the fineness ratio (SL/BD) between juveniles and adults indicating an increase in body depth with size.

samples of *R. altipinna* of the other rivers surveyed (Hoteling generalized T^2-test, Wilks' $\lambda = 0.295$, df = 28, 176, $P < 0.001$), differences among juveniles, females, and large males from the Santa Rosa River remained the clearest source of divergence among samples of *R. altipinna* indicating that allometry and sexual dimorphism are likely more important sources of variation in body shape than river drainage among populations in southwestern Ecuador. The paratype series of *Rhoadsia minor* on the other hand, was generally segregated (with only slight overlap) from all samples of *R. altipinna* examined including the sample of juvenile specimens from the Santa Rosa River. This indicates that the difference in body shape between the described species is substantial and transcends allometry. *Rhoadsia minor* differ in body shape and are more streamlined than *R. altipinna* from southwestern Ecuador, including *R. altipinna* of comparable size.

Genetic diversity and population genetic structure.—PCR and subsequent editing of sequences yielded a fragment of the COI gene that was 606 bp in length that was used in the genetic analysis. Among the 47 specimens sequenced, there were only seven haplotypes. All of the mutations were transitions and all involved third base pair positions. None of the mutations resulted in amino acid substitutions. Haplotypes appeared closely related to one another, and the maximum pairwise

