

Effects of Genetic and Environmental Variation on the Morphology of  
*Pimelodella chagresi*, a Neotropical Catfish Species

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Handed in to  
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### **Name and Coordinates of Host Institution**

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### **Information about Host Institution**

My internship was hosted by the Smithsonian Tropical Research Institute (STRI), with most of the laboratory work and data analysis being done in their Naos facilities. I was under the direct supervision of Andrew Moeser, a McGill M.Sc candidate. The laboratory space and equipment, as well as some of the data, were provided by Dr. Eldredge Bermingham, Mr. Moeser's co-supervisor and STRI researcher.

Mr. Moeser's research lies in the field of evolutionary biology, which is described as the sub-discipline of biology interested in understanding how organisms diversify, at what rate, and through which mechanisms. The two main goals of his research, as stated in his 2002 M.Sc proposal, are the following:

1. To determine the spatial distribution of mitochondrial DNA lineages of *Pimelodella chagresi* (a Neotropical catfish species).
2. To use nuclear markers to verify mitochondrial DNA phylogeny, in order to determine if hybridization and genetic introgression are occurring between lineages of *P. chagresi*, as well as to investigate genetic parameters of this species.

### **Distribution of Work Load**

Field Work: 13 full days

Laboratory Work: 7 full days

Office and Analysis Work: 25 days

Total: 45 days

### **Acknowledgements**

-Dr. Eldredge Bermingham  
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## Introduction

The biodiversity of freshwater fishes found today in Lower Central America (Costa Rica and Panama) is very much linked to the diversity of the geological landscape, and its recent history. The isthmus of Panama was formed 3 to 4 million years ago, being completely emergent around 3 million years ago (Martin and Bermingham, 2000). The emergence of a land bridge between North and South America coincided with the first invasion of primary freshwater fishes from South America. Primary freshwater fish are completely intolerant of saltwater, and were thus limited from dispersing into Central America by the physical barrier of saltwater, rather than by a climatic barrier, as was the case of freshwater fish from North America (Myers, 1966).

Although the region of Lower Central America (LCA) is still today dominated by non-obligatory freshwater fish species (Moyle and Cech, 1988), there is a high diversity of primary freshwater species. Twelve families are endemic to LCA, representing 74 species of fish. Pimelodidae are the second largest family, with 23 recognized species to date. All families originate from a common source region in Northwestern South America (Martin and Bermingham, 2000), as supports the evidence of a much lower freshwater fish diversity found north of LCA, leading to the idea that species have not had time to disperse further (Myers, 1966). The formation of the Central Cordillera of Panama created yet another challenge to dispersion, and led to a stepping stone pattern of dispersion along both slopes, although the conditions are rather different among the slopes. The Pacific slope, which will be discussed in this study, presents extreme variation in river flow, relatively steep river gradients, short river courses, and an altitude that varies between 200 to 600m (Martin and Bermingham, 2000).

A common, very widespread species is *Pimelodella chagresi*, a Neotropical catfish inhabiting isolated drainage basins of Lower Central America (Costa Rica and Panama) and Northwestern South America. Little is known of the ecology and behavior of *P. chagresi*. It is known to be mainly carnivorous, feeding on bottom invertebrates in clear or sandy bottom streams (Martin and Bermingham, 1998), and to sometime exhibit schooling behavior. Easily recognizable morphological traits include 3 pairs of very long

barbells, pectoral spines with venom glands, smooth skin, and 2 pairs of black stripes on their back (Moyle and Cech, 1988). Described by Steindachner in 1877, its status as a species remained unchallenged until Martin and Bermingham (2000) used mitochondrial DNA analysis on individuals from various watersheds over the species' distribution range and found that a high level of genetic diversity existed within *Pimelodella chagresi*. They identified five different haplotypes over the species' range, and thus recommended that the species be recognized as a species complex. In Panama, two main haplotypes, or lineages, were identified (Lineages A and B). These two lineages apparently arose from distinct ancestors that invaded LCA at different periods, from a common source region in Northwestern South America (Martin and Bermingham, 2000; Myers, 1966). The first wave of invasion possibly took place 3-4 million years before present, coinciding with the formation of the isthmus of Panama, and resulting in the evolution of the ancestor of Lineage A. The second wave apparently took place 1 million years before present, and led to the evolution of the Lineage B ancestor (Martin and Bermingham, 2000). These distinct invasion times have been proposed as explanation for the current distribution of the lineages over Panama watersheds, the limited distribution of Lineage B individuals being linked to its more recent arrival (Mooser, unpublished). Other factors proposed to explain the distribution of the lineages include hybridization of lineages living in sympatry, competitive exclusion in the case of lineages using the same resources, and random processes leading to extinction such as variation in sea levels and climate, leading to stream morphology changes and destruction of habitat (Martin and Bermingham, 2000).

