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Niche overlap in sympatric Rocio (Teleostei: Cichlidae) of Guatemala

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Niche overlap in sympatric *Rocio* (Teleostei: Cichlidae) of Guatemala

A thesis presented in partial fulfillment of the
requirements for the degree of Master of Science

by

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August 2022

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III. ACKNOWLEDGEMENTS

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Faber est suae quisque fortunae. NSE.

IV. ABSTRACT

Rocio is a small genus of Neotropical freshwater fishes that is distributed in Atlantic drainages of northern Middle America. Two species in the genus exhibit sympatry in the Río Dulce basin (in Izabal, Guatemala), where one species is endemic (*R. spinosissima*), while the other only includes this area as part of a larger geographic range (*R. octofasciata*). Unfortunately, the ecology of these species has been poorly studied. This study sought to determine the ecological and morphological differences between these two closely related sympatric freshwater fishes. We hypothesized that *R. octofasciata* would exhibit greater ecological and morphological variation, showing an overlap with *R. spinosissima*. We also hypothesized that morphological divergence would be associated with character displacement. Local-scale environmental data suggest habitat characteristics overlap between the two species, with a greater use of slow-flowing to lentic, poorly oxygenated and with a larger range of pH, environments by *R. spinosissima*, and *R. octofasciata* inhabiting areas with a greater range of environmental characteristics. Drainage-scale data also indicate that land cover, soil, precipitation and temperature largely define the distribution of both species, with some noticeable differences. Additionally, the mean body shape is different between specimens from the two species when they are in sympatry, while specimens from outside of the sympatric area tend to show similar shape characteristics between the two species, following a pattern of character displacement.

Chapter I

I. GENERAL INTRODUCTION

Ichthyology, the study of fishes, is often supported by detailed assessments of the ecology and evolution of these animals. The present work provides such approach to obtain a better understanding a group of understudied fishes from Guatemala, Central America. The rest of this chapter (Ch. 1) consists of a literature review that covers important concepts and topics that provide context for the present research. Herein, background on fundamental information for the project is provided. First, the concept of the ecological niche, along with its relationship to niche overlap and niche conservatism is presented. This is also linked with ecomorphology and the patterns of niche divergence among sympatric species. A detailed description of the study area in eastern Guatemala is provided and contextualized using a baseline of ichthyological studies. Lastly, the fish genus *Rocio* is described, highlighting the two species used for the present work. The next chapter (Ch. 2) pertains to the research paper, where all the procedures and methods are described, along with the main findings and a discussion of their significance.

II. REVIEW OF THE LITERATURE

The ecological niche

The ecological niche is a central concept in ecology, in which every species has unique ecological properties that allow them to occupy a specific place and carry out particular functions in an *n* dimensional ecological space (Grinell, 1917; Elton, 1927; Hutchinson, 1957; Holt, 2009). The classical approaches have focused either on the environmental requirements that allow a species to occur (Grinell, 1917), or the functions that the species fulfill in each ecosystem (Elton, 1927). However, the concept involving the unique properties of each species in the environment and the community to which they belong has been widely used because of its explanatory capacity (Hutchinson, 1957). Moreover, discussions about the concept of the niche and its use have constantly appeared in the literature, including new methods of study and perspectives on its nature and importance (Soberón, 2007; Holt, 2009; Schoener, 2009).

The ecological properties of the niche are related to the species' natural history and many processes that result in the evolution of species (Hutchinson, 1957; Holt, 2009; Wiens et al., 2010). Studying the ecological niche can result in a greater comprehension of the ecology and evolution of species. Abiotic conditions are an important component of the ecological niche of species, and can provide insight into their physiological properties. Biotic conditions are associated with the relationships with other species, with many variable outcomes such as predation or competition (Wiens et al., 2010; Pianka, 2011).

Freshwater fish constitute a major component of the biological diversity of vertebrates in Neotropical ecosystems. The distribution and abundance of fish is affected by multiple factors that operate at different scales (Pease et al., 2012). For example, environmental characteristics, habitat availability and biogeochemical variation in water bodies affect the diversity of fish assemblages (Bogotá-Gregory et al., 2020), and species responds differently to this environmental variation (Pease et al., 2012; Barbosa et al., 2019). Understanding these interactions is of great importance given that they can have a direct effect on the abundance, distribution and conservation of species experiencing the impacts of global change, including climate change (McMahan et al., 2020). Describing and comparing the environmental interactions of freshwater fishes is essential for conservation planning and ecological studies.

Niche overlap and niche conservatism

The ecological niche of each species is a unique property and is not shared with any other species. Nonetheless, many similarities and shared characteristics between species exist, which might be considered as a partial overlap in the ecological niche (MacArthur & Levins, 1964, 1967; May & MacArthur, 1972; Hurlbert, 1978). These overlaps might happen at random, given the distribution of each species, where the assemblage is interacting and only responds to current processes. This means that niche overlap can occur given random coincidences in geographically and phylogenetic distantly related groups. However, niche overlap can also happen due to a shared evolutionary history of closely related species.

Niche conservatism, the tendency for closely related species to retain similar aspects of their niches, represents an area of ongoing research in which phylogenetic methods are used to study the implications of the ecological niche (Losos, 2008; Wiens, 2008; Wiens et al., 2010; Pyron et al., 2015). The main empirical explorations of NC have involved the use of ecological niche models and large phylogenies where tests for phylogenetic signal are performed using particular traits (Wiens et al., 2010; Crisp & Cook, 2012). However, the study of NC has declined in the recent literature apart from comparisons with ecological niche models or tests for phylogenetic signal using groups at higher-classification levels than species (Broennimann et al., 2012; Culumber & Tobler, 2016; Hamlin et al., 2017; Olalla-Tárraga et al., 2017). Nonetheless, NC remains as an interesting framework in evolutionary ecology.

Ecomorphology

Ecomorphology or ecological morphology is a comparative discipline that studies the interaction between the morphological and ecological diversity of organisms, both in the present and over evolutionary time (Motta & Kotrschal, 1992; Motta et al., 1995). However, this concept has been commonly included in the ecological literature on the use of "functional traits", along with "life histories" or "morphometry" (Luiz et al., 2019). Common applications include the study of shape variation related to functional and trophic ecology, phylogenetics, fisheries, and the long-term analysis of invasion ecology and climate change (Luiz et al., 2019). Despite being an important field, there are still gaps in knowledge related to population and community ecology, as well as in relation to freshwater habitats and hydrogeomorphology (Luiz et al., 2019).

Tools like geometric morphometrics are often employed to explain patterns of morphological variation observed in species as a function of variables such as geographic distance, habitat type, or geology (Aguirre & Jiménez-Prado, 2018; McMahan et al., 2017b). Geometric morphometrics is also used to analyze morphological variation in different ecosystems and geographic areas (Bower & Piller, 2015, Figure 1.1), and to examine morphological variation between sexes and age groups in species (Aguirre et al., 2016; Aguirre et al., 2019).

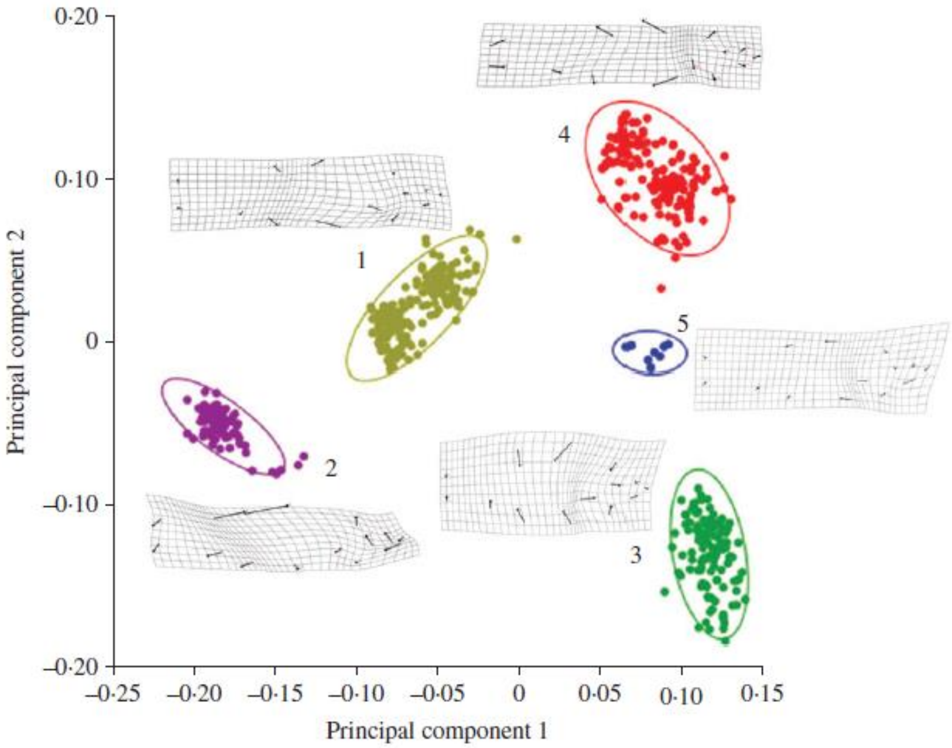

Figure 1.1. Principal component analysis of fish species from the Tickfaw River, describing up to five ecomorphotypes (Bower & Piller, 2015).

Niche divergence among sympatric species

Evolutionary ecology examines how intra and interspecific interactions are related to their evolutionary history (Pianka, 2011). This involves the study of traits or characters of biological importance for the ecology of organisms. Ecological character displacement is described as the depletion of shared resources by sympatric and morphologically similar species that favors the exploitation of new resources to reduce interspecific competition, leading to divergence (Schluter & McPhail, 1992; Schluter, 2000; Losos, 2000). Given the case where two species live in the same habitat, their characters will show a divergent state and will be different compared to those places where only one species is distributed and has the whole range of variation occupied (Brown & Wilson, 1965; Stuart et al., 2017) (Figure 1.2). This is of special interest in closely related sympatric species because it can illuminate the selective pressures and adaptations that have allowed them to coexist.

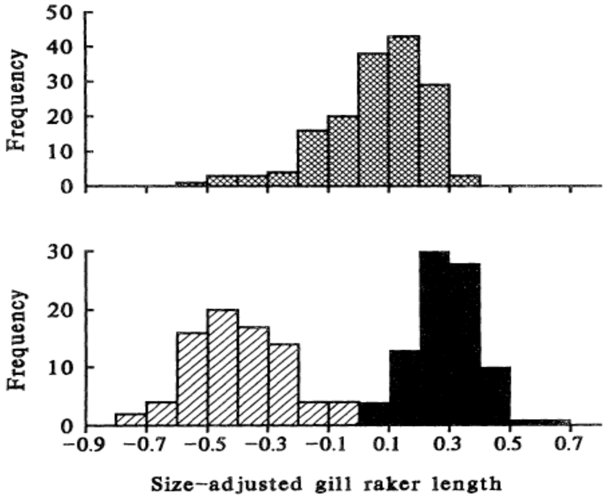

Figure 1.2. Frequency distribution of size-adjusted gill raker lengths of fish in lakes with one (top) and two (bottom) species (Schluter & McPhail, 1992).

Sympatric species are those which have the same or overlapping geographic distributions, regardless of whether or not they occupy the same macrohabitat (Rivas, 1964; Fitzpatrick et al., 2008). Sympatric speciation is usually considered rare for Neotropical freshwater fishes (Albert et al., 2020), but there are described examples, particularly within the family Cichlidae (Galis & Metz, 1998; Barluenga et al., 2006; Matschiner et al., 2020; Ronco et al., 2021). Studying sympatric species is of particular interest given that many dynamics involve limited resources that must be distributed among species, often leading to competition. Considering that this has to be sustainable over time for sympatric divergent populations to allow species to evolve (Foote, 2017), it raises interest for this area of study.

Study Area: Lago de Izabal-Rio Dulce and Polochic river basins

As part of the Convention on Biological Diversity, Guatemala is a Like-Minded Megadiverse Country with high levels of biodiversity (CONAP, 2013), and fishes constitute an important component of this diversity. There are over 240 species of fishes distributed in inland waters, 18 of which are endemic (Kihn-Pineda et al., 2006; Kihn-Pineda & Cano, 2012). Studying these vertebrates is of great importance given the ecological roles (e.g. trophic chains, nutrient cycling) they play in lentic (standing, slow flowing waters) and lotic (running, fast flowing waters) systems (Wetzel, 2001). They are also important for the local economy and as a source of food for people in the area. Guatemala has 38 hydrographic basins, divided into three versants: Gulf of Mexico, Pacific and Atlantic (Suárez, 2011).

Figure 1.3. Lago de Izabal merging with Río Dulce in Izabal, Guatemala (Fuentes, 2020).

The Lago de Izabal-Río Dulce basin spans approximately 340,000 hectares and constitutes an important input to the availability of surface water in the Guatemalan Atlantic versant (Figures 1.3 and 1.4). It provides valuable human services such as a place for sporting activities (e.g. sailing, swimming, and paddling) and tourism, a thriving fishing industry that provides food, and a source of water for local populations (Suárez, 2011). This basin includes Izabal Lake, the largest lake in Guatemala with a surface area of 717 km^2 and a depth of up to 17 m (Brinson & Nordile, 1975; Barrientos & Allen, 2008; Barrientos & Quintana, 2012). In addition, the Río Dulce National Park is located within the basin, occupying an area of 80 km^2 of flooded areas and 9 km^2 of floodable areas that flow towards the Atlantic (Quintana et al., 2011). Upstream, we can find the Río Polochic basin with approximately 281,100 hectares of extension, adding up to about 621,100 hectares together with Lago de Izabal-Río Dulce. Together, they connect in a way that the water flows from west to east, until draining into the Caribbean. Both basins are part of the Polochic-Cahabón area of endemism (Elías et al., 2020; Matamoros et al. 2015), which also includes the Cahabón river (about 245,900 ha), that has been recognized by a particular composition of fish species, particularly by endemics.

Figure 1.4. Typical streams located within the Lago de Izabal-Río Dulce basin (Fuentes, 2020).

The waterbodies within the Lago de Izabal-Río Dulce basin are part of the Polochic-Cahabón Area of Endemism, where up to 34 freshwater fish species are distributed, seven of which are endemic to this area (Matamoros et al., 2015; Elías et al., 2020). Of these endemic species, four species are cichlids and three are poeciliids. Even though research on fishes in the region dates back to the mid-20th century (Bigelow & Schroeder, 1948; Thorson et al., 1966) and that there have been recent studies (Pérez Alvarado et al., 2003; Barrientos & Allen, 2008; Quintana et al., 2011), there are still some important gaps in knowledge of the ecology of fish species in the area. In addition, recent studies provide some environmental data, such as water quality, in the Lago de Izabal-Río Dulce basin (Robledo et al., 2014; Aguirre Cordón et al., 2016; Hernández et al., 2020), but there are no empirical analyses that relate the distribution and abundance of freshwater fish with the environmental characteristics in the region.

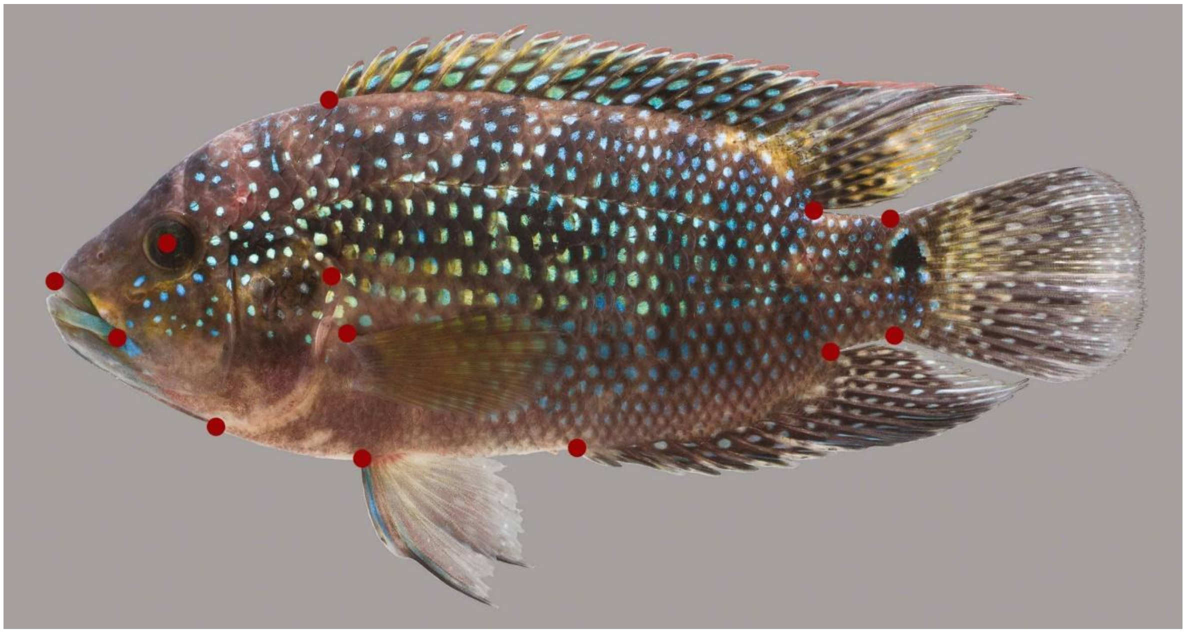

Figure 1.5. Homologous landmarks placed on a specimen of *Rocio octofasciata* following McMahan et al. (2011). Photograph from Robins et al. (2018).