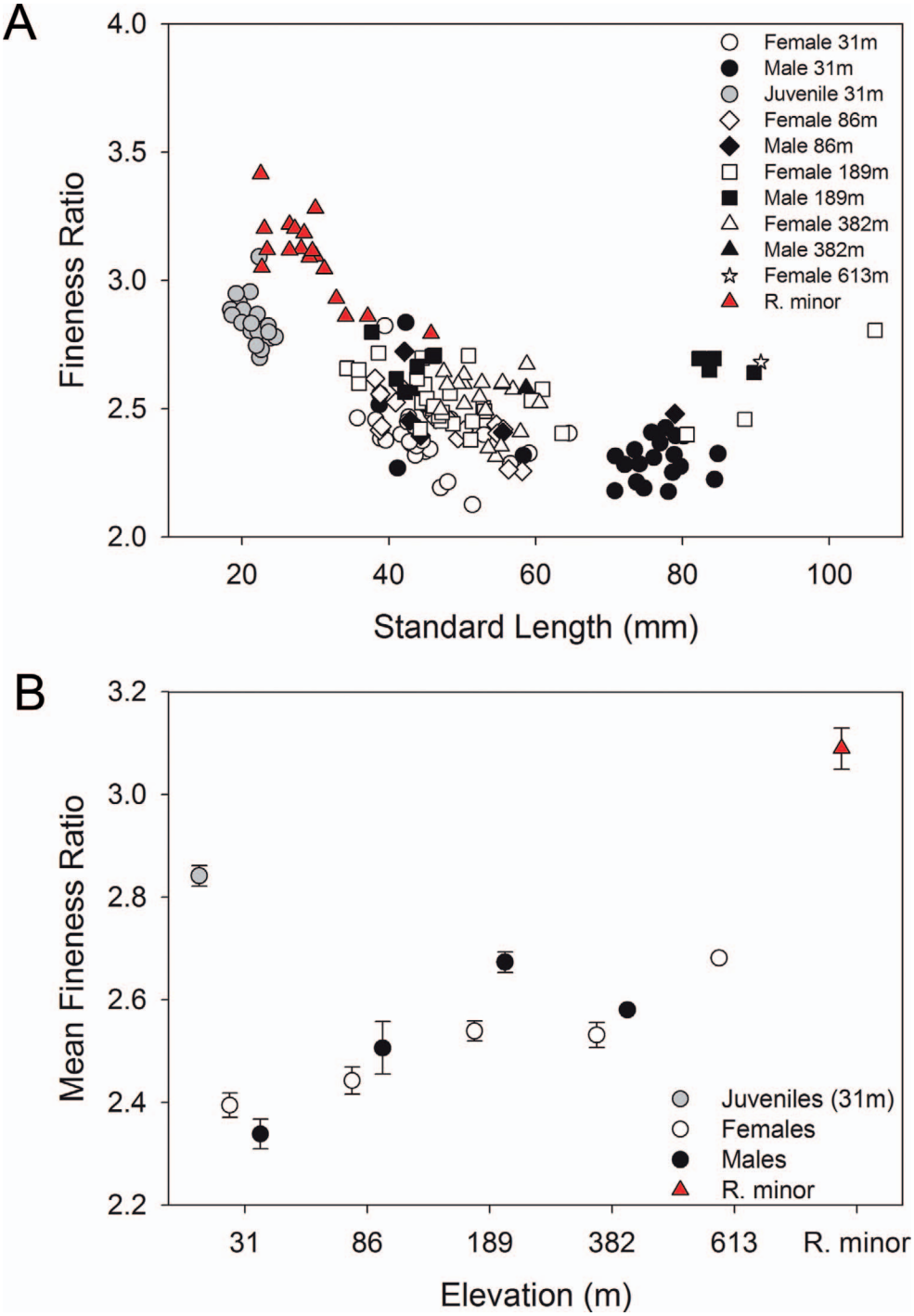

Fig. 5. Variation in the fineness ratio (SL/BD) among Santa Rosa drainage *Rhoadsia*. (A) Standard length vs. fineness ratio for all specimens with specimens of *R. minor* included for reference. (B) Mean fineness ratio of Santa Rosa samples (±standard error) plotted against sample elevation. Mean of paratype series of *R. minor* included for reference.

number of nucleotide differences was 5 (0.8%). Genetic diversity was relatively low within samples with the number of haplotypes per sample ranging from 2 to 4 and haplotype diversity ranging from 0.248 to 0.516 (Table 3). The number of private haplotypes per sample was relatively high ranging from 50–66.7%, although the relatively small sample sizes probably contributed to this. The Santa Rosa sample was not recovered as a monophyletic lineage relative to the Guayas drainage samples. Haplotype H_4 was shared by four Santa Rosa specimens and three Palenque specimens. However, there was substantial divergence of haplotype frequencies between the Guayas and Santa Rosa samples. The most common haplotype in the two Guayas River drainage samples, H_1, with frequencies of 86.7% and 68.8% for the Jauneche and Palenque populations, respectively, was not present in the Santa Rosa sample (Table 3, Fig. 7). Similarly, the most common haplotype in the Santa Rosa sample, H_5, with a frequency of 68.8%, was not present in either of the Guayas River samples. Pairwise F_{ST} values indicated large and significant genetic divergence between the Santa Rosa and Jauneche ($F_{ST} = 0.762$, $P < 0.001$) and Santa Rosa and Palenque samples ($F_{ST} = 0.707$, $P < 0.001$), but not between the Jauneche and Palenque samples from the Guayas River ($F_{ST} = 0.005$, $P = 0.261$). This was confirmed by AMOVA, with 70.2% of the genetic variation segregating between the Guayas River and Santa Rosa drainages, and the variation between populations within the Guayas River and among individuals within populations not being significant (Table 4).