Current species concepts include various characters in order to distinguish a species from another, mainly genetic, ecological, behavioral, and morphological. The study of morphology has been the historical basis for the sciences of taxonomy and evolution, using morphometric and meristic measurements to distinguish between species (Schreck and Moyle, 1990; Rohlf, 1990; Mayr, 1970). In the case of *Pimelodella chagresi*, preliminary morphometric data from Martin and Bermingham (2000) for four traits measured on preserved specimens revealed significant differences between lineages A and B for two of those traits, namely caudal peduncle depth and the proportion of the

pectoral spine covered with posterior projecting teeth. This study thus aims at describing the morphological variation that exists among individuals of *Pimelodella chagresi* across three Panamanian watersheds, to test the hypothesis that there is a statistically significant relationship between the genetic variation and the morphological variation demonstrated by Martin and Bermingham (2000).

However, genetics is but one aspect of phenotype expression. Environmental conditions, as well as the relationship between environmental conditions and the genotype, may also have a strong influence on phenotype expression (Schlichting, 1986). This change in expressed phenotype of a genotype in response to environmental factors has been named phenotypic plasticity (Scheiner, 1993). As Schreck and Moyle (1990) described, there are two components to the development of variation within a same species: first, the variation that arises from the different phenotypic responses to environmental factors, depending on the genotype; second, the existence of random, stochastic within population variations for a species. In view of this concept of phenotypic plasticity, this study also aims at determining if there is a statistically significant relationship between morphological variation in *P. chagresi* and geographic location, used as a proxy for environmental variation. This should enable me to decipher the respective roles of genetics and environmental characters, as well as the role of their interaction, on the expression of *P. chagresi* phenotypes (Vidalis, Markakis and Tsimenides, 1996; Dobzansky, 1970).



### **Research Question**

My main research question is: Are there measurable morphological differences between individuals of *Pimelodella chagresi* inhabiting the Rio Santa Maria, Rio Cocle del Sur and Rio Bayano watersheds? If there exist morphological variation, can it be statistically linked to the genetic variation identified in *P.chagresi* by Martin and Bermingham (2000)? Finally, can the morphological variations, if there are any, be correlated with environmental factors represented by the diversity of drainage basins, and with the interaction between genetics and the environment?

### **Objectives**

The objectives of this study are (1) to describe the morphological variations that occur between individual specimens of *Pimelodella chagresi* in three different watersheds of Panama, using a range of morphometric and meristic characters; (2) to test the hypothesis that there is a statistically significant relationship between the genetic variation identified among specimens by Martin and Bermingham (2000) and the distribution of morphological characters in *P. chagresi*; (3) to test the hypothesis that there is a statistically significant relationship between the morphological characters and geographic location.

### **Relevance and Significance of the Study**

This research will generate new information on the morphological distribution of an otherwise poorly studied species, *Pimelodella chagresi*. It will test if there are statistically significant links between the morphological trait distribution, the genetic variation distribution, and the environment. It will also open the door to further ecological research on *Pimelodella chagresi*, allowing for eco-morphological and behavioral studies to be undertaken. This study, in helping to determine whether *P. chagresi* is in fact a species complex and should be recognized as such by taxonomists, has implications for freshwater fish diversity. Morphology is still recognized by many as being the starting point of species designation and identification, but this study might show that for freshwater fish, diversity may be measured at various scales and may not be visible at first glance. It might thus be that little morphological variation may exist side by side with wide genetic variation, and this will have implications on how we define the concept of species.

This possible discrepancy between the scales of morphological versus genetic variation also has conservation implications, in that many species which were previously thought to be unique due to morphological similarity might need to be reassessed for genetic similarity. This may mean that the diversity estimates for freshwater fishes in Lower Central America will need to be reviewed and increased, and as endemism might higher than estimated, nature reserve designs will need to be rethought to take in this newly acknowledged diversity.