Rocio Schmitter-Soto, 2007 (Teleostei: Cichlidae)

The genus *Rocio* is a small Neotropical fish genus (Figure 1.5) distributed in Atlantic drainages of northern Middle America (see Figure 1.6), with four species currently considered valid (Schmitter-Soto, 2007a, 2021; Říčan et al., 2016; Artigas-Azas, 2018). Two of the species share their geographic range in the Lago de Izabal-Río Dulce basin (located in Izabal, Guatemala), in which one is endemic (*Rocio spinossisima*), while the other only includes this area as part of a larger geographic range (*R. octofasciata*) (see Figure 1.6). These are likely sister species as evidenced in recent phylogenetic analyses (Říčan et al., 2016), even though the systematics of the genus has not been recently assessed (Schmitter-Soto, 2007a, 2007b). However, little is known about their ecological interactions, or their abundance and distribution in the Lago de Izabal-Río Dulce basin, despite this basin being of special interest for conservation (Schmitter-Soto, 2019; Lyons et al., 2020b) and biogeographical (Matamoros et al., 2015; Elías et al., 2020) matters. Given their geographic ranges, it can be hypothesized that *R. octofasciata* can occupy a larger range of environments than *R. spinosissima*, while sharing some aspects of its niche. This hypothesis can be assessed under the concept of niche overlap and NC.

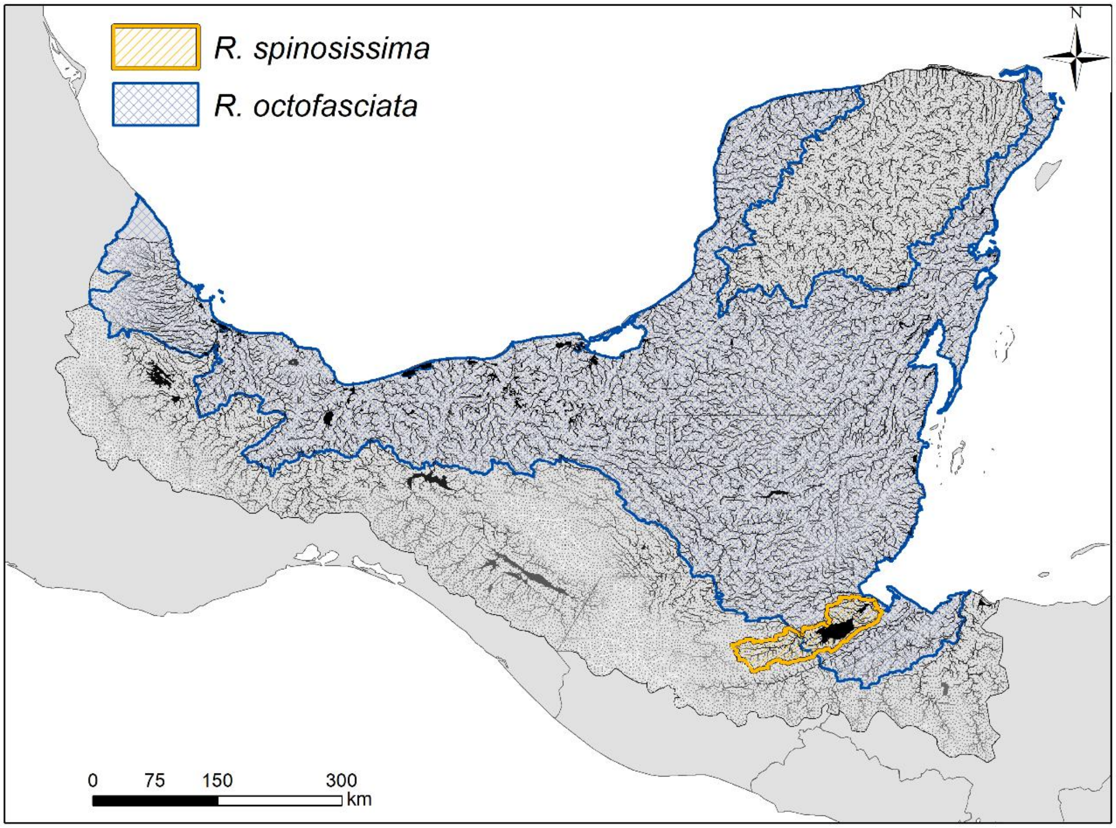

Figure 1.6. Distribution of two sympatric species of the genus *Rocio*. Layers available from Schmitter-Soto (2019) and Lyons et al. (2020b).

Chapter II.

Niche overlap between sympatric species of the genus *Rocio* (Teleostei: Cichlidae) in Guatemala

INTRODUCTION

Sympatric species are those which have the same or overlapping geographic distributions, regardless of whether or not they occupy the same macrohabitat (Rivas, 1964; Fitzpatrick et al., 2008). Understanding how closely related sympatric species coexist has been a topic of intense study (Bolnick & Fitzpatrick, 2007; Fitzpatrick et al., 2008). Most closely related sympatric species are thought to have evolved allopatrically, with ecological and or morphological differences arising while the populations are geographically isolated (Bolnick & Fitzpatric, 2007; Foote, 2017). The geographic isolation allows a straightforward mechanism for incipient species to diverge. A range expansion or shift of one or both species then results in the observed sympatry (Mayr, 1942, 1963). Although sympatric speciation is usually considered rare for Neotropical freshwater fishes (Albert et al., 2020), there are some examples in which barriers to reproduction and mechanisms for coexistence evolve in species occupying the same geographic area, including freshwater fishes like the Cichlidae (Galis & Metz, 1998; Barluenga et al., 2006; Matschiner et al., 2020; Ronco et al., 2021). Studying closely related sympatric species can illuminate the mechanisms that allow morphologically and ecologically similar species to coexist and overcome the negative consequences of competition for limited resources and shared ecological interactions.

The ecological niche is the concept describing that every species has unique ecological properties that allow them to occupy a specific place and carry out particular functions in an *n* dimensional ecological space (Grinnell, 1917; Elton, 1927; Hutchinson, 1957). Despite every species having a unique ecological niche, there are shared aspects of the niche that allow species to exhibit partial ecological overlap. Niche conservatism is the tendency for closely related species to retain niche-related fundamental traits over time (Wiens et al., 2010). Niche divergence describes the ecological differentiation from closely related species, reducing resource competition (Macarthur & Levins, 1967; Losos, 2000), which is expected to commonly occur in non-coexisting species (McCormack et al., 2010). This results in a pattern in which closely related species retain ecological properties from their ancestors, in addition to resource partitioning and non-coexistence of species with similar niches. Those properties commonly match morphological attributes of species.

The morphology of species is greatly linked to their ecology and evolutionary history (Gatz, 1981; Cooper, 2018; Keppeler & Winemiller, 2020; Magalhães de Oliveira et al., 2020). Ecomorphology studies morphological traits or biologically important characters and their relationship with ecological properties of organisms (Motta & Kotrschal, 1992; Motta et al., 1995; Luiz et al., 2019). Additionally, divergence of sympatric and morphologically similar species has been attributed to interspecific competition, caused by a reduction of resources, often tested under the concept of ecological character displacement (Schluter & McPhail, 1992; Schluter, 2000; Losos, 2000). Given the case where two species live in the same habitat, their characters will show a non-overlapping distribution, compared to those places where only one species is distributed and occupies all of the ecological space. This is of special interest in closely related sympatric species because it can illuminate the selective pressures and adaptations that have allowed them to coexist, restricting where they can live.

The distribution and abundance of fishes are affected by multiple factors that operate at different scales (Pease et al., 2012). For example, environmental characteristics, habitat availability and biogeochemical variation in water bodies affect the diversity of fish assemblages (Bogotá-Gregory et al., 2020), and species respond differently to this environmental variation (Pease et al., 2012; Barbosa et al., 2019). Understanding these interactions is of great importance as they can have a direct effect on the abundance, distribution and conservation of species experiencing the impacts of global change, including climate change (McMahan et al., 2020) and habitat loss due to various factors (Aguirre et al., 2021). Describing and comparing the environmental interactions of freshwater fishes is essential for ecological studies and conservation planning, particularly in a changing and threatened region such as the Neotropics (Reis et al., 2016; Dudgeon, 2019; Albert et al., 2020; Lyons et al., 2020b).

The genus *Rocio* Schmitter-Soto, 2007 (Teleostei: Cichlidae) is a small Neotropical genus distributed along Atlantic drainages of northern Middle America (Schmitter-Soto, 2007a; Říčan et al., 2016, see Supp. Figs. 1-2). Two of the species in the genus exhibit sympatry in the Río Polochic and Lago de Izabal-Río Dulce river basins (from now on referred to as Dulce River basin as a unique system), located in Izabal, Guatemala (Suarez, 2011). One of them, *R. spinosissima, which is* endemic to the Dulce River basin, has been considered rare due to scarce collections and unknown population size, being these some of the reasons for which its conservation status is assessed as endangered (Lyons et al., 2020b). The other species, *R. octofasciata*, occupies the Dulce River basin as part of a larger geographic range that includes basins across southeastern Mexico, Guatemala, Belize, and Honduras, being described as abundant and having stable populations, leading to its conservation status assessed as a species of least concern (Schmitter-Soto, 2019). Despite the systematics of the genus has not been assessed recently (Schmitter-Soto, 2007a, 2007b), phylogenetic analysis indicates that they are likely sister species (Říčan et al., 2016). Unfortunately, the ecology of these species has been poorly studied, representing a great gap in the knowledge of the genus that is worsened by the conservation threats that these species face in the region (Schmitter-Soto, 2019; Lyons et al., 2020a; Lyons et al., 2020b).

To fill these gaps in knowledge, this study sought to determine the ecological and morphological differences between these two closely related sympatric species of *Rocio* in Guatemala. We address two specific objectives: 1) assess the environmental characteristics of the sites inhabited by each species to determine the characteristics that allow these two species to coexist in sympatry, and 2) evaluate to what degree their morphology differs and whether there is any evidence of character displacement. This study provides critical information on the environmental requirements of species from the genus *Rocio*, as well as the factors associated with niche evolution in these closely related species.

MATERIALS AND METHODS

Surveying

We did an exploratory sampling trip to corroborate potential sampling localities during August 2021 mainly in sites along the south shore of Lake Izabal, and sites closer to Río Dulce. This allowed us to confirm accessibility throughout the basin due to infrastructure and weather, considering that the Dulce River basin and the Izabal department show a marked rain seasonality (García-Oliva and Pazos, 2021) which hampers access to the sampling sites and makes water conditions highly variable. We aimed to cover only the dry season which generally goes from December through April. Based on the observations made and previous registered collections, field surveys were conducted during January 2022. We sampled 18 localities, all within the Polochic-Cahabón area of endemism (sensu Elías et al., 2020), across the Dulce River. Those were distributed among Ciénaga (seven), Juan Vicente (six), Polochic (two), Sauce (two), and Tunico (one) Rivers (see Fig. 2.1). Elevation for all localities ranged from 11 to 201 meters above sea level, overall considered a lowland elevation.

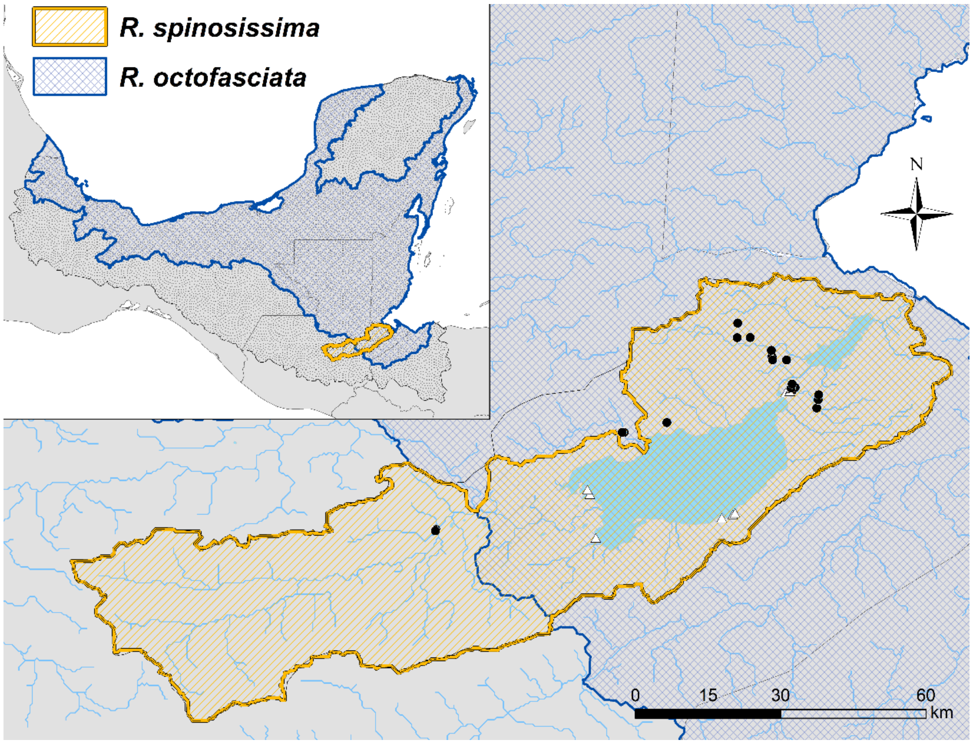

Figure 2.1. Geographic ranges of the two species of *Rocio* (Schmitter-Soto, 2019; Lyons et al., 2020b) along with exploratory sampling sites (white triangles) and sampling sites (black circles).

Environmental data acquisition

In order to assess the specific habitats in which the species of *Rocio* were located, an array of in-site environmental and habitat characteristics were measured immediately after arriving at each site. We categorized the sites as a pond (small lentic waterbodies), stream (narrow, often walkable across and mostly slow flowing lotic waterbodies) or river (wide, often difficult to walk across and mostly fast flowing lotic waterbodies). Each sampling event consisted of 20m sections of the river, stream, or pond that were accessible and displayed a particular set of habitat characteristics, different from adjacent sites. When sampling rivers or streams, we obtained triplicates of the data 0.5 m from the border of the section, and when sampling ponds, the triplicates were made closer to the edge or towards the middle section based on accessibility, always trying to capture any variation within the sampling area.

We used a YSI Professional Plus Multiparameter to obtain water quality data including temperature, pressure, dissolved oxygen (DO) and pH, submerging the probe midway through the column of water. We used a previously measured and marked PVC pipe tube to record depth, a measuring tape to measure width, and a Forestry Suppliers spherical concave densiometer to measure the canopy cover. We visually categorized the dominant substrates based on the size of grain of each substrate to identify the percentage of clay (0.002 mm), mud (0.002-0.06), sand (0.06 - 2.0 mm), gravel (2mm - 6 cm), rocks (6 - 25 cm) and boulders (>25 cm). All measurements were annotated and later on recorded as an average for each variable at each site.

Fish sampling

Several active collecting methods were used. We used an ABP-4-MR backpack electrofisher from ETS Electrofishing Systems, LLC that was fed energy by a 12V and 12 Ah Steren battery, with a general equipment setting of 100-400 Volts range, with a 20% duty cycle at a 60 Hz rate. If the water conductivity was appropriate, we used a backpack electrofisher for a maximum of 45 minutes, and if the water conductivity did not allow for its use, we focused on the remaining methods. The latter consisted of one person using a cast net and two persons using a seine net, where we used a maximum of 10 seine passes and 10 castnet throws per site.

Fish counts were done at each site, and fish specimens were collected in order to corroborate the taxonomic accuracy and abundance of the two species of *Rocio*. Specimens were euthanized using concentrated clove oil solution, and fin and muscle clips were taken as tissue samples for a selection of specimens. We preserved fish specimens using 10% formalin for a minimum period of 72 hours. Specimens were then rinsed multiple times in water to remove residual formalin, and then transferred to 25%, 50%, and finally 70% ethanol for long-term storage. All collected specimens were deposited at the Field Museum of Natural History (FMNH) Fish collection. All methods were carried out in accordance with relevant guidelines and regulations in Guatemala with permits granted by Consejo Nacional de Áreas Protegidas (CONAP), and all fieldwork protocols were approved and conducted under Institutional Animal Care and Use Committee (IACUC) approval FMNH 2020-2 at the Field Museum of Natural History.