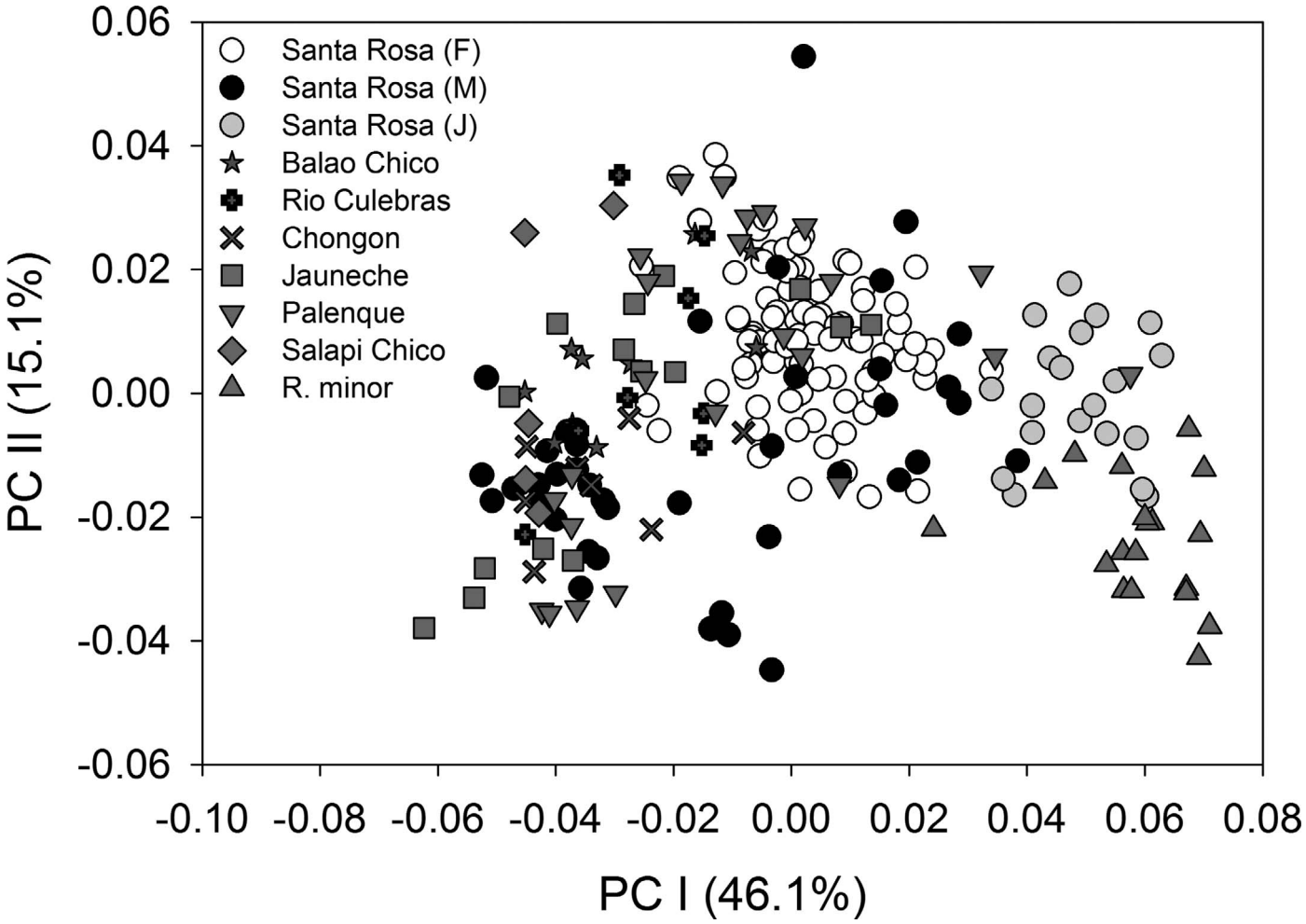

Fig. 6. Principal Component Analysis of all specimens of *Rhoadsia* examined. Santa Rosa River specimens are coded as male, female, and juvenile but not by sampling site to reduce the number of symbols used. Percentages of variance in body shape explained by PC axes are listed in parentheses.

DISCUSSION

Abundance and distribution of* R. altipinna *in the Santa Rosa River.—Fish diversity in the rivers of western Ecuador is lower than that of other major rivers of South America (Albert et al., 2011; Barriga, 2012). The Santa Rosa River is a particularly small, high gradient river that exhibits low fish diversity even when compared to other rivers in western Ecuador (Barriga, 2012; Jiménez Prado et al., 2015). Only 19 species of fishes were collected across all sites and both collection seasons. In this low diversity, clear water, high gradient system, *R. altipinna* appears to be thriving. It was the second most abundant fish collected and was one of only three species collected at every elevation. Although like other characids it was more common in pool mesohabitats, it also occurred in riffles in good numbers. In the Santa Rosa River, it appears to be most common at lower elevations, following the general trend for fishes in the river. Given that it was common at the 31 m site, which was highly impacted by humans (next to a farm and having cars crossing the river in a shallow area close to the collection site), *R. altipinna* also appears to tolerate significant environmental degradation. The presence of *R. altipinna* at all elevations, in both pools and riffles, and at environmentally impacted sites, suggests that this species possesses the potential for broad ecological tolerance, which may help explain why it is a common species in western Ecuador.

Allometry and sexual dimorphism.—*Rhoadsia altipinna* exhibits substantial intraspecific variation in body shape associated with allometry and sexual dimorphism. Although greater

Table 2. MANOVA of body shape variation of *Rhoadsia altipinna* in Santa Rosa drainage. Juveniles from the 31 m site for which sex was not available, other individuals for which sex could not be determined, and the single female from 613 m, were not included. Wilks' λ is a multivariate test statistic with smaller values implying greater significance. Wilks' η^2 is a measure of the strength of the effect of a particular factor that takes into account differences in the number of groups for different variables. Larger numbers imply greater importance of a particular factor. All factors were statistically significant ($P < 0.001$).