### *Ethical Considerations*

My first ethical consideration in performing this study regarded animal welfare and ecosystem disturbance. Many methods of capturing and killing catfish were available, and I aimed to use the one which lessened the stress felt by the animal before killing it. I also aimed at performing the sampling while causing as little disturbance in the stream system as I could. Therefore, I chose to use seine nets rather than electro-fishing. Electro-fishing is a method consisting of two electrified rods being lowered into the water on each side of a stream, creating a wall of electric current through which organisms pass. While it is known to usually stun fish, I was worried about the possible effects on other macro- and micro-fauna. By using seine nets, I hoped to limit the ecosystem disturbance, and avoid useless animal deaths, in that I was able to release accidentally caught species not related to this study.

As to the killing method, putting the fish in a cold water environment is the fastest, least intrusive method we found. It avoids the use of chemicals which would then be released into the environment, while also avoiding excessive stress on the animal.

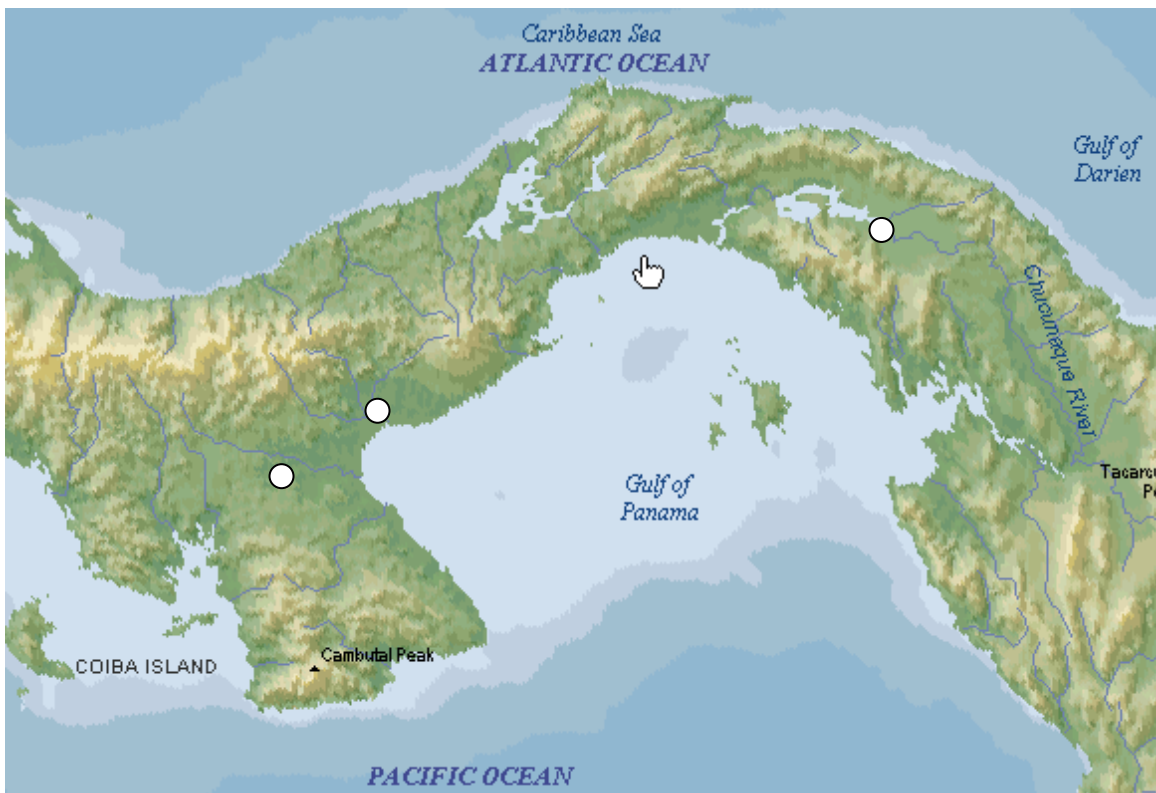
Other ethical considerations, not directly related to the study but corollaries of doing research in Panama, included respecting Panamanian customs and traditions. As a researcher in a foreign country, I have the double responsibility of respecting my hosts while representing adequately my home institution. Concretely, this meant speaking Spanish whenever possible, respecting Panamanian dress codes, adopting the proper tone when dealing with Panamanian people, whether they were members of the authorities or locals. It also included making sure that the sampling was done without invading people's properties, and respecting the beliefs which might be linked to the sampling areas. Finally, it implied being able to answer the unavoidable question: Why are you here? with as much simplicity and understanding as possible.