Environmental analysis: quantitative analysis at fine scale

Environmental niche overlap was assessed using two different scales and approaches. Most of our analysis were done using R 4.0.3 (R Core Team, 2020) and RStudio (RStudio Team, 2022). First, we used the function *rda* from the package *vegan* 2.5-7 (Oksanen et al., 2020) to perform a Redundancy Analysis (RDA) as a way to assess the relationship between the fine scale environmental variables (Borcard et al., 2011; Legendre & Legendre, 1998) and the presence of species of *Rocio*. To do this, we created a matrix that contained the mean value for all environmental variables measured at each site (independent variables), and another matrix with abundance per species (dependent variable). In order to make the variables comparable and avoid outliers, we used a Hellinger transformation for the environmental data (Legendre & Gallagher, 2001), while the abundance was standardized by using a capture per unit effort (CPUE = number of individuals captured / time of effective sampling in hours) method as a way to homogenize different sampling times for the given conditions at each site. Additionally, since environmental data are commonly correlated, we aimed to reduce the influence of collinearity among variables by obtaining a parsimonious RDA for each species. We assessed performance of different subsets of environmental variables and the capability to explain the abundance by using the function *ordistep* in the package *vegan* to show a reduced set of variables. We used only variables that showed a Variance Inflation factor (VIF) lower than 10 (Dormann et al., 2013). A new RDA was done only using those environmental variables chosen by the forward selection procedure. Finally, we compared the biplots obtained for the response of abundance to remaining non-collinear environmental variables for each species.

Environmental analysis: modeling at basins scale

In order to assess environmental niche overlap at a large scale, we used a maximum entropy modeling approach (Phillips et al., 2006), considering that distribution for both species extend to places where our sampling could not reach. Ecological niche models (ENMs), along with species distribution models (SDMs) were estimated using Maxent v.3.4.1 (Phillips et al., 2022), which overlays presence data onto environmental layers and characterizes those conditions most suitable for a species. We used a set of 21 freshwater-specific environmental variables (Earth Environment – EarthEnv; Domisch et al., 2015) to estimate the suitable habitat for each species. All layers were clipped to both a large and small extension range. The large range included all Caribbean basins ranging from throughout southern Mexico, using the Papaloapan River as a westernmost edge, Belize, and Guatemala, up to the Ulúa River in Honduras. The small range only included the sympatric area of the Dulce River, including the Polochic River, Izabal Lake and Dulce River basins. All layers had a 30 arc second (~ 1 km) spatial resolution. Since only the distribution of *R. octofasciata* goes outside of the sympatric area, we only generated models with two different ranges for that species, while only one the small range was use for *R. spinosissima*. Using a delimited range reduced the potential for pseudoabsences detected in the analyses (McMahan et al., 2017a; McMahan et al., 2020).

We tested for correlation among variables using a Pearson's correlation test, and when two layers were correlated (using a threshold of 0.8) we retained the climate layer that appeared most biologically meaningful and excluded layers with multiple correlations. The remaining variables (11 for large range *R. octofasciata*, seven for small range *R. octofasciata*, eight for small range *R. spinosissima*) were used to estimate contemporary suitable habitat (Supplementary Table 1). In addition to this, we ran models without elevation, since it consistently appeared as the highest contributing variable for all models. Comparisons of the full, partially reduced, and reduced datasets with non-correlated variables demonstrated clear overestimation of distribution and suitable habitat in the models built using fewer variables

(Supplementary Figs. 3-4), which was highly supported by previous collections (both museum specimens and our empirical field and habitat data). Although some perspectives argue that correlated variables favor overestimation in the models, the robustness of Maxent in optimizing collinearity among variables has been supported (De Marco Júnior & Nóbrega, 2018; Feng et al., 2019; McMahan et al., 2020). Therefore, removing highly correlated variables from the complete dataset has little impact. Given this, we used the full dataset for analyses as this most accurately matched biology and distribution of the species, particularly working at such a fine scale, thus allowing Maxent to choose the most informative variables among all predictors for modeling distribution (Bagley et al., 2013).

Prior to generating the ENMs and SDMs, we prepared an input matrix with presence records for both species (at two scales for *R. octofasciata*). We used the coordinates of the presence records from our sampling, in addition to previous collections with specimen vouchers available through GBIF (GBIF, 2021) and housed at Universidad de San Carlos de Guatemala (USAC), creating a merged matrix with coordinates for all the known presence records of *R. spinosissima* and *R. octofasciata*, which was curated for accuracy, clipped into the grid cells extent of the environmental layers, and cleaned to avoid duplicate records. With this dataset, we ran Maxent under a convergence threshold of 10−6 and used 10,000 iterations with bootstrap resampling and 10 replicates (Pearson et al., 2007; Wisz et al., 2008). We included a total of 357, 14, and 18 independent occurrence records for *R. octofasciata*, large and small range, and *R. spinosissima*, respectively for each replicate, and used a 25% random test percentage of these records to assess model performance. In order to assess the contribution of individual environmental variables, we set a jackknife analysis in Maxent when running the models to assess the overall contribution of each variable for each model as a percentage, and compared the top six contributing variables.

We used the receiver-operating characteristic curve (AUC) as an evaluation of model performance (Phillips et al., 2006). The AUC values were between 0 and 1, with higher values indicating a better model performance. When the AUC was below 0.5, the model performed worse than random, and the closer the AUC was to 1 the better the model performed (Elith et al., 2007). We additionally used the True Skill Statistic (TSS) as an independent assessment of model performance, with values ranging between − 1 and + 1, where + 1 indicated perfect agreement and values ≤ 0 indicated a performance no better than random (Allouche et al., 2006). We then reclassified each replicate into binary (presence/absence) maps to evaluate suitability using the Maximum Training Sensitivity Plus Specificity threshold, which minimizes false-presence and false-absence errors (McMahan et al., 2017; Jiménez-Valverde, A. & Lobo, 2007; Liu et al., 2015), and stacked them to produce a single map. As a way to compare the species distribution models, we plotted all presence points for the two species within the Dulce River, then contrasted them with our sampling sites and all available ichthyological collections (all fish samplings recorded) with voucher specimens in the area available in GBIF (GBIF 2022) and at USAC. This allowed us to corroborate the accuracy and prediction capacity of our models, in addition to comparing the known distribution of the two species with all of the sampling effort that has been done throughout the area.

Geometric morphometrics

Patterns of variation in body shape were assessed using a geometric morphometrics approach. In addition to specimens collected during field surveys, museum specimens from prior collecting events were included in the analysis. This included specimens of the genus *Rocio* from the entire native distribution (with an emphasis on the eastern drainages of Guatemala) that were available at the following museum collections (code names following Sabaj, 2020): El Colegio de la Frontera Sur, Chetumal (ECO-CH), El Colegio de la Frontera Sur, San Cristóbal de las Casa, Chiapas (ECO-SC-P), Field Museum of Natural History (FMNH), University of Michigan Museum of Zoology (UMMZ), and Colección Ictiológica de la Escuela de Biología de la Universidad de San Carlos de Guatemala (USAC). The full list of catalog numbers and locality information for specimens can be found in Supplementary Table 2. We only used specimens that were well preserved, not bent and easy to distinguish the landmarks used. We took 2D photographs of the left side of the specimens and used a scale next to each specimen for size reference.

To capture body shape variation, we used fifteen (15) homologous landmarks and thirty (30) sliding semi-landmarks (see Fig. 2.2) following previous proposals (McMahan et al., 2011; Gilbert et al., 2020). We manually digitized and placed the landmarks over the photographed specimens using the package *Stereomorph* 1.6.4 (Olsen & Westneat, 2015), and processed and analyzed the data using the package *Geomorph* 4.0.0 (Adams et al., 2022; Baken et al., 2021), following general geometric morphometrics procedures by Ardón et al. (2022) and Nash et al. (2022). We used the function *gpagen* to perform a generalized Procrustes analysis (GPA) in order to align the scaled digitized landmarks. The GPA eliminates the variation in landmark position that is attributed to rotation, translation, and scaling, but keeps important shape information (Zelditch et al., 2012). This allows us to make comparisons of shape among specimens.

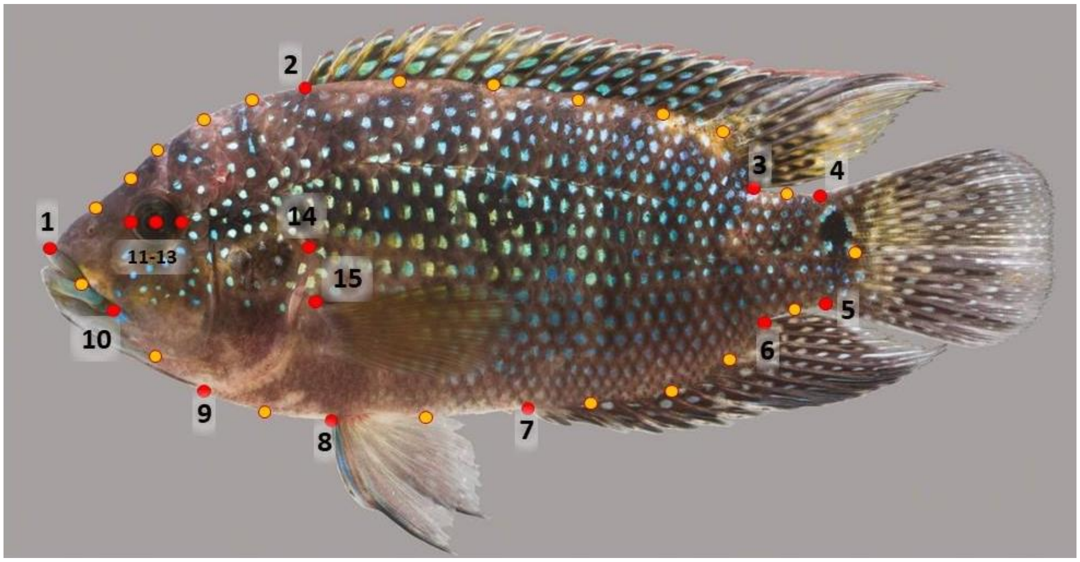

Figure 2.2. Landmarks used for geometric morphometrics with homologous (15) landmarks in red, and sliding semilandmarks (30) in yellow) placed on a specimen of *Rocio octofasciata*. Photograph from Robins et al. (2018).

We analyzed the geometric morphometrics data from an initial set of specimens (N=369) that were taxonomically differentiated. This set included 63 specimens of *R. spinosissima*, 268 specimens of *R. octofasciata*, and a group of three specimens from the other two species in the genus (*R. gemmata* and *R. ocotal*) to use as a reference. Later, in order to standardize the size of specimens we used a reduced dataset (N=341) with only specimens larger than 30 mm in Standard Length (SL). Preliminary analysis of shape data showed that size had an effect on body shape variation, and considering that life history studies for the genus are not published and we are unaware of the size at maturity for the two species, we limited the specimens used to only those with a SL equal or larger than 30% of the maximum SL registered for each species (maximum SL 110 mm for *R. spinosissima* and 250 mm for *R.*

octofasciata) (Ardón et al., 2022). This left us with a final set of what we considered adult specimens (N=124, 59 for *R. spinosissima* over 33 mm, and 65 for *R. octofasciata* over 75 mm). For each dataset (all, >30 mm, and >30% SL) we performed a multivariate analysis of covariance (MANCOVA) in order to determine the relationship between body shape, used as dependent variable, and sympatric state (i.e. whether the specimens came from the area of sympatry or not), used as independent variable, adding size as a covariate. To do the latter, we used the function *procD.lm* with 10,000 iterations, selecting a residual randomization permutation procedure (Collyer & Adams, 2021; Collyer & Adams, 2018). Finally, we ran a principal component analysis (PCA) with the function *gm.prcomp*, using the GPA aligned landmark coordinates and a matrix of classifiers corresponding to each specimen plotted, in order to visualize the grouping by species and sympatric state. Lastly, thin-plate grid wraps were obtained using the function *picknplot.shape* for the negative and positive ends of each PCA axis (PC1, PC2). This allowed us to understand the distribution of all *Rocio* specimens in morphospace and how body shape changed across PCA axes.

We evaluated the difference between sympatric state groups by calculating the Euclidean distance of the mean coordinates (using only specimens above 30% of SL) of each group, along with the differences over each axis. The latter was done by calculating the difference between mean coordinates of each axis separately, in order to assess whether the main differences were seen over PC1 or PC2. To corroborate if the differences over morphospace axis reflected a pattern of character displacement, we measured different size independent traits that included the body finess ratio (FR = SL/Body depth), head ratio (HR = Head length/Head depth) and peduncle size ratio (PS = Mean peduncle length/Mean peduncle depth). This was computed individually for specimens within each group and compared their mean values. We used the comparison suggested by Stuart et al. (2017) in which the difference between the mean value for non-sympatric groups is contrasted with the difference for sympatric groups. If the difference of sympatric groups is greater than non-sympatric groups, the pattern of character displacement can be suggested.

RESULTS

Fine-scale environmental niche

The parsimonious RDA for *R. octofasciata* (Fig. 2.3) explained 47.56% of the variation (r2=0.476, adjusted r2=0.109), of which 47.54% was explained by axis 1 and 0.02% was explained by axis 2. The constrained variance was 47.57%, while the unconstrained variance was 52.43%. The forward selection procedure selected seven environmental variables as contributing the most to the output model: sand, gravel, rock, boulders, canopy, stream and pond. The results suggest that the presence of *R. octofasciata* is limited to relatively soft substrates, open canopy and associated with a variety of both streams and (mainly) ponds. Sites lacking the species are clustered in the lower left section of the RDA biplot.

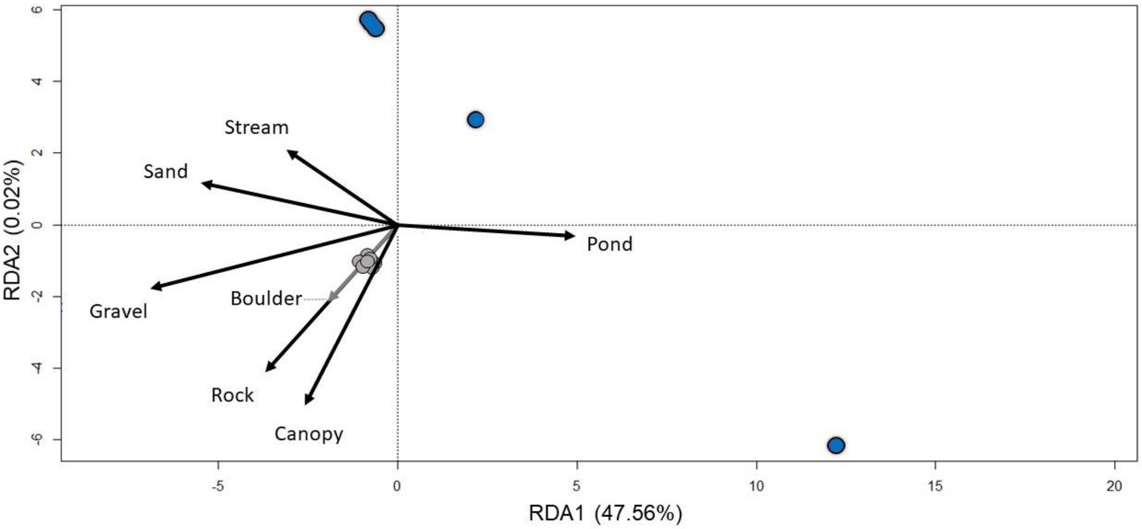

Figure 2.3. Biplot showing the output of the redundancy analysis (RDA) for *Rocio octofasciata*. Blue circles represent sites *where R. octofasfciata* is present and grey circles represents sites where the species is absent.

The parsimonious RDA for *R. spinosissima* (Fig. 2.4) explained 55.57% of the variation, of which 55.54% was explained by axis 1 and 0.03% was explained by axis 2. The constrained variance was 55.58%, while the unconstrained variance was 44.42%. The forward selection procedure selected six environmental variables as contributing the most to the output model: Mud, pH, boulders, width, canopy cover and sand. These results suggest that the presence of *R. spinosissima* is generally associated to muddy and soft substrates, narrow and canopy open spaces with a large range of pH values (below 6 and above 7.5). Sites at which this species is absent clustered in the lower left section of RDA biplot.