Factor	Wilks' λ	F_s	df_1	df_2	Wilks' η^2
Centroid size	0.292	8.312	28	96	0.708
Elevation	0.084	4.408	84	288.1	0.561
Sex	0.474	3.801	28	96	0.526
Elev. X Sex	0.279	1.824	84	288.1	0.347
Total	0.001	4.794	224	756.9	—

Table 3. Haplotype frequency and genetic diversity of samples of *Rhoadsia altipinna* analyzed. *n* is the number of specimens per sample, H is the number of haplotypes per sample, H_d is the haplotype diversity, and H_p is the number (and percentage) of private haplotypes per sample.

Site	*n*	H_1	H_2	H_3	H_4	H_5	H_6	H_7	H	H_d	H_p (%)
Jauneche	15	13	—	—	—	—	—	2	2	0.248	1 (50%)
Palenque	16	11	1	1	3	—	—	—	4	0.516	2 (50%)
Santa Rosa	16	—	—	—	4	11	1	—	3	0.492	2 (66.7%)
n	47	24	1	1	7	11	1	2			
Frequency (%)		51.1	2.13	2.13	14.9	23.4	2.13	4.26			

than typically seen in other Neotropical characids, similar patterns of allometric body shape change and sexual dimorphism do occur in other characiforms like the African Alestidae (e.g., Zanata and Vari, 2005). Size and sexual dimorphism are somewhat confounded in *R. altipinna* because males are larger than females and body shape changes substantially with size. Adult males are the most distinct morphologically (and quite striking), exhibiting the deepest bodies, largest mouths, and dramatic secondary sexual characteristics like elongation of the dorsal and anal fins and the presence of bright red and orange breeding colors. However, the distinctively deep body characterizing adult *R. altipinna* is a feature that develops as individuals grow. Deeper bodies tend to be associated with fish species inhabiting slower moving waters or pools, and favor maneuverability and acceleration over sustained swimming (Webb, 1984; Langerhans and Reznick, 2010). This would be generally consistent with the greater abundance of *R. altipinna* in pool mesohabitats than in riffle mesohabitats. The magnitude of allometric change in body shape in this species suggests significant evolutionary potential for body shape divergence among populations because the developmental machinery for generating highly divergent body shapes already exists within the species. Studying the modification of developmental trajectories as a mechanism for producing evolutionary diversity has a rich history in evolutionary biology (e.g., Gould, 1977) and may be an avenue worth pursuing in future studies of this species.

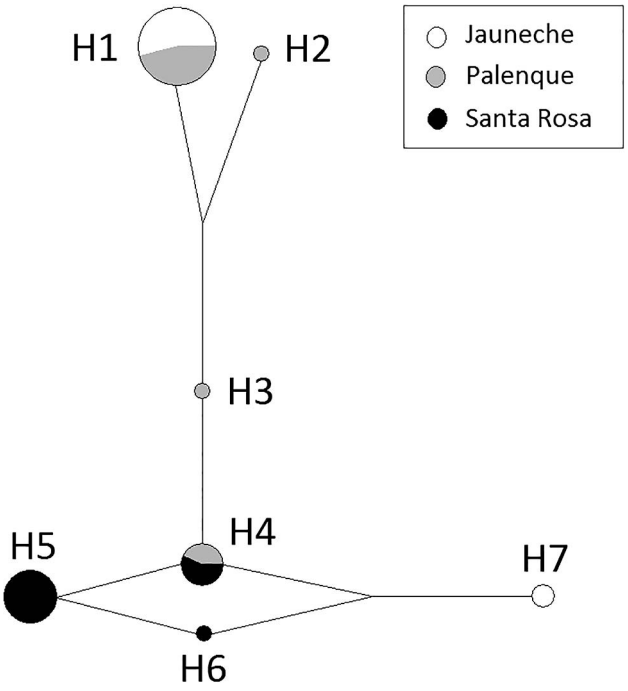

Fig. 7. Haplotype network created with Network 4.6.1.2. Circles are proportional to haplotype frequencies, and colors depict haplotype frequencies for each population. Jauneche and Palenque are part of the Guayas River drainage and are labeled as sites 9 and 11 in Figure 1, while Santa Rosa is site 2 in the Santa Rosa River.