One last consideration involved scientific ethics. I aimed at presenting unbiased results based on unbiased methodology, and at acknowledging the limits of my study.

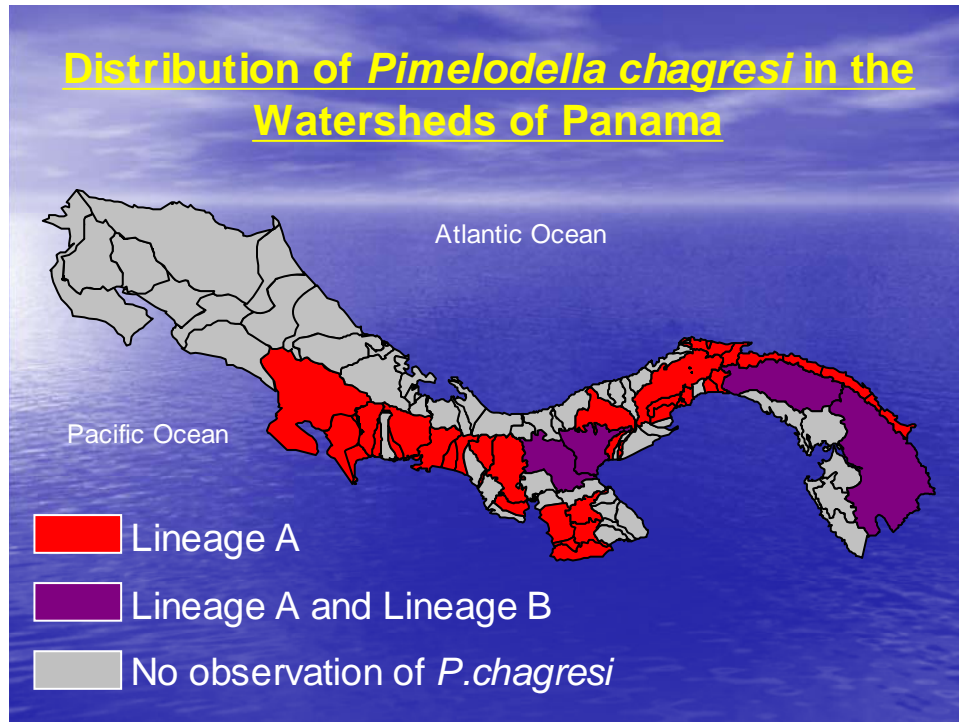
## Methodology

### *Sampling area*

I performed the sampling in three different watersheds, all draining the Pacific side of the Cordillera Central of Panama (Figure 1). Preliminary data from Martin and Bermingham (2000) shows the Rio Bayano watershed to contain only *Pimelodella* haplotype B, while Rio Santa Maria and Rio Cocle del Sur watersheds contain both haplotypes A and B. However, unpublished data from Moeser (2004) shows that both lineages are in fact present in the Bayano watershed (Figure 2). Sampling was performed on several rivers and streams within these watersheds, chosen as randomly as accessibility allowed. Sampling was also done at several points along each stream.



**Figure 1. Topographic map of Panama. Watersheds sampled in this study (left to right): Rio Santa Maria, Rio Cocle del Sur, Rio Bayano.**



**Figure 2. Distribution of *P. chagresi* lineages in Panamanian watersheds.**

#### *Sampling methods*

Sampling was done using seine nets. Whenever possible, a minimum of 20 individuals from each site were collected, identified using Eigenmann (1922). Exact sampling locations were recorded using GPS referencing and topographic maps. The fish were killed by immersing them in a mix of water and ice for 5 minutes. Following death, a gill tissue sample was taken for future genetic analysis, and the fish was tagged with a STRI identification number. Identified specimens were preserved in buffered formalin, being later transferred to 70% ethanol for preservation in the STRI collection (Martin and Bermingham, 2000).

#### *Morphometric analysis*

Six morphometric measurements and 2 meristic measurements were made on each specimen using dial calipers (to the nearest 0.01mm), magnifying glass and microscope. Body measurements consisted of (i) Standard Length (SL), (ii) Caudal Peduncle Depth (CPD), (iii) Caudal Peduncle Length (PeL), and (iv) Length between the Adipose Fin and the End of the Body (A-HP). Pectoral fin measurements consisted of (v) Length of

Pectoral Spine (SpL), and (vi) Length of