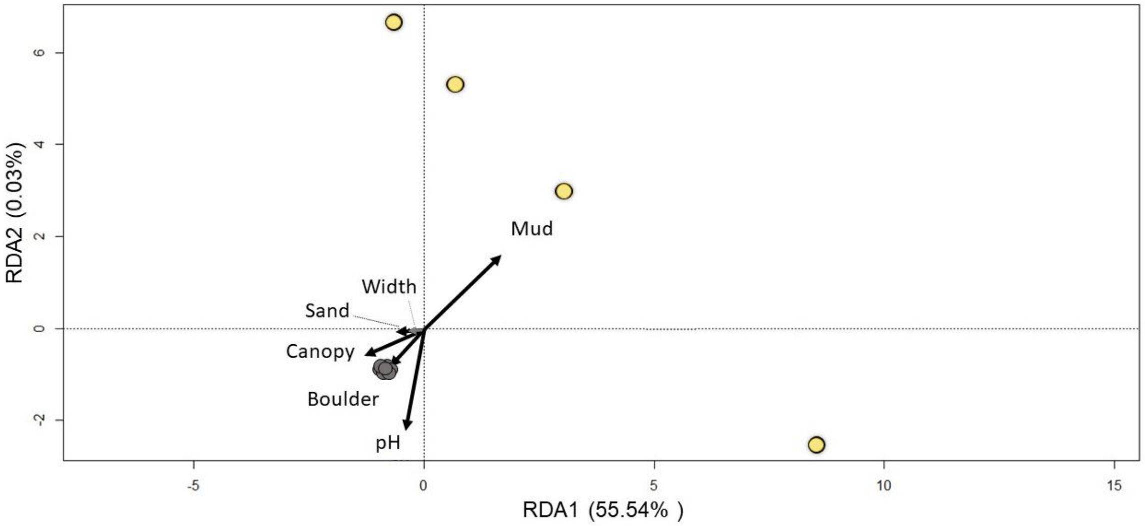

Figure 2.4. Biplot showing the output of the redundancy analysis (RDA) for *Rocio spinosissima*. Yellow circles represent sites *R. spinosissima* is present and grey circles represents sites where the species is absent.

Drainage scale environmental niche

Fish sampling allowed us to identify the presence of *Rocio* over six of the 18 collection sites, three of which were inhabited by both species. Our collection raised the known localities for *R. octofasciata* and *R. spinosissima* to 14 and 18 sites throughout the Dulce River basin (Fig. 2.5). Based on the jackknife analysis, the ENMs suggest that the environmental ecological niche of both species is strongly impacted by elevation (at any scale), and that under the Dulce River small scale, *R. spinosissima* is affected by LC_ran (33.8%), Flow (4.5%), Soil_min, Soil_avg, and Tmin_wavg (4.8% contribution together), while *R. octofasciata* is also affected by Pre_wsum (12.8%), Soil_wavg (11.3%), LC_max, Tmax_avg, and Soil_ran (25.4 % contribution together) (Table 1). The ENM’s for *R. octofasciata* at the large scale display a greater contribution from Pre_wsum (18.2%), Tmax_wavg (9.5%), Flow, Slope, and Tmax_avg (18.9% contribution together).

Table 1. Top six contributing variables for ENM's used. Contributing percentage and permutation importance is included for each variable.

Species	*Rocio spinosissima*			*Rocio octofasciata*					
Range	Small			Small			Large		
Contribution order	**Variable**	**Contrib. (%)**	**Permut.**	**Variable**	**Contrib. (%)**	**Permut.**	**Variable**	**Contrib. (%)**	**Permut.**
1	DEM	53.4	55	DEM	36.9	43	DEM	30.2	14
2	LC_ran	33.8	21	Pre_wsum	12.8	0	Pre_wsum	18.2	8.6
3	Flow	4.5	0.1	Soil_wavg	11.3	13	Tmax_wavg	9.5	6.8
4	Soil_min	2.1	5	LC_max	11.1	1.5	Flow	6.7	7.7
5	Soil_avg	1.6	8.7	Tmax_avg	9.2	8.6	Slope	6.1	4.6
6	Tmin_wavg	1.1	0.7	Soil_ran	5.1	4.5	Tmax_avg	6.1	8
Added contribution		96.5	91		86.4	71		76.8	50

The SDMs predict highly suitable environments over the lower sections of the Dulce River basin for *R. octofasciata* (Fig. 2.5), with an overall medium to low habitat suitability prediction at lower reaches of rivers across the basin. The SDMs for *R. spinosissima* predict suitable environments in a disjunct pattern, with the Polochic and Dulce rivers sections predicted to be medium to highly suitable, in contrast to very low to null suitability elsewhere in the Dulce River basin. The large-scale SDM for *R. octofasciata* predicts highly suitable environments in the majority of the basin, with a wide range of predictions over different basins (Fig. 2.6). The average AUC value for *R. octofasciata* small range models was 0.451 ± 0.316, and 0.427 ± 0.070 for the large range models, while it was 0.433 ± 0.121 for *R. spinosissima*. Lastly, the average TSS value for the *R. octofasciata* small range models was 0.897 ± 0.054, and 0.819 ± 0.009 for the large range model, while it was 0.803 ± 0.051 for *R. spinosissima* (see Supp. Table 2 for full AUC and TSS values).

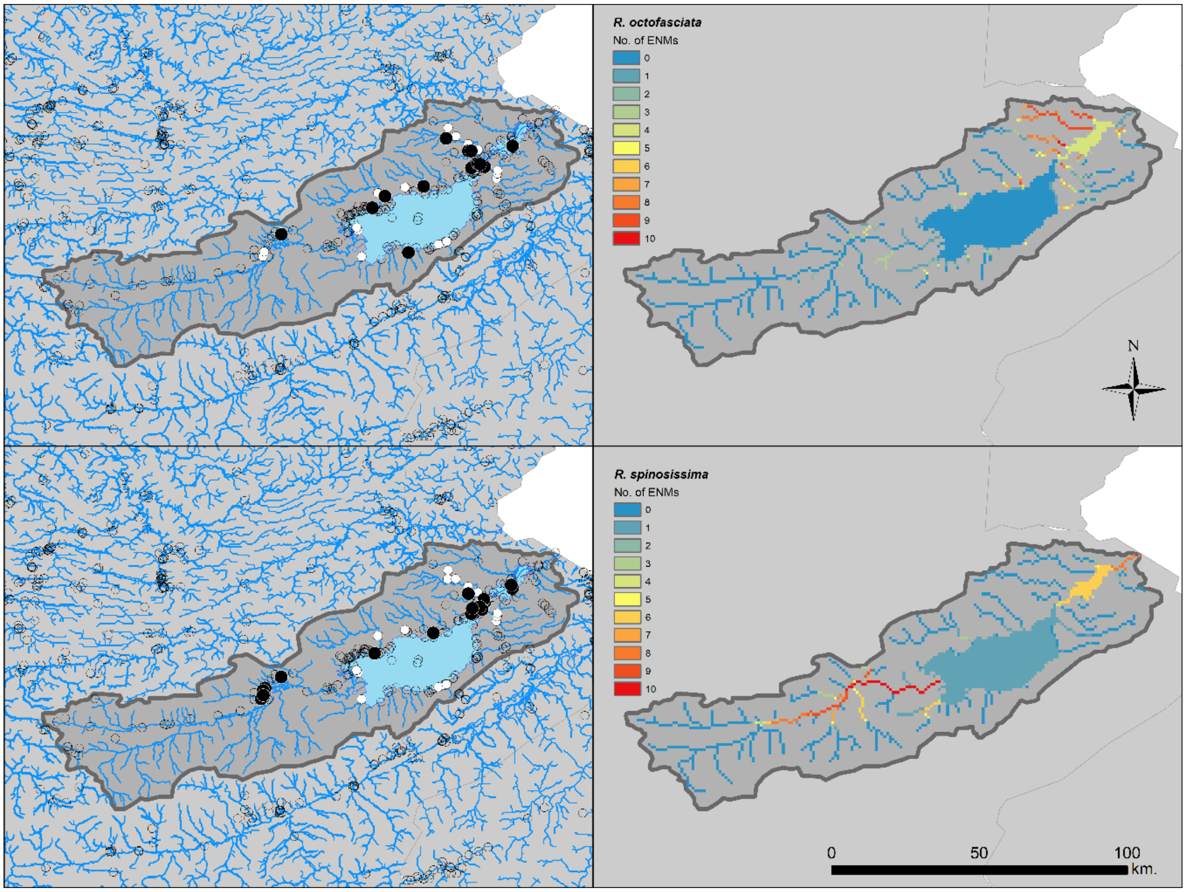

Figure 2.5. Maps of the Dulce River basin and distribution of *Rocio*, with the upper section for *R. octofasciata* and the lower section for *R. spinosissima*. Left panels show their presence (black circles), sampling field sites (white circles) and ichthyological collecting events (open circles). Right panels show suitable habitat based on SDM's under EarthEnv variables.

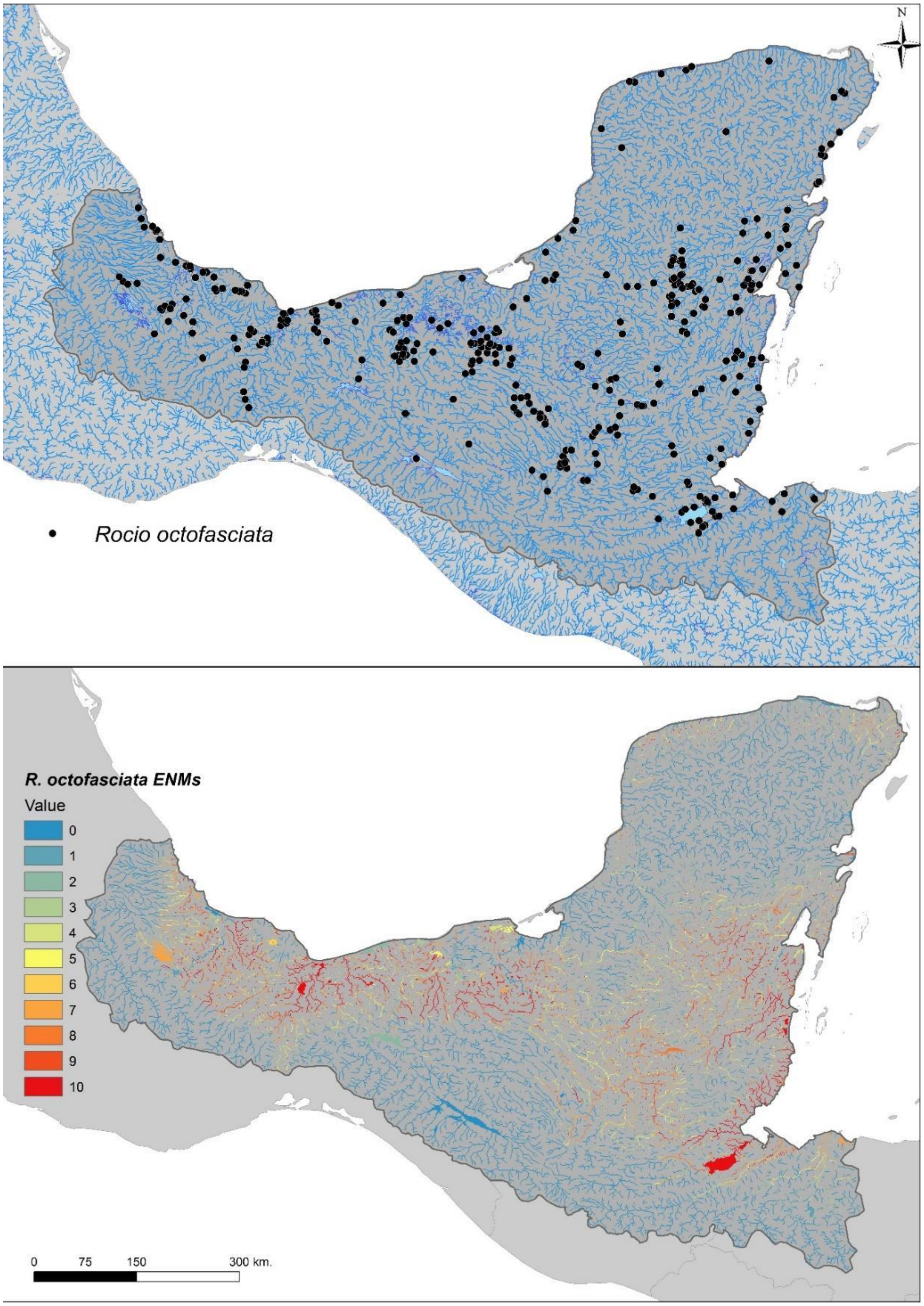

Figure 2.6. Maps of the Caribbean river basins and distribution of *Rocio octofasciata*. Upper panel show their presence (black circles), and lower panel show suitable habitat based on SDM's under on EarthEnv variables.

Body shape morphometrics

The first two Principal Components (PC) explained most of the body shape variation in all analyses. The PCA for all specimens (Fig. 2.7) shows that PC1 explained 41.71% as a primary axis, with an elongated body towards the negative values of the axis, while a deeper and more rounded body occurs towards the positive end of that axis. The PC2 explained 18.52% of the variation, and was associated with a steep forehead, terminal mouth and narrow caudal peduncle towards the negative end of the axis, while a longer caudal peduncle and taller forehead occur towards the positive end of the axis. Most specimens of *R. spinosissima* exhibited more rounded, deeper bodies, while most *R. octofasciata* had more elongated bodies, and exhibited greater variation on both PC1 and PC2. Convex hulls delimiting the two species (with both sympatric states for *R. octofasciata*) largely overlapped in the morphospace.

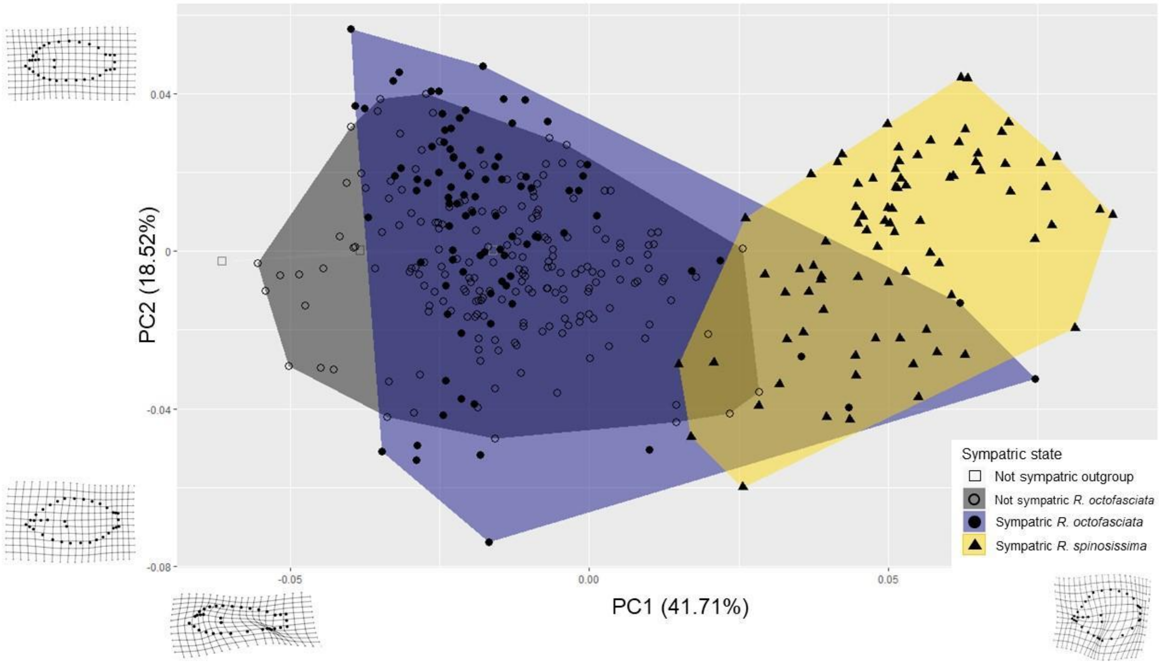

Figure 2.7. Principal component analysis of all (n=369) specimens.

The PCA for specimens larger than 30 mm (Fig. 2.8) shows that PC1 explained 42.70% of the variation in body shape, with an elongated body towards the negative end of the axis, while a deeper and more rounded body was associated with the positive end of that axis. The PC2 explained 16.70% of the variation, and was associated with tall forehead and tall caudal peduncle towards the negative end of that axis, while a steep forehead, terminal mouth and more posterior insertion of anal fin are associated with the positive end of that axis. All the specimens for *R. spinosissima* had a round and deep body, while most specimens of *R. octofasciata* had a more elongated body shape, and exhibited greater variation on both PC1 and PC2. Convex hulls delimiting the two species (with both sympatric states for *R. octofasciata*) indicate an overlap in morphospace between sympatric and non-sympatric specimens of *R. octofasciata*, while the convex hull for specimens of *R. spinosissima* shows no overlap with the other species.