Variation in body depth with elevation.—Relative body depth declined with elevation in the Santa Rosa River such that specimens collected at higher elevations exhibited more streamlined bodies. The trend was similar in both sexes. This result was somewhat surprising because our highest elevation site was only 613 m above sea level and *Rhoadsia* are known to occur up to at least 1,260 m in other rivers (Böhlke, 1958). The morphological gradient may have been stronger if we sampled at higher elevations.

Whether the differences in body shape with elevation reported here are due to phenotypic plasticity or have a genetic basis is not clear. Phenotypic plasticity is very common in fishes, and many species can exhibit substantial changes in morphology when exposed to different environmental conditions during development (e.g., Meyer, 1987; West-Eberhard, 2003; Wund et al., 2008). Plasticity is often the cause of phenotypic differences among populations within the same species because it does not require genetic changes, just differences in the environmental conditions that populations experience and flexibility in developmental pathways (Hendry et al., 2008). Local adaptation on microgeographical scales is also relatively common in fishes, especially in systems in which species diversity is relatively low and habitat heterogeneity is high (e.g., Hendry et al., 2002; Aguirre, 2009), making it conceivable that some component of the difference in body depth associated with elevation is due to local adaptation. Common garden experiments or similar approaches would be needed to disentangle the potential contributions of phenotypic plasticity and genetic divergence.

What are the environmental factors that could be leading to the differences in body depth among *Rhoadsia* inhabiting different elevations? There are a number of factors that vary with elevation in Neotropical streams. One factor that seems particularly worth further examination is water velocity. Although flow regimes can be complex and vary temporally and spatially, and water velocity is sometimes as great in large lowland rivers as it is in upland streams (e.g., Leopold, 1953), high-gradient streams originating in mountains are often narrower, have faster running water, and

Table 4. AMOVA of COI haplotype data.

Source	df	SS	Variation (%)	*P*
Among drainages	1	26.603	70.23	<0.001
Among populations within drainages	1	0.682	0.64	0.296
Within populations	44	22.417	29.14	0.306

consist of more riffle habitat at higher elevations than at lower elevations (Hauer and Lamberti, 2006). Water velocity is also known to impact fish body shape in accordance with the pattern observed in this study. Fishes that inhabit faster running water tend to be more streamlined and exhibit higher fineness ratios than fishes in habitats with slower running water or pools (e.g., Webb, 1984; Langerhans and Reznick, 2010). More streamlined bodies are more energetically efficient for fishes inhabiting rivers with fast running water because they reduce drag during the prolonged periods of sustained swimming that are required in these habitats. If water velocity is generally greater (or riffle habitats are more abundant) at higher elevations in the Santa Rosa River, this might account for the greater fineness ratios and more streamlined bodies seen among *Rhoadsia* inhabiting higher elevation sites. Furthermore, because of the deep body that adult *Rhoadsia* exhibit relative to other characids in the region, *Rhoadsia* spp. might be under particularly strong pressure for a more streamlined body form when in habitats with faster running water, like those presumably present at higher elevations. Unfortunately, the water velocity data collected in this study is not appropriate for addressing whether the water velocity that *R. altipinna* experience at higher elevations is typically greater than at lower elevations because the sampling sites were not selected at random; we specifically looked for high velocity riffle habitat near pool habitat at all elevations. The data collection was also too limited to characterize general conditions in the river.

Local adaptation to elevational variation in predation regimes is another potential cause. Greater body depth has long been known to provide fishes with greater maneuverability, which can help with predator avoidance (e.g., Webb, 1984; Langerhans and Reznick, 2010). Greater body depth itself also provides protection from gape-limited predators like piscivorous fishes (e.g., Werner, 1974). For example, in an experimental study of prey selectivity conducted by Hambright (1991), largemouth bass, *Micropterus salmoides*, were given the choice of two prey species differing substantially in body depth, shallow-bodied fathead minnows *Pimephales promelas* and deep-bodied pumpkinseeds *Lepomis gibbosus*. The bass exhibited preference for individuals of similar body depth in both species, indicating that body depth is more important than body length for prey selectivity, and never fed on fishes with body depths that were greater than their own external mouth width. Although we do not have data on fish predation intensity in the Santa Rosa River, large piscivorous fishes are often more common at lower elevations than at higher elevations in mountain streams (e.g., Vannote et al., 1980). Consistent with this, we collected only one specimen of *Hoplias microlepis*, a major fish predator in western Ecuador, and this specimen was collected at the 31 m site. If fish predation intensity is greater at low elevations in the Santa Rosa River, the greater body depth of *R. altipinna* at lower elevations may also be a defensive adaptation.