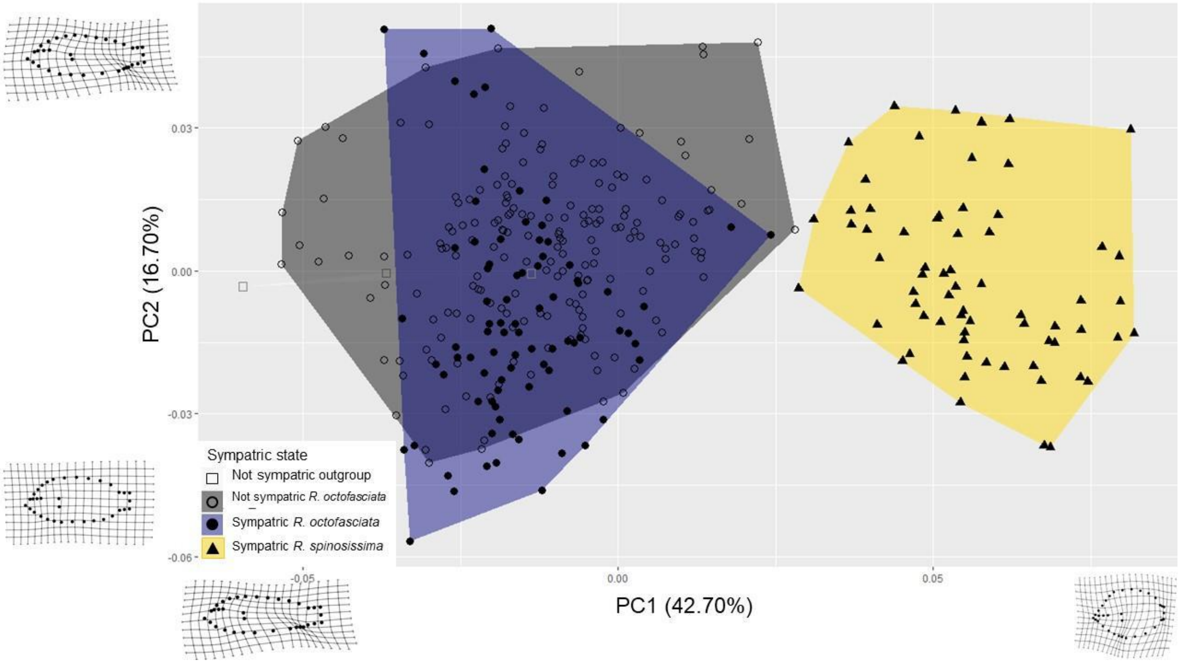

Figure 2.8. Principal component analysis of specimens over 30 mm of SL (n=341).

The PCA for specimens larger than 30% of the SL for each species (Fig. 2.9) shows very similar results to that of specimens with over 30 mm, with PC1 and PC2 explaining 42.70% and 16.70% of the variation, respectively. Convex hulls delimiting the three groups (two species, including both sympatric states for *R. octofasciata*) display an overlap in morphospace of specimens of *R. octofasciata* (sympatric and allopatric), while all *R. spinosissima* specimens are separated from the other species. The non-sympatric outgroup is almost entirely within the non-sympatric *R. octofasciata* portion of the space. The morphospace used by *R. spinosissima* across PC2 broadly overlaps with that of non-sympatric *R. octofasciata*, while the sympatric specimens of *R. octofasciata* overlap only partially with *R. spinosissima*.

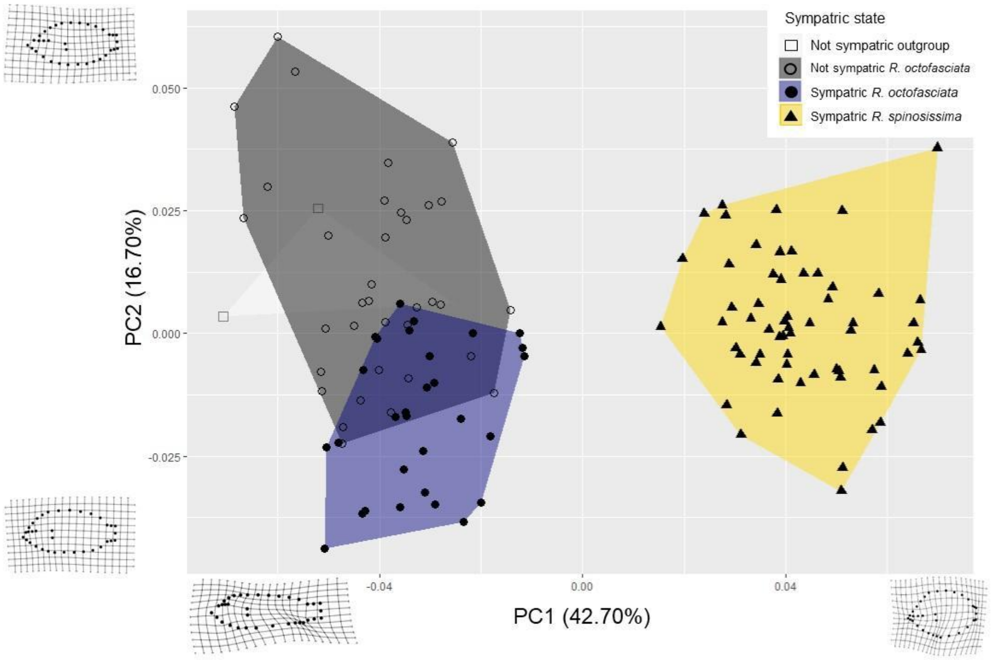

Figure 2.9. Principal component analysis of specimens over 30% of SL (n=124).

The PCA for specimens larger than 30% of the SL of *R. octofasciata* only (Fig. 2.10) shows that PC1 explained 37.43% of the variation in body shape, with a tall forehead and a narrow caudal peduncle towards the negative end of the axis, while a steep forehead, very terminal mouth and more posterior insertion of pectoral and anal fins was associated with the positive end of the axis. The PC2 explained 12.18% of the variation, showing a more elongated and depressed posterior body shape towards the negative end of the axis, while a deeper and more rounded body occurred towards the positive end of the axis. The majority of morphospace is occupied by non-sympatric specimens, while sympatric specimens fall mainly towards the negative side of PC1 and closer to the center of PC2. The convex hulls delimiting the two sympatric states show a partial overlap in response to the variation on PC1, with sympatric specimens reaching the most positive section of PC1.

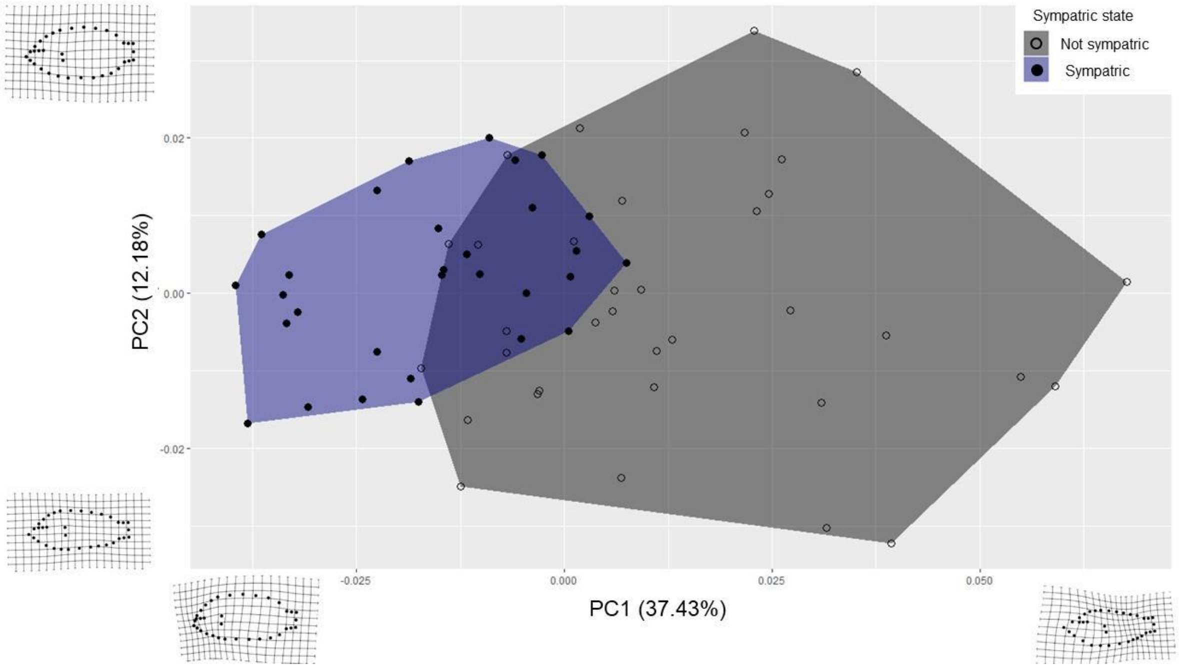

Figure 2.10. Principal component analysis of *R. octofasciata* over 30% of SL (n=65).

The multivariate regression of shape on size explained between 13.5% and 37.4% of the variation (constantly $p < 0.0001$), when using a sample with a mix of the two species. For the sample set with all specimens, sympatric state explained 10.8% of the variation, with the interaction of sympatric state and size explaining 2.6% of the variation ($p < 0.001$). For the sample set with specimens above 30 mm, the sympatric state explained 10.9%, with the interaction of sympatric state and size explaining 2.5% of the variation ($p < 0.0001$). For the sample set with specimens over 30% of SL the sympatric state explained 26.7% of the variation, with the interaction of sympatric state and size explaining up to 6.1% ($p < 0.0001$). When the specimens were analyzed by species, only the shape from *R. spinosissima* exhibited a significant size effect (10.6%, $p < 0.0001$), while size did not have an effect on shape in *R. octofasciata* (3.2%, $p = 0.055$). Both sympatric state (16.2%, $p < 0.0001$) and the interaction of sympatric state and size (2.7%, $p < 0.01$) had an effect on shape for the *R. octofasciata*.

Table 2. Results for multivariate regressions for body shape, size and sympatric state.

Sample used	Equation	Variable	R^2	Pr(>F)
All specimens	Shape ~ Size	Size	0.16534	9.999e-05***
		Residuals	0.83466	
Sample used	Equation	Variable	R^2	Pr(>F)
All specimens	Shape ~ Sympatry * Size	Sympatry	0.10831	0.001 **
		Size	0.15556	0.001 **
		Sympatry:Size	0.02574	0.001 **
		Residuals	0.71039	
Sample used	Equation	Variable	R^2	Pr(>F)
Specimens above 30 mm	Shape ~ Size	Size	0.1348	9.999e-05 ***
		Residuals	0.8652	
Sample used	Equation	Variable	R^2	Pr(>F)
Specimens above 30 mm	Shape ~ Sympatry * Size	Sympatry	0.10876	9.999e-05 ***
		Size	0.13526	9.999e-05 ***
		Sympatry:Size	0.02466	9.999e-05 ***
		Residuals	0.73132	
Sample used	Equation	Variable	R^2	Pr(>F)
All specimens above 30% total SL	Shape ~ Size	Size	0.37417	1e-04 ***
		Residuals	0.62583	
Sample used	Equation	Variable	R^2	Pr(>F)
All specimens above 30% total SL	Shape ~ Sympatry * Size	Sympatry	0.26728	9.999e-05 ***
		Size	0.19794	9.999e-05 ***
		Sympatry:Size	0.06092	9.999e-05 ***
		Residuals	0.47386	
Sample used	Equation	Variable	R^2	Pr(>F)
R. octofasciata specimens above 30% total SL	Shape ~ Size	Size	0.0323	0.05459
		Residuals	0.9677	
Sample used	Equation	Variable	R^2	Pr(>F)
R. octofasciata specimens above 30% total SL	Shape ~ Sympatry * Size	Sympatry	0.16241	9.999e-05 ***
		Size	0.03491	0.008299 **
		Sympatry:Size	0.02738	0.032097 *
		Residuals	0.7753	
Sample used	Equation	Variable	R^2	Pr(>F)
R. spinosissima specimens above 30% total SL	Shape ~ Size	Size	0.10635	9.999e-05 ***
		Residuals	0.89365	
Signif. Codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' '				

Lastly, the difference between each sympatric state group shows that the mean coordinates for specimens of *R. spinosissima* have a lower Euclidean distance from the mean coordinates of specimens of sympatric *R. octofasciata*, compared to those coordinates for specimens of non-sympatric *R. octofasciata* (Table 3). However, the difference on the PC2 axis for the mean coordinates is lower between *R. spinosissima* and non-sympatric *R. octofasciata* than when compared with sympatric *R. octofasciata*. This difference is larger than that between sympatric and non-sympatric specimens of *R. octofasciata*. We also found that the difference between means for morphological traits such as FR and PS, but no HR, show a greater difference between specimens from sympatric groups compared to the difference between specimens of non-sympatric groups. The distribution of values can be found in Figs. 2.11-2.12. This suggests that a character displacement pattern over FR and PS is seen in populations of *Rocio*.

Table 3. Divergence between sympatric states groups of Rocio specimens over morphospace and in morphological traits.

<table>
<tr><td colspan="5">Euclidean distance</td></tr>
<tr><td></td><td colspan="2"><i>R. spinosissima</i></td><td><i>R. octofasciata</i> non-sympatric</td><td><i>R. octofasciata</i> sympatric</td></tr>
<tr><td><i>R. spinosissima</i></td><td colspan="2">0.0000</td><td>Higher</td><td>Medium</td></tr>
<tr><td><i>R. octofasciata</i> non-sympatric</td><td colspan="2">0.0844</td><td>0.0000</td><td>Lower</td></tr>
<tr><td><i>R. octofasciata</i> sympatric</td><td colspan="2">0.0775</td><td>0.0292</td><td>0.0000</td></tr>
<tr><td colspan="5">Coordinates comparison in morphospace</td></tr>
<tr><td>Groups compared</td><td colspan="2">$|\bar{X}1-\bar{X}2|$</td><td>$|\bar{Y}1-\bar{Y}2|$</td><td>Comparison</td></tr>
<tr><td>A-<i>R. spinosissima</i> vs <i>R. octofasciata</i> non-sympatric</td><td colspan="2">0.0839</td><td>0.0093*</td><td>A < B</td></tr>
<tr><td>B-<i>R. spinosissima</i> vs <i>R. octofasciata</i> sympatric</td><td colspan="2">0.0752</td><td>0.0186*</td><td>B < C</td></tr>
<tr><td>C-<i>R. octofasciata</i> sympatric vs <i>R. octofasciata</i> non-sympatric</td><td colspan="2">0.0086</td><td>0.0279</td><td>NC</td></tr>
<tr><td colspan="5">Morphological traits comparison</td></tr>
<tr><td>Groups compared</td><td>|FR1-FR2|</td><td>|HR1-HR2|</td><td>|PS1-PS2|</td><td>Comparison</td></tr>
<tr><td>A-<i>R. spinosissima</i> vs <i>R. octofasciata</i> non-sympatric</td><td>0.3523*</td><td>0.1175</td><td>0.1547*</td><td>A < B</td></tr>
<tr><td>B-<i>R. spinosissima</i> vs <i>R. octofasciata</i> sympatric</td><td>0.3950</td><td>0.0874</td><td>0.1713</td><td>B < C</td></tr>
<tr><td>C-<i>R. octofasciata</i> sympatric vs <i>R. octofasciata</i> non-sympatric</td><td>0.0427</td><td>0.0301</td><td>0.0167</td><td>NC</td></tr>
<tr><td colspan="5">Bold and asterisk indicate the comparison is correct. FR= Fineness ratio; HR=Head ratio; PS=Peduncle size; NC = Not compared.</td></tr>
</table>

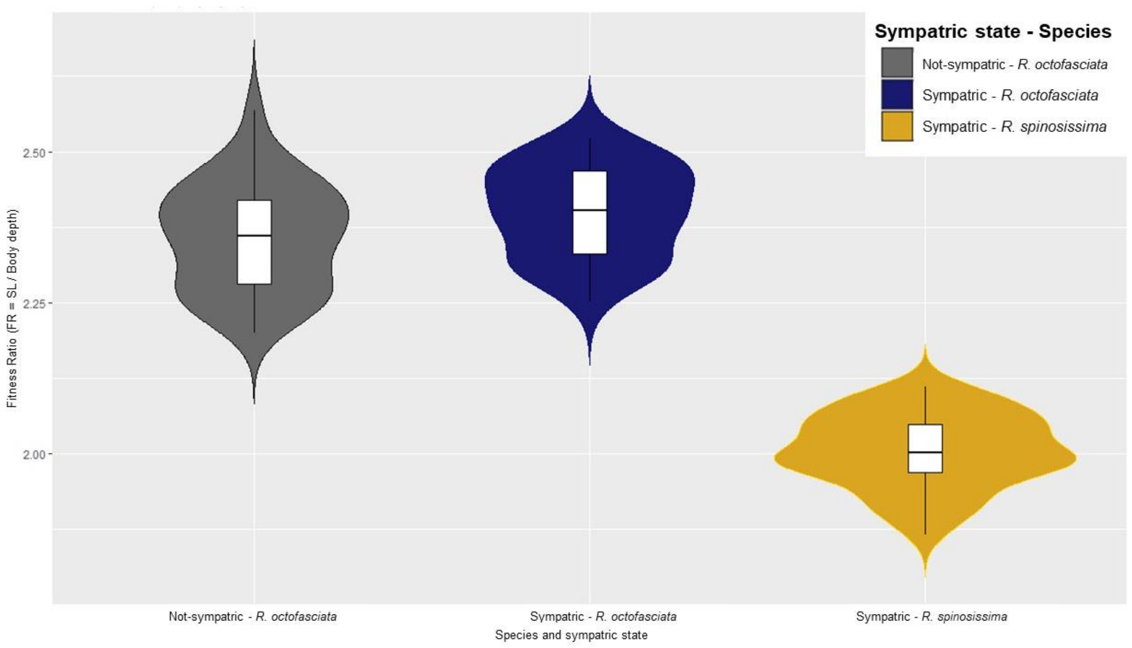

Figure 2.11. Distribution of fineness ratio (FR) values for each sympatric state group.