Sexual selection is one of the most important processes influencing the evolution of biological diversity (Andersson, 1994), and sexually selected traits are often among the most rapidly evolving (e.g., Yeh, 2004). Like other animals, freshwater fishes frequently exhibit exaggerated secondary sexual characteristics that differ between closely related species or even populations (Seehausen et al., 2008; Elmer et al., 2009). The magnitude of sexual dimorphism in *R. altipinna* is unusual for Neotropical characids and suggests that this species experiences strong sexual selection. This raises the possibility that body depth could be a trait subjected to sexual selection in as much as increased body depth in males would tend to make the elongation of the dorsal and anal fins appear even more exaggerated and also make males appear generally larger. Differences in the intensity of sexual selection on body shape at different elevations could thus also be contributing to the variation in body depth seen, although directed studies of the reproductive strategies of this species are necessary since we are not aware of any published accounts describing its reproductive habits.

Regardless of the cause, associations between body form and elevation have been reported previously for fishes in Neotropical ecosystems. Sidlauskas et al. (2006) found that specimens of the characid *Bryconops* sp. cf. *melanurus* were significantly more streamlined at higher elevation (>200 m) sites than in lower elevation sites in rivers of the Brazilian Pantanal and suggested that differences in water velocity (and/or temperature) between the high and low elevation sites might be playing a role. We anticipate that other examples will likely appear in the future as more detailed morphological studies along elevational gradients are performed. Because of the relatively deep bodies and the broad altitudinal distributions that they exhibit, *Rhoadsia* spp. may be a good system for future studies on the impacts of variables associated with elevation on body shape variation in fishes. Sampling across broader altitudinal gradients, better data on the variation of ecological variables with elevation, and experimental tests of individuals differing in body shape may help elucidate the causes of the variation in body depth seen in *R. altipinna*.

Comparison to other populations in southwestern Ecuador.—In his list of the fishes of Ecuador, Barriga (2012) indicates an undescribed species of *Rhoadsia* occurring in southwestern Ecuador in the Catamayo system, to which the Santa Rosa River belongs. This suggested the possibility that a third undescribed species of *Rhoadsia* occurs in western Ecuador. Our analysis of body shape variation found that although there was significant heterogeneity between *Rhoadsia* in the Santa Rosa River drainage and samples from other drainages in southwestern Ecuador, this divergence was relatively small and was dwarfed by variation attributable to allometry and sexual dimorphism within samples. More importantly, our analysis of COI sequence data did not recover a monophyletic lineage in the Santa Rosa River relative to the two Guayas River samples analyzed. In fact, genetic diversity and divergence among haplotypes was relatively low. There were only seven distinct haplotypes recovered among 47 specimens sequenced, the most divergent haplotypes were less than 1% distinct, and all mutations were located at third codon positions and synonymous. Both the body shape and DNA sequence data were more consistent with population level divergence within a single, geographically structured species than with the occurrence of two distinct species. Nonetheless, there was strong population genetic structure between the Santa Rosa and Guayas river samples suggesting the occurrence of distinct evolutionary units in these drainage systems and the potential for local adaptation. This was evidenced by the large divergence in the frequencies of haplotypes between the Santa Rosa and the two Guayas river samples and correspondingly high F_{ST} values. This population genetic structure is consistent with the geographic isolation be-

tween the populations in the two river systems. The Santa Rosa River runs independently into the Pacific Ocean approximately 200 km south of the larger Guayas River drainage and is one of many such rivers south of the Guayas that run between the Andes and the Pacific Ocean. The high salinity in the Gulf of Guayaquil makes migration between rivers through the ocean difficult for freshwater characids like *R. altipinna*. Sampling in the Gulf typically yields a distinct estuarine and marine fish fauna from that in continental waters. Although gene flow is theoretically possible among geographically neighboring rivers during floods in the rainy season in southwestern Ecuador (~January–April), and over geological time some gene flow between the Guayas and Santa Rosa Rivers might occur either through migration or stream capture events, the magnitude of the difference in haplotype frequencies between the Guayas River and Santa Rosa River samples indicates that there is significant isolation between them.