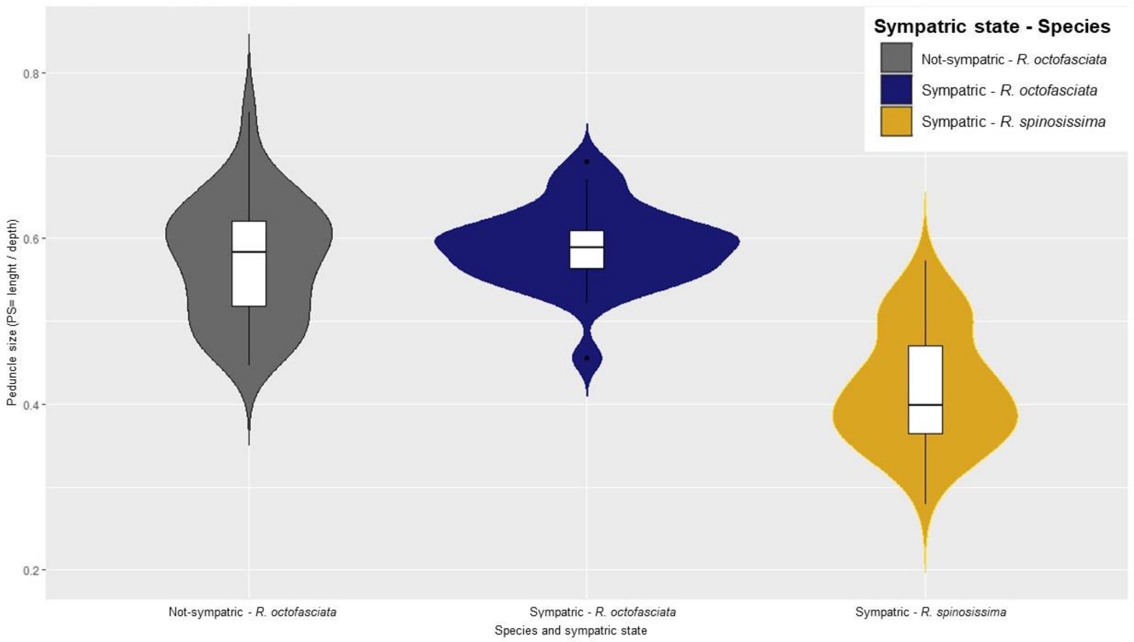

Figure 2.12. Distribution of peduncle size (PS) ratios for each sympatric state group.

DISCUSSION

Distribution, abundance and habitat characteristics

The genus *Rocio* has been described as omnivorous-detritivorous and is commonly found throughout the entire Caribbean slope of northern Middle America (Miller, 2005; Hinojosa-Garro et al., 2013; Říčan et al., 2016). However, this has long been based on the assumption that species in this genus all have similar characteristics and ecology to that type species (*R. octofasciata*), since descriptions of the habitats for the other species of *Rocio* are limited. A great example of this is the endemic *R. spinosissima*, which has been considered rare and difficult to find throughout its narrow range (Lyons et al., 2020b), with almost no description of its habitat and ecology until now. The limited field explorations and restricted annotation of aquatic habitats and environments in natural populations for both *R. spinosissima* and *R. octofasciata*, even since their original descriptions (Vaillant & Pellegrin, 1902; Regan, 1903), might have blinded the understanding of the habitats and interactions of the species. Our results provide a new perspective into the ecology of these two species and how they differ.

Despite its wide distribution, *Rocio* should not be considered common. For example, *R. octofasciata* has one of the larger ranges for northern Middle American cichlids, but collections within the Dulce River basin have been scarce. The rareness and difficulty of finding these species within the Dulce River basin can be clearly linked to the specificity of their habitats by comparing our results with previous work done. Barrientos & Allen (2008) carried out extensive sampling efforts applying rotenone inside blocknets throughout the shores of Lake Izabal with no captures for either species of *Rocio*. Barrientos et al. (2012) used the same methods over the three major lakes in Guatemala, including Lake Izabal, and were able to capture a total of 24,832 fish, but only managed to capture two (2) specimens of *R. spinosissima* and none of *R. octofasciata*. Similarly, Quintana et al. (2011) used the same methodology in the Rio Dulce National Park, collecting 7,590 fish with only two (2) specimens of *R. spinosissima* and none of *R. octofasciata*. Further, SDMs show more suitable habitats for both species in lower reaches of the rivers and specific subsections of the Dulce River basin, with Río Dulce and El Golfete as coincident suitable areas for both species (Fig 11), while highlighting the north side of Rio Dulce for *R. octofasciata* and the Polochic River for *R. spinosissima*. Suitable habitats were not predicted in the Izabal Lake. When comparing our sampling effort and the actual presence records (Fig. 2.5), along with our quantitative environmental analysis depicted by the parsimonious RDA for the two species (Figs. 2.3-2.4) also highlight the habitat specificity of these species throughout the Dulce River basin.

Not only should commonness *per se* be treated carefully, but also the status of 'generalist' might not be accurate for the genus. We now know that the two species are quite unusual in every kind of habitat throughout the Dulce River basin. Fast flowing water and densely covered canopy are characteristics that will not provide the kind of habitat where either species is found. On the other hand, many ponds or shallow lakes possessing different environmental characteristics (as in Richardson et al., 2022) are found in the Dulce River basin and could provide an appropriate space for *Rocio*. The uneven distribution of these fish, along with their observed abundances, suggests that they have a clumped distribution and are limited to those habitats where not many other cichlid species live. Interestingly, the whole area is prone to flash flooding which sets a dynamic landscape for both *R. spinosissima* and *R. octofasciata*.

Environmental similarities and differences

Our sampling demonstrated that the two species can be found in the same localities, sharing habitat characteristics but with slight differences. The parsimonious RDA in the quantitative environmental analysis for both species used Canopy, Sand and Boulders for their predictions, broadly describing the sites where both species were found. These variables mainly influenced the RDA in a negative proportion, describing the sites as places with little canopy cover and small-sized grain on the substrate. These sites reflect open and slow-flowing habitats. The parsimonious RDA for *R. octofasciata* also included Gravel and Rock with a negative contribution, added to Pond and Stream, highlighting the different habitats where this species occurs, especially sites with soft bottoms. On the other hand, the parsimonious RDA for *R. spinosissima* included pH, Width, Mud, and Sand, highlighting the narrow and slow flowing habitats where this species lives, but also pointing out the characteristic wide range of pH values at sites in which *R. spinosissima* can be found. Miller et al. (2005) described *R. octofasciata* to also inhabit wetlands, springs and ditches in addition to ponds and streams as we found for the Dulce River basin. However, they did not explicitly describe a relationship to any type of substrate, and described an abundant vegetation. Despite this, they mention that no to slow flowing waters are often seen at sites where *R. octofasciata* lives, which coincides with what we observed and can be also described for *R. spinosissima* as well.

The larger scale environmental analysis provides a basin-scale perspective that can be linked to the quantitative analysis. While all ENMs show DEM as the main contributor for the models (Table 1), the second most contributing variable to *R. spinosissima* models is the LC_ran, which almost doubles the second most contributing variable for *R. octofasciata* (Pre_wsum) at either scale. This links the open spaces with little canopy and certain land use where we found *R. spinosissima* to the ENMs, and the precipitation amounts to the type of waterbodies where we found *R. octofasciata*, being those mainly ponds and streams, instead of rivers. Another variable that appears as a top contributor is Flow, which generally describes the slow flowing waterbodies where *Rocio* can be found. This adds to various soil and temperature characteristics that cover the remaining top contributing variables for the ENMs, reflecting the soft bottoms and high temperatures among the sites with presence for the genus. Although DEM represents much of the variation across the basin, it also matches with the low elevations where we recorded both species, backing up the fact that both *R. spinosissima* and *R. octofasciata* can be found in waterbodies located in lowlands and plains across the Dulce River basin.

Finding some differences in habitat characteristics for these species makes sense, since habitats for cichlids are quite diverse (Miller et al., 2005; Montaña & Winemiller, 2009; Albert et al., 2020). It becomes even more noticeable in some cases where, in similar regions, one species may display high habitat specificity while another may be more flexible in it habitat use (Barrientos & Allen, 2008; McMahan et al., 2020). Not only are their habitats are different, but habitat partitioning has also been associated with divergent morphologies (Langerhans et al., 2003; Albertson, 2008; López-Fernández et al., 2013; Ford et al., 2016; Conith et al., 2020). Therefore, making a connection between the environments where we found *Rocio* and their varying morphologies will add support for ecomorphological models linking divergence in cichlid habitats with divergence in morphology.

Body shape variation and character displacement

Our results show a clear differentiation in body shape between the two species of *Rocio* living in sympatry within the Dulce River basin (Figs. 2.7-2.9), with *R. spinosissima* having a rounder, deeper body and *R. octofasciata* having an elongated body shape. Body shape, particularly body elongation, has been recovered as a main effector over morphospace in other fish studies (López-Fernández et al., 2013; Claverie & Wainwright, 2014; Aguirre et al., 2016; Malato et al., 2017; Ardón et al., 2022), where body shape varies along the main axis. This variation is also greatly linked to water flow and the habitats resulting from flow changes (Langerhans, 2008). This is consistent with the greater variability on habitats seen for *R. octofasciata* compared to those of *R. spinosissima*, where an elongated body shape could facilitate inhabiting areas with different flow regimes. Body shape variation has also been associated with hypoxia levels as a plastic response to pressures in the environment, where deeper bodies were observed in fish reared under hypoxic conditions (Crispo & Chapman, 2011). This coincides with the direction of body shape divergence observed for *R. spinosissima*, being this species the one we found in sites with surprisingly low oxygen levels (1.9-45.7 DO%), compared to *R. octofasciata* (21.0-54.6 DO%). Another possible cause for the observed body shape pattern can be attributed to the presence of predators (Andersson et al., 2006; Johansson & Andersson, 2009), which has been linked to the development of deeper bodies. This could be the case for *R. spinosissima*, where predation in their habitats might be a factor influencing their body shape, but considering that trophic dynamics in the region are poorly known, more research is be needed to assert this.

Interestingly, the group including a small sample from the other two currently valid species (*R. gemmata* and *R. ocotal*) fell closer to *R. octofasciata* than to *R. spinosissima*, with the convex hulls completely overlapping (Figs. 2.7-2.9). This similarity in body shape might be due to ecological similarities between these species, phylogenetic relatedness or doubtful taxonomic delimitation. A recent proposal for a taxonomic change, in which the validity of *R. gemmata* was in dispute (Schmitter-Soto, 2021; Artigas-Azas, 2018), may be worth further research given our results. In addition, after assessing the variation using different sized specimens, we acknowledge an allopatric effect for the genus *Rocio*, where smaller sized individuals display a more similar body shape even among specimens from different species (Figs. 2.7-2.9) as seen with other neotropical freshwater fish (Aguirre et al., 2016), and more clearly with cichlids (Ardón et al., 2022). This could also mean that different developmental stages in the genus could show different levels of ecological divergence.

Changes along the second axis in morphospace describe differences related to mouth positioning, forehead shape and slight differences on fins and caudal peduncle, which is more evident in those specimens from *R. octofasciata*. Having this intraspecific variation is marked by the comparison between specimens from sympatric and non-sympatric basins. If we use *R. spinosissima* as a reference, the body shapes of sympatric vs. non-sympatric *R. octofasciata* are significantly different (Fig. 2.10; Table 2). Other geometric morphometrics examples using northern Middle American fish show that intraspecific body shape variation is common (Ardón et al., 2022; Macossay-Cortez et al., 2022; McMahan et al., 2017b), and might lead to understanding the kinds of habitats where those species are found (Aguilar-Contreras et al., 2021). However, previous assessments of body shape variation in freshwater sympatric fish species have shown no clear pattern of character displacement in body shape (Garita-Alvarado et al., 2021; Elmer et al., 2010; Klingenberg et al., 2003). Here, we provide an example of character displacement using the body shape variation in the genus *Rocio* from the Dulce River basin. Although the Euclidean distance was less between sympatric groups, the difference between PC2 coordinates show a greater separation between them (Table 3). Specifically, FR and PS were more divergent between sympatric groups than those in allopatry.

Ecomorphological and evolutionary implications

Our analysis provides a new perspective and brings insight into the ecology of both *R. spinosissima* and *R. octofasciata* by understanding their environments and habitats, along with their body shape. Since the morphology of organisms reflects their biotic and abiotic interactions (Wiens et al., 2010; Pianka, 2011; Pease et al., 2012; Barbosa et al., 2019), we can infer that the body elongation plays a role in movement and the use of spaces by each species. However, sympatric specimens differ more than non-sympatric *R. octofasciata* and *R. spinosissima*, particularly in mouth position, head shape, fin insertions and caudal peduncle over morphospace, and for traits such as FR and PS. This is likely linked to their roles in the ecosystem (Violle et al., 2007) and their divergent ecological characteristics such as distinct feeding habits and trophic positioning (Soria-Barreto et al., 2019; Pease et al., 2018; Pease et al., 2012).

Ecomorphological variation has been recorded in fish, even within a single region (Luiz et al., 2022), suggesting that functional traits could have a different impact from individual to individual, rather than only from species to species. Since our results indicate that morphological variation could be enough to separate groups within a species, displaying a divergent body shape would be an example of how specific traits would have a different impact in the ecosystem with specific peculiarities within groups. Some of these peculiarities would probably set *Rocio* spp. in clear guilds within the fish assemblage (Córdova-Tapia & Zambrano, 2016). This raises the question of how much do *Rocio spp.* differ from other species in the fish assemblage, particularly in the Dulce River basin.

Both species share certain habitats but their body shapes differ markedly. This is exemplified in specimens from the Dulce River basin sympatric area, where the species tend to diverge rather than overlap in morphospace. Instead, *R. octofasciata* not in sympatry overlap almost entirely with *R. spinosissima* across PC2 (Fig. 2.9). Considering that character displacement is usually referred to the pattern of divergence in sympatry relative to allopatry between species (Stuart et al., 2017; Brown & Wilson, 1965), we can understand that the observed morphologies are linked not only to the ecology but also the evolution of the genus *Rocio*. Some processes that have been suggested as an explanation for character displacement include resource competition or predation, ecological sorting or even phenotypic plasticity (Stuart et al., 2017). Although data are lacking to explain causative agents driving character displacement documented, accelerated feeding trait evolution has been suggested for northern Middle American cichlids (Arbour, & López-Fernández, 2016) and could be at play here. Ecomorphology can be linked to adaptive radiation (López-Fernández et al., 2013) and diversification of functional groups (Arbour & López-Fernández, 2014). Thus, consideration of processes involving ecological divergence in allopatry before the two *Rocio* species coexisted in the Dulce River basin, or both species competing for resources should not be ruled out, especially since sympatric speciation is considered rare (Albert et al., 2020).

Conservation implications

The region of study has seen historically varying climatic and hydrologic patterns (Obrist-Farner et al., 2022; Obrist Farner et al., 2020), particularly showing rising and decreasing water levels as seen in the geological record. Floodplains have been suggested to result in high connectivity with rising water levels (Hurd et al., 2016), a pattern that could explain some of the evolution of *Rocio* in the Dulce River basin. This is important since pulsating and dynamic changes in water levels have been demonstrated to play a major role in freshwater Neotropical ecosystems (Winemiller et al., 2014), even displaying marked changes in niche breath for some species (Quirino et al., 2017). Many examples of fish living in fast changing floodplains have been described using ephemeral ponds (Abrantes et al., 2020; Polačik & Podrabsky, 2015; Berois et al., 2014), where some similarities in habitats to those used by *Rocio* have been observed. However, considering that environmental variables have a key influence in the fish assemblages rather than spatial factors (Lopez-Delgado et al., 2020), the characteristics of these environments should also be considered. Thus, the characteristics that define the habitats must be prioritized in conservation planning.

Coastal wetlands, such as those within and next to the Dulce River basin, are expected to suffer migration inland due to climate change (Osland et al., 2022), while future ecological models predict changes for freshwater fish species in the Caribbean slope of northern Middle America (McMahan et al., 2020). Large storms such as hurricanes have been registered to have a clear effect over the area (Cochran et al., 2009) and even have recently affected the Dulce River basin area as well (IFRC, 2022). Those changes might also influence effectors from biocides or even incidental releases of non-native fish species that affect the local ecosystems. Palm plantations (*Elaeis guineensis*) for oil extraction have increased through the region in the last 20 years, and they are settled in places with great proximity to water sources (Camacho-Valdez et al., 2022). Some sites where we detected the presence of *Rocio* are within the palm matrix, raising concern of a potential extirpation of those populations due to rapid environmental changes related to the plantation. In addition, some sites where the two *Rocio* species were found have other agricultural uses (mainly livestock pastures) and might also be threatened if land management changes abruptly. Although floodplains with agricultural fields are used by fish (Katz et al., 2017), details on the population dynamics for

Rocio spp., particularly the endemic *R. spinosissima*, and how they manage to survive in those environments remains unknown.

Both species of *Rocio* are probably not rare, but occupy a specific niche and habitat, which overlaps possibly due to niche conservatism (Losos, 2008; Wiens, 2008; Wiens et al., 2010; Pyron et al., 2015). Despite the many ichthyological sampling efforts that have been done throughout the basin, it is only now that we have a better sense of where these species live in the Dulce River basin. The data from this study represents the most important recent collections of the species, which may be more common within the basin than previously thought. Recent IUCN assessments proposed that *R. spinosissima* should be listed as Endangered (Lyons et al., 2020b), and our data will be of great importance for further re-assessment of the species. On the other hand, the IUCN assessment for *R. octofasciata* listed it as a species of Least Concern (Schmitter-Soto, 2019), and our data supports the idea that their populations might be stable, despite several threats present. The more variable habits of *R. octofasciata* even allow its relatively easy establishment in those places where it has been introduced outside of its native range (Nico & Neilson, 2022; Pashkov & Zvorykin, 2009), which might give ease of thought regarding their conservation status. However, since *R. octofasciata* has a wide distribution range, it may warrant some attention since this species might not be as common as previously thought, at least within the Dulce River basin. Special consideration should be given to those habitats where these species live, especially since they are susceptible to threats such as climate change, land use and other anthropogenic factors (Su et al., 2021).

CONCLUSIONS

The genus *Rocio*, particularly the endemic *R. spinosissima*, had been neglected in ecological studies and recent collections were scarce in the Dulce River basin. Our work provides a better understanding of the distribution, abundance, habitats and environmental niche for both *R. spinosissima* and *R. octofasciata*. Our quantitative analysis indicates that the two species share some environmental with similar characteristics, including soft bottoms (related to slow water flow) and open spaces (low canopy cover). The quantitative analysis also provides evidence of the specific characteristics of the environmental niche of each species. These differences include greater variation in types of waterbodies that serve as habitat for *R. octofasciata*, and a greater variation in pH levels and a higher association with narrow and slow flowing habitats for *R. spinosissima*. Additionally, ENMs highlighted the importance of Elevation as a main driver at a basin scale, but other major contributing variables for ENMs were identified at a finer spatial scale. The latter includes Land Cover for *R. spinosissima* and Precipitation for *R. octofasciata*, among other variables describing Land Cover, Soil and Temperature characteristics for both species. SDMs indicate there are sections with the greater suitable habitat for each species, having a major overlap in the low reaches of the Dulce River basin, which is where many of our collections came from. Therefore, we provided a clear assessment of the environmental characteristics of the sites where *Rocio spp*. live in sympatry.

The body shape is quite different between the two species of *Rocio* examined. *Rocio spinosissima* possesses a deep and rounded body shape, while *R. octofasciata* has an elongated body. The second axis of variation for the geometric morphometrics analysis indicates variation associated with head shape, mouth position, insertion of fins and caudal peduncle shape, all of which likely represent a response to trophic ecology rather than water flow regimes. We found high similarity in body shape from an outgroup with the set of specimens from *R. octofasciata*, suggesting that further taxonomic assessment must be done. An allometric effect was observed in all groups, suggesting that smaller sized individuals (i.e. juveniles) have a more similar shape between species, as seen in other cichlid species in the region. Finally, the body shape variation seen in non-sympatric *R. octofasciata* largely overlaps with that seen in *R. spinosissima*, but the shape variation in sympatric *R.*

octofasciata only overlaps partially with *R. spinosissima*. After assessing the effect of their sympatric state, a statistically significant difference was observed when comparing sympatric and non-sympatric specimens of *R. octofasciata*. Therefore, we provide evidence of character displacement between our study species in the Dulce River basin.

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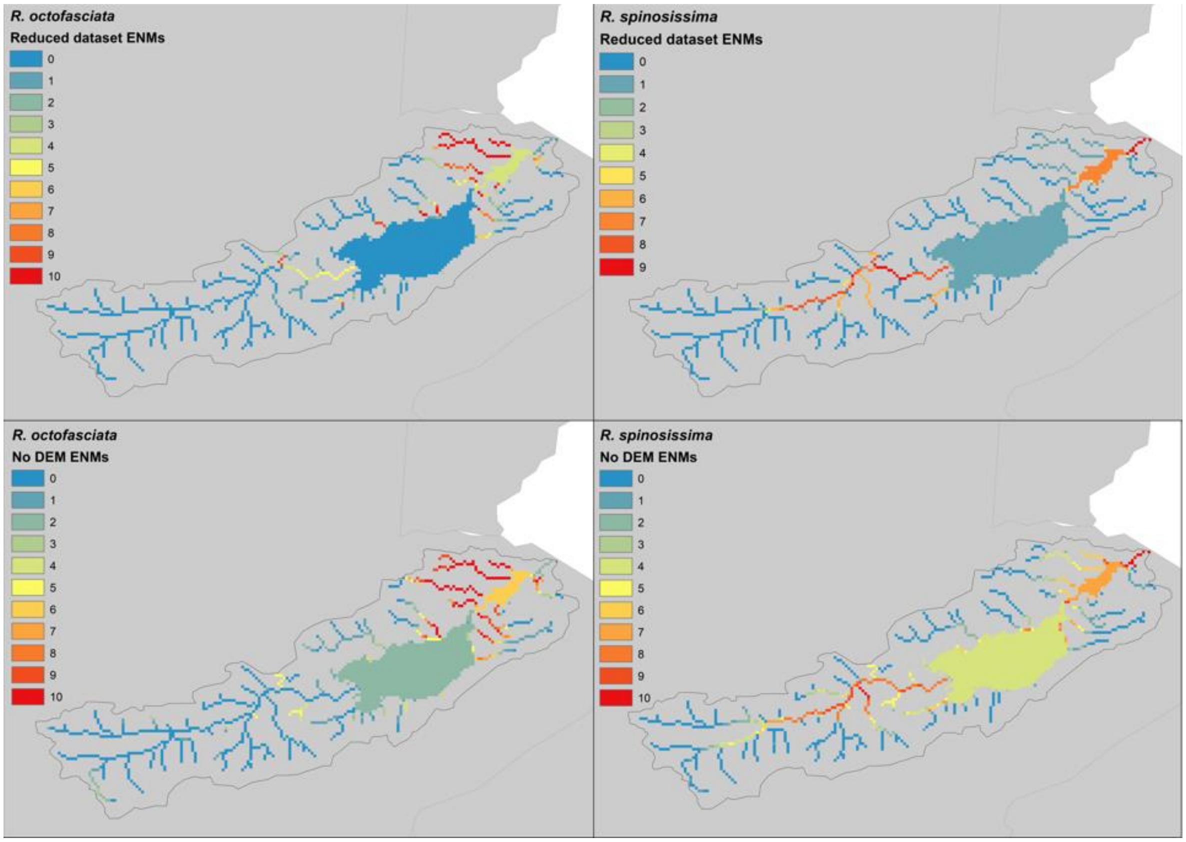
SUPPLEMENTAL MATERIAL

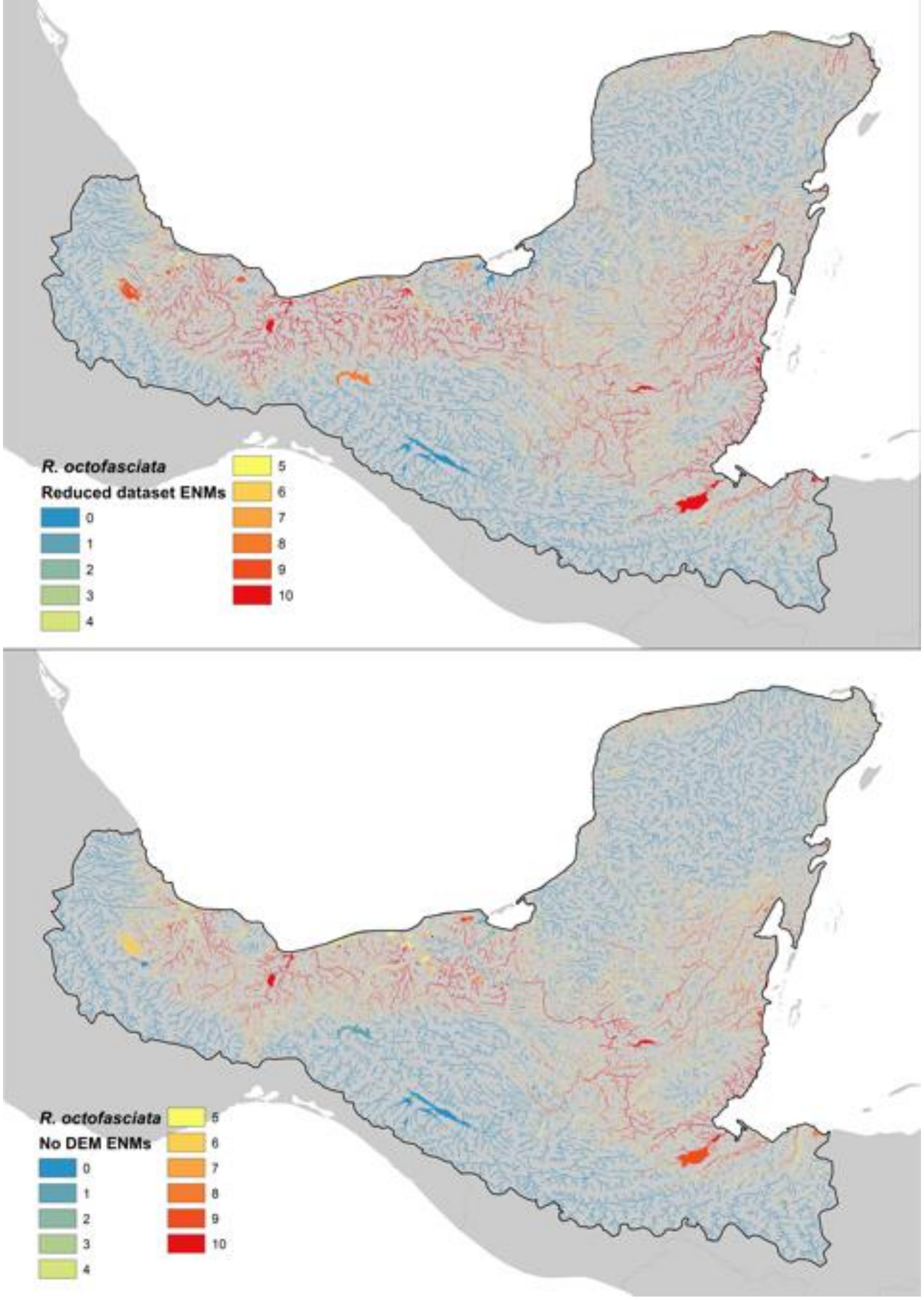
Supplementary Figures

Supplementary Figure 1. Example individual of *Rocio spinosissima* from the Dulce River basin.

Supplementary Figure 2. Example individual of *Rocio octofasciata* from the Dulce River basin.



Supplementary Figure 3. Maps of the suitable habitat in the Dulce River basin based on SDMs of *Rocio*, with the left panels for *R. octofasciata* and the right panels for *R. spinosissima*. Upper panels show SDMs using reduced datasets, while lower panels show SDMs using partially reduced dataset with no elevation.



Supplementary Figure 4. Maps of the suitable habitat in the Caribbean river basins based on SDMs of *Rocio octofasciata*. Upper panel show SDMs using reduced datasets, while lower panel show SDMs using partially reduced dataset with no elevation.

Supplementary Figure 5. Photographs from surveying events throughout the Dulce River Basin.

Supplementary Figure 6. Photographs from sampling sites throughout the Dulce River Basin. Upper panels show sites where *Rocio* individuals were not collected, lower panels show sites where specimens were collected.

Supplementary Tables

Supplementary Table 1. List of variables used for the ENMs for each species, per range size and setting.

Variable abbreviation	Full name and description	*Rocio octofasciata*						*Rocio spinosissima*		
		Large range			Small range			Small range		
		All layers	No DEM	Reduced dataset	All layers	No DEM	Reduced dataset	All layers	No DEM	Reduced dataset
DEM	Upstream elevation	X		X	X		X	X		X
Flow	Upstream catchment and stream length (sum)	X	X	X	X	X	X	X	X	X
Hydavg	Hydroclimatic variables (average and sum)	X	X		X	X		X	X	X
Hydwavg	Hydroclimatic variables (distance-weighted average and sum)	X	X		X	X		X	X	
LC_avg	Upstream landcover coverage (average)	X	X		X	X		X	X	
LC_max	Upstream landcover coverage (maximum)	X	X	X	X	X		X	X	
LC_min	Upstream landcover coverage (minimum)	X	X	X	X	X		X	X	
LC_ran	Upstream landcover coverage (range)	X	X		X	X		X	X	X
LC_wavg	Upstream landcover coverage (distance-weighted average)	X	X	X	X	X	X	X	X	
Pre_sum	Monthly upstream precipitation (sum)	X	X		X	X		X	X	
Pre_wsum	Monthly upstream precipitation (distance-weighted sum)	X	X	X	X	X		X	X	
Slope	Stream length and flow accumulation	X	X	X	X	X	X	X	X	X
Soil_avg	Upstream soil (average)	X	X		X	X		X	X	
Soil_wavg	Upstream soil (distance-weighted average)	X	X	X	X	X		X	X	
Soil_max	Upstream soil (maximum)	X	X	X	X	X	X	X	X	
Soil_min	Upstream soil (minimum)	X	X	X	X	X	X	X	X	X
Soil_ran	Upstream soil (range)	X	X		X	X		X	X	
Tmax_avg	Monthly maximum temperature (average)	X	X	X	X	X	X	X	X	X
Tmax_wavg	Monthly maximum temperature (distance-weighted average)	X	X		X	X		X	X	X
Tmin_avg	Monthly minimum temperature (average)	X	X		X	X		X	X	
Tmin_wavg	Monthly minimum temperature (distance-weighted average)	X	X		X	X		X	X	
Total variables used		21	20	11	21	20	7	21	20	8

Supplementary Table 2. ENMs assessment after 10k maximum iterations, using 10 replicates and 25% of records to test						
Species	Range	Variables set	TSS		AUC	
			Average	Standard deviation	Average	Standard deviation
Rocio octofasciata	Large	All variables	0.4269	0.06989	0.8192	0.00885
		No Elevation (DEM)	0.44154	0.04352	0.8058	0.01117
		Reduced dataset	0.41352	0.04596	0.791	0.01558
Rocio octofasciata	Small	All variables	0.451	0.31595	0.8975	0.05393
		No Elevation (DEM)	0.37022	0.32383	0.8758	0.05439
		Reduced dataset	0.53948	0.21058	0.8706	0.04828
Rocio spinosissima	Small	All variables	0.43269	0.1212	0.8033	0.05054
		No Elevation (DEM)	0.41226	0.3323	0.8163	0.07373
		Reduced dataset	0.40629	0.26277	0.8272	0.03677

Supplementary Table 3. List of specimens used for geometric morphometrics.