***Comparison to* R. minor.**—A sample of specimens from the paratype series of *R. minor* were included in the morphological analyses for comparative purposes. *Rhoadsia minor* was described from elevations above 1,200 meters in the Esmeraldas River drainage in northwestern Ecuador as being a smaller, more streamlined species than *R. altipinna* (Eigenmann and Henn, 1914). Although most of the specimens analyzed in this study were relatively small, they were comparable in size or larger than specimens from the sample of juvenile specimens of *R. altipinna* collected at 31 m, allowing us to account for allometry in body shape. The specimens of *R. minor* were indeed more streamlined than all samples of *R. altipinna* measured, including the juveniles from the Santa Rosa 31 m sample. In the PCA analysis of body shape variation for all samples, the specimens of *R. minor* also clustered in a separate portion of the space, highlighting their morphological distinctiveness. Does this morphological difference ratify their status as distinct species? Not necessarily, especially given the decrease in relative body depth found in specimens of *R. altipinna* collected between 31 and 613 m in the Santa Rosa River. If body depth continues to decline in *Rhoadsia* collected at higher elevations in southwestern Ecuador and body depth increased at lower elevations in *Rhoadsia* in the Esmeraldas River, the two described species could blend into each other morphologically, at least in terms of their body shape. Broader sampling along altitudinal gradients throughout the range of the genus is needed to resolve this question. Back in 1958, Böhlke indicated that the distinction between *R. altipinna* and *R. minor* was not as clear as suggested by Eigenmann and Henn (1914) when they described *R. minor* and compared it to *R. altipinna*. Géry (1977) also questioned the validity of *R. minor*, suggesting that it may be a high elevation dwarf form of *R. altipinna*. Our finding of variation in body depth of *R. altipinna* with elevation, even along a gradient of less than 600 m, supports Böhlke and Géry's contentions that a more thorough analysis of the relationship among species of *Rhoadsia* is needed. For example, it is currently not clear whether the low elevation populations of *Rhoadsia* in the Esmeraldas and Santiago-Cayapas rivers in northwestern Ecuador should be considered *R. altipinna* or *R. minor*. Barriga (2012) lists all Esmeraldas *Rhoadsia* as *R. minor*. However, Böhlke (1958) indicated that low elevation *Rhoadsia* in the Esmeraldas and Santiago Rivers are similar to *R. altipinna* from the Guayas River system. A major goal for future research is to broaden the sampling efforts to examine the evolutionary relationships among *Rhoadsia* spp. throughout western Ecuador. Further analysis of morphological and genetic variation of *Rhoadsia* will help elucidate the evolutionary dynamics of this ecologically important freshwater fish.

MATERIAL EXAMINED

Catalog numbers, sample size, and standard length (mm; mean SL±standard deviation, min SL–max SL) listed for each lot. Institutional abbreviations follow Sabaj Pérez (2014), with the addition of MECN (Museo Ecuatoriano de Ciencias Naturales [Ecuador]); WAM-XXX indicates field numbers for uncataloged specimens.

Rhoadsia altipinna.—Santa Rosa River samples (listed by elevation in meters): 31 m: FMNH 122407, 20 juveniles (21.6±1.8, 18.4–24.5), 21 females (46.9±6.6, 38.8–64.6), 20 males (75.9±5.7, 58.3–84.9); MECN-DP-2647, 3 males (41.6±3.1, 38.7–44.9); WAM-310, 7 females (40.9±3.6, 35.7–45.8), 1 male (42.3). 86 m: MECN-DP-2626, 8 females (46.4±7.5, 38.8–55.6), 6 males (51.3±14.4, 42.1–79.0); WAM-301, 7 females (48.5±7.7, 38.1–58.2). 189 m: MECN-DP-2641, 10 females (52.4±21.6, 34.3–106.2), 10 males (59.7±22.0, 37.7–89.8); WAM-307, 10 females (56.3±15.6, 45.2–88.5); WAM-308, 9 females (46.7±3.0, 43.9–53.0). 382 m: FMNH 122431, 6 females (53.1±3.9, 47.0–57.9); MECN-DP-2647, 8 females (54.0±3.5, 50.2–60.5); WAM-305, 5 females (51.8±5.0, 47.5–58.8), 1 male (58.7). 613 m: FMNH 122423, 1 female (90.7). Samples for comparative analysis of body shape variation: FMNH 71867, 9, Estación Científica Río Palenque (45.7±16.5, 24.7–76.7); FMNH 79077, 10, Estación Científica Río Palenque (56.9±16.0, 36.1–93.2); FMNH 79080, 5, Estación Científica Río Palenque (98.7±4.7, 91.0–103.4); MUGT P-198, 8, Chongón (73.6±6.8, 64.81–88.29); MUGT P-0011, 5, Salapi Chico (96.2±16.2, 77.3–120.6); MUGT P-0226, 3, Jauneche (76.7±23.8, 57.7–103.5); MUGT P-0300, 10, Río Culebras (62.2±6.5, 53.5–72.0); MUGT P-0380, 5, Jauneche (97.8±10.6, 85.8–113.4); MUGT P-0404, 7, Jauneche (50.2±7.3, 40.8–61.2); MUGT P-0416, 10, Balao Chico (59.3±8.2, 43.2–69.0).