	Species	Country	Department or State	Drainage	Catalog number	SL (mm)
1	R.gemmata	Mexico	Quintana Roo	Yucatan	ECO-CH-3145	52.4
2	R.gemmata	Mexico	Quintana Roo	Yucatan	ECO-CH-4054	31.3
3	R.ocotal	Mexico	Chiapas	Grijalva-Usumacinta	UMMZ-245583	104.9
4	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-6989	76.8
5	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-6989	88.8
6	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7101	77.2
7	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7101	54.9
8	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7101	53.3
9	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7101	62.1

10	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7101	118.4
11	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7111	65.4
12	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7111	98.7
13	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7111	94.4
14	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7111	105.1
15	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7111	68.8
16	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7111	73.7
17	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7111	74.8
18	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7111	77.0
19	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7111	83.5
20	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7111	79.7
21	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7111	86.3
22	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7111	92.1
23	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7660	111.1
24	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7660	111.3
25	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7660	125.3
26	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-7660	128.3
27	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-986	61.7
28	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-986	83.0
29	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-986	76.9
30	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	ECO-SC-986	116.5
31	R.octofasciata	Belize	Belize	Belize	FMNH-104555	29.8
32	R.octofasciata	Belize	BZ	BZ	FMNH-104556	47.6
33	R.octofasciata	Belize	BZ	BZ	FMNH-104556	40.4
34	R.octofasciata	Belize	BZ	BZ	FMNH-104556	49.4
35	R.octofasciata	Belize	BZ	BZ	FMNH-104556	40.5
36	R.octofasciata	Belize	BZ	BZ	FMNH-104556	35.2
37	R.octofasciata	Belize	BZ	BZ	FMNH-104556	38.7
38	R.octofasciata	Belize	BZ	BZ	FMNH-104556	33.3

39	R.octofasciata	Belize	BZ	BZ	FMNH-104556	39.1
40	R.octofasciata	Belize	BZ	BZ	FMNH-104556	36.4
41	R.octofasciata	Belize	BZ	BZ	FMNH-104556	34.9
42	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-109030	41.3
43	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-109031	57.3
44	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-109031	58.7
45	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-109031	60.3
46	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-109031	50.4
47	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-109031	62.0
48	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-109032	39.5
49	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-109033	49.3
50	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-109033	46.1
51	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-109034	40.6
52	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-109035	37.0
53	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-109035	37.6
54	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-109035	36.3
55	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-109036	70.5
56	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-109037	46.0
57	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-130811	78.1
58	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-130811	63.3
59	R.octofasciata	Guatemala	Alta Verapaz	Grijalva-Usumacinta	FMNH-131843	107.0
60	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-131844	78.7
61	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-134255	59.3
62	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-134314	56.1
63	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	FMNH-134314	56.8
64	R.octofasciata	Guatemala	Izabal	Motagua-Ulua	FMNH-56194	54.7
65	R.octofasciata	Guatemala	Izabal	Motagua-Ulua	FMNH-7951	69.4
66	R.octofasciata	Belize	BZ	BZ	FMNH-82025	33.6
67	R.octofasciata	Belize	BZ	BZ	FMNH-82025	34.7

68	R.octofasciata	Belize	Belize	BZ	FMNH-82122	44.5
69	R.octofasciata	Belize	Belize	BZ	FMNH-82122	40.2
70	R.octofasciata	Belize	Belize	BZ	FMNH-82122	43.6
71	R.octofasciata	Belize	Belize	BZ	FMNH-82122	38.6
72	R.octofasciata	Belize	Belize	BZ	FMNH-82122	42.0
73	R.octofasciata	Belize	Toledo	BZ	FMNH-82224	63.8
74	R.octofasciata	Belize	Toledo	BZ	FMNH-82224	51.1
75	R.octofasciata	Belize	Toledo	BZ	FMNH-82224	53.8
76	R.octofasciata	Belize	Toledo	BZ	FMNH-82224	49.5
77	R.octofasciata	Belize	Toledo	BZ	FMNH-82224	54.0
78	R.octofasciata	Belize	Toledo	BZ	FMNH-82224	45.9
79	R.octofasciata	Belize	Toledo	BZ	FMNH-82224	56.7
80	R.octofasciata	Belize	BZ	BZ	FMNH-82293	25.2
81	R.octofasciata	Belize	BZ	BZ	FMNH-82293	32.5
82	R.octofasciata	Belize	BZ	BZ	FMNH-82304	35.9
83	R.octofasciata	Belize	Orange_Walk	BZ	FMNH-82331	51.1
84	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	39.3
85	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	41.7
86	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	34.7
87	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	55.3
88	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	34.3
89	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	35.9
90	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	30.6
91	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	36.1
92	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	48.2
93	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	38.6
94	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	39.2
95	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	37.1
96	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	36.8

97	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	55.0
98	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	36.0
99	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	35.0
100	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	39.1
101	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	28.6
102	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	44.2
103	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	42.3
104	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	37.3
105	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	36.0
106	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	28.5
107	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	36.9
108	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	33.0
109	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-82394	40.8
110	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-83010	56.4
111	R.octofasciata	Belize	Stann_Creek	BZ	FMNH-83010	71.5
112	R.octofasciata	Belize	Belize	BZ	FMNH-83020	55.2
113	R.octofasciata	Belize	Belize	BZ	FMNH-83020	44.8
114	R.octofasciata	Belize	Belize	BZ	FMNH-83020	41.5
115	R.octofasciata	Belize	Belize	BZ	FMNH-83020	34.3
116	R.octofasciata	Belize	Belize	BZ	FMNH-83039	51.3
117	R.octofasciata	Belize	Belize	BZ	FMNH-83039	43.5
118	R.octofasciata	Belize	BZ	BZ	FMNH-83110	48.2
119	R.octofasciata	Belize	BZ	BZ	FMNH-83110	62.3
120	R.octofasciata	Belize	BZ	BZ	FMNH-83110	54.8
121	R.octofasciata	Belize	Belize	BZ	FMNH-97662	71.5
122	R.octofasciata	Belize	Belize	BZ	FMNH-97662	57.6
123	R.octofasciata	Belize	Belize	BZ	FMNH-97662	41.4
124	R.octofasciata	Belize	Belize	BZ	FMNH-97662	44.8
125	R.octofasciata	Belize	Belize	BZ	FMNH-97662	42.7

126	R.octofasciata	Belize	Toledo	BZ	FMNH-97667	55.3
127	R.octofasciata	Belize	Toledo	BZ	FMNH-97667	80.9
128	R.octofasciata	Belize	BZ	BZ	FMNH-97681	60.1
129	R.octofasciata	Belize	BZ	BZ	FMNH-97681	75.8
130	R.octofasciata	Belize	BZ	BZ	FMNH-97681	57.5
131	R.octofasciata	Belize	BZ	BZ	FMNH-97681	64.3
132	R.octofasciata	Belize	BZ	BZ	FMNH-97681	66.6
133	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147153	126.2
134	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147153	37.1
135	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147153	30.6
136	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147153	36.3
137	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147153	48.4
138	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147153	38.7
139	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147153	28.5
140	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147153	29.7
141	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147153	30.6
142	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147153	31.6
143	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147152	108.6
144	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147152	89.0
145	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147152	76.3
146	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147152	91.9
147	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147152	84.7
148	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147152	105.9
149	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147152	111.9
150	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147152	91.3
151	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147152	125.8
152	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147353	54.2
153	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147354	23.7
154	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	37.6

155	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	40.6
156	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	39.0
157	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	48.4
158	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	45.7
159	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	38.2
160	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	38.8
161	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	74.8
162	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	65.8
163	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	69.9
164	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	67.4
165	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	36.7
166	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	40.4
167	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	60.5
168	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	75.0
169	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	75.5
170	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	60.9
171	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	27.9
172	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	36.9
173	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	51.7
174	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	35.9
175	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	37.1
176	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	43.8
177	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	36.4
178	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147357	36.5
179	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	77.9
180	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	75.8
181	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	73.1
182	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	81.4
183	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	87.6

184	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	66.1
185	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	82.7
186	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	53.3
187	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	81.2
188	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	78.4
189	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	70.8
190	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	100.0
191	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	75.8
192	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	69.7
193	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	57.8
194	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	69.8
195	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	71.8
196	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	56.3
197	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	86.1
198	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	80.5
199	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	72.8
200	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	89.4
201	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	70.8
202	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	71.5
203	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	76.3
204	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	79.3
205	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	62.1
206	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	66.2
207	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	61.9
208	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	64.3
209	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	94.6
210	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	82.3
211	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	69.2
212	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	92.7

213	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	70.6
214	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147358	70.9
215	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147360	27.2
216	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147360	39.8
217	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147360	29.1
218	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147360	31.3
219	R.octofasciata	Guatemala	Izabal	Dulce River	FMNH-147360	30.2
237	R.octofasciata	Mexico	Oaxaca	West_Tehuan	UMMZ-162472	84.7
238	R.octofasciata	Mexico	Oaxaca	West_Tehuan	UMMZ-162472	128.3
239	R.octofasciata	Honduras	Cortes	Motagua-Ulua	UMMZ-173281	33.7
240	R.octofasciata	Honduras	Cortes	Motagua-Ulua	UMMZ-173281	32.0
241	R.octofasciata	Mexico	Oaxaca	West_Tehuan	UMMZ-176671	51.3
242	R.octofasciata	Mexico	Oaxaca	West_Tehuan	UMMZ-176671	56.5
243	R.octofasciata	Mexico	Oaxaca	West_Tehuan	UMMZ-176671	36.7
244	R.octofasciata	Mexico	Oaxaca	West_Tehuan	UMMZ-176671	54.7
245	R.octofasciata	Guatemala	Izabal	Dulce River	UMMZ-179895	45.0
246	R.octofasciata	Mexico	Veracruz	West_Tehuan	UMMZ-181301	51.0
247	R.octofasciata	Mexico	Veracruz	West_Tehuan	UMMZ-181301	50.2
248	R.octofasciata	Mexico	Veracruz	West_Tehuan	UMMZ-181301	40.2
249	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	UMMZ-187976	47.5
250	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	UMMZ-187976	62.1
251	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	UMMZ-187976	64.0
252	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	UMMZ-187976	74.9
253	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	UMMZ-187976	54.9
254	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	UMMZ-187976	53.4
255	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	UMMZ-187976	70.0
256	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	UMMZ-187976	50.3
257	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	UMMZ-187976	50.3
258	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	UMMZ-187976	51.9

259	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	UMMZ-190726	38.6
260	R.octofasciata	Guatemala	Peten	Grijalva-Usumacinta	UMMZ-190726	42.2
261	R.octofasciata	Guatemala	Alta Verapaz	Polochic	UMMZ-190736	61.0
262	R.octofasciata	Guatemala	Alta Verapaz	Polochic	UMMZ-190736	49.3
263	R.octofasciata	Mexico	Campeche	Yucatan	UMMZ-190859	82.1
264	R.octofasciata	Mexico	Campeche	Yucatan	UMMZ-190859	64.3
265	R.octofasciata	Mexico	Campeche	Yucatan	UMMZ-190859	86.4
266	R.octofasciata	Mexico	Campeche	Yucatan	UMMZ-190859	91.4
267	R.octofasciata	Mexico	Campeche	Yucatan	UMMZ-190859	93.2
268	R.octofasciata	Mexico	Campeche	Yucatan	UMMZ-190859	89.3
269	R.octofasciata	Mexico	Campeche	Yucatan	UMMZ-190859	65.0
270	R.octofasciata	Mexico	Campeche	Yucatan	UMMZ-190859	58.2
271	R.octofasciata	Mexico	Campeche	Yucatan	UMMZ-190859	50.1
272	R.octofasciata	Mexico	Campeche	Yucatan	UMMZ-190859	70.7
273	R.octofasciata	Guatemala	Izabal	Motagua-Ulua	UMMZ-193998	71.7
274	R.octofasciata	Guatemala	Izabal	Motagua-Ulua	UMMZ-193998	88.0
275	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	UMMZ-196430	50.9
276	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	UMMZ-196430	50.7
277	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	UMMZ-196430	50.7
278	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	UMMZ-196430	44.4
279	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	UMMZ-196430	46.4
280	R.octofasciata	Mexico	Yucatanatan	Yucatan	UMMZ-196567	33.3
281	R.octofasciata	Mexico	Yucatanatan	Yucatan	UMMZ-196567	61.4
282	R.octofasciata	Mexico	Yucatanatan	Yucatan	UMMZ-196567	57.2
283	R.octofasciata	Mexico	Campeche	Yucatan	UMMZ-196618	56.9
284	R.octofasciata	Mexico	Campeche	Yucatan	UMMZ-196618	58.2
285	R.octofasciata	Mexico	Campeche	Yucatan	UMMZ-196618	53.7
286	R.octofasciata	Mexico	Campeche	Yucatan	UMMZ-196618	58.5
287	R.octofasciata	Guatemala	Izabal	Dulce River	UMMZ-197245	92.7

220	R.octofasciata	Guatemala	Izabal	Motagua-Ulua	UMMZ-197316	48.1
221	R.octofasciata	Guatemala	Izabal	Motagua-Ulua	UMMZ-197316	44.5
222	R.octofasciata	Guatemala	Izabal	Motagua-Ulua	UMMZ-197316	42.4
223	R.octofasciata	Guatemala	Izabal	Motagua-Ulua	UMMZ-197316	43.7
224	R.octofasciata	Guatemala	Izabal	Motagua-Ulua	UMMZ-197316	52.0
225	R.octofasciata	Guatemala	Izabal	Motagua-Ulua	UMMZ-197316	48.2
226	R.octofasciata	Guatemala	Izabal	Motagua-Ulua	UMMZ-197324	60.4
227	R.octofasciata	Mexico	Yucatanatan	Yucatan	UMMZ-201745	86.2
228	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	UMMZ-209319	73.0
229	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	UMMZ-209319	60.7
230	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	UMMZ-209319	63.8
231	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	UMMZ-209358	100.8
232	R.octofasciata	Guatemala	Izabal	Motagua-Ulua	UMMZ-225010	99.4
233	R.octofasciata	Guatemala	Izabal	Motagua-Ulua	UMMZ-225018	58.6
234	R.octofasciata	Guatemala	Izabal	Motagua-Ulua	UMMZ-225018	53.7
235	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	UMMZ-225048	40.6
236	R.octofasciata	Mexico	Chiapas	Grijalva-Usumacinta	UMMZ-225048	43.1
288	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147154	54.0
289	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147154	55.8
290	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147154	59.2
291	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147154	32.2
292	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147154	31.5
293	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147154	27.3
294	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147154	32.7
295	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147154	28.0
296	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147352	56.2
297	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147352	42.9
298	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147352	44.6
299	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147352	36.9

300	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147352	33.3
301	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147352	52.2
302	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147352	48.3
303	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147352	63.5
304	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147352	64.4
305	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147352	63.0
306	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147352	45.5
307	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147352	55.6
308	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147352	47.9
309	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147355	57.3
310	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147359	62.2
311	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147359	29.3
312	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147359	26.0
313	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147359	25.2
314	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147359	21.9
315	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147359	25.8
316	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147359	28.1
317	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147359	39.2
318	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147359	30.4
319	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147359	32.8
320	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147359	29.5
321	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147359	25.2
322	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147359	39.0
323	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147359	33.3
324	R.spinosissima	Guatemala	Izabal	Dulce River	FMNH-147359	24.3
325	R.spinosissima	Guatemala	Alta Verapaz	Polochic	FMNH-147361	70.1
326	R.spinosissima	Guatemala	Alta Verapaz	Polochic	FMNH-147361	28.2
327	R.spinosissima	Guatemala	Alta Verapaz	Polochic	FMNH-147361	29.0
328	R.spinosissima	Guatemala	Alta Verapaz	Polochic	FMNH-147361	41.1

329	R.spinosissima	Guatemala	Alta Verapaz	Polochic	FMNH-147361	41.8
330	R.spinosissima	Guatemala	Alta Verapaz	Polochic	FMNH-147361	40.2
331	R.spinosissima	Guatemala	Alta Verapaz	Polochic	FMNH-147361	30.2
332	R.spinosissima	Guatemala	Alta Verapaz	Polochic	FMNH-147361	38.3
333	R.spinosissima	Guatemala	Alta Verapaz	Polochic	FMNH-147361	31.3
334	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-146086	33.5
335	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-146086	38.0
336	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-146086	34.6
337	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	51.0
338	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	53.9
339	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	46.9
340	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	51.3
341	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	49.0
342	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	52.8
343	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	50.0
344	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	46.1
345	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	47.4
346	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	50.6
347	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	47.2
348	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	47.4
349	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	54.0
350	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	50.5
351	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	51.2
352	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	49.8
353	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	50.4
354	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	48.2
355	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	51.4
356	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	48.2
357	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	46.3

358	R.spinosissima	Guatemala	Alta Verapaz	Polochic	UMMZ-190737	54.3
359	R.spinosissima	Guatemala	Izabal	Dulce River	UMMZ-197199	33.0
360	R.spinosissima	Guatemala	Izabal	Dulce River	UMMZ-197249	53.5
361	R.spinosissima	Guatemala	Izabal	Dulce River	UMMZ-197249	42.1
362	R.spinosissima	Guatemala	Izabal	Dulce River	UMMZ-197249	42.1
363	R.spinosissima	Guatemala	Izabal	Dulce River	UMMZ-197249	45.6
364	R.spinosissima	Guatemala	Izabal	Dulce River	UMMZ-197249	37.2
365	R.spinosissima	Guatemala	Izabal	Dulce River	UMMZ-225023	43.6
366	R.spinosissima	Guatemala	Izabal	Dulce River	UMMZ-225023	46.2
367	R.spinosissima	Guatemala	Izabal	Dulce River	USAC-0272	34.3
368	R.spinosissima	Guatemala	Izabal	Dulce River	USAC-2279	48.8
369	R.spinosissima	Guatemala	Izabal	Dulce River	USAC-2282	62.5