Rhoadsia minor.—CAS 32457, 19, Mindo, paratypes (29.3±5.6, 22.5–45.7).

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Appendix 1. Species abundance at sampling sites in the Santa Rosa River.

		December 2012											July 2013											Grand
Genus	Species	31P	31R	86P	86R	189P	189R	382P	382R	613P	613R	Total	31P	31R	86P	86R	189P	189R	382P	382R	613P	613R	Total	total
Rhoadsia	*altipinna*	46	1	14	—	23	66	15	11	—	—	176	145	59	68	13	68	10	7	1	1	—	372	548
Brycon	*atrocaudatus*	—	1	1	13	—	12	16	44	6	16	109	—	32	—	10	18	21	18	21	57	19	196	305
Eretmobrycon	*brevirostris*	13	2	14	2	—	—	—	—	—	—	31	2	6	—	—	—	—	—	—	—	—	8	39
Eretmobrycon	*dahli*	7	2	4	—	—	1	3	3	—	—	20	—	22	—	1	8	—	9	4	4	—	48	68
Iotabrycon	*praecox*	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1	1
Hoplias	*microlepis*	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1	1
Lebiasina	*bimaculata*	—	—	1	—	1	—	—	—	—	—	2	2	—	—	—	2	—	—	—	—	—	4	6
Saccodon	*wagneri*	1	6	5	70	—	15	—	7	—	—	104	3	7	—	92	—	15	1	1	—	—	119	223
Poecilia	sp.	47	1	—	—	—	—	—	—	—	—	48	59	7	—	—	—	—	—	—	—	—	66	114
Pseudopoecilia	sp.	2	—	—	—	3	1	—	—	—	—	6	2	1	—	—	—	—	—	—	—	—	3	9
Andinoacara	*rivulatus*	2	4	6	1	48	11	1	1	—	—	74	17	14	5	3	59	2	—	—	—	—	100	174
Mesoheros	*festae*	1	—	—	—	1	—	—	—	—	—	2	2	17	—	—	5	3	—	—	—	—	27	29
Sicydium	*rosenbergii*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1	1
Astroblepus	sp.	—	—	—	—	—	4	—	59	—	54	117	—	—	—	—	—	9	13	129	9	115	275	392
Ancistrus	*clementinae*	1	6	—	—	—	—	—	—	—	—	7	—	37	—	—	—	—	1	—	—	—	38	45
Transancistrus	*aequinoctialis*	—	31	—	5	—	—	—	—	—	—	36	2	497	—	29	—	1	—	—	—	—	529	565
Transancistrus	*santarosensis*	—	—	—	2	—	—	—	3	—	—	5	—	—	—	4	—	—	—	—	—	—	4	9
Pimelodella	*modestus*	1	—	2	5	8	20	13	1	—	—	50	3	92	—	13	19	10	5	3	—	—	145	195
Ituglanis	*laticeps*	—	1	—	3	—	—	2	1	—	—	7	—	—	—	1	—	—	1	2	—	—	4	11
Num indiv=		121	55	47	101	84	130	50	130	6	70	794	238	792	73	166	179	72	55	161	71	134	1941	2735
Num species=		10	10	8	8	6	8	6	9	1	2		11	13	2	9	7	9	8	7	4	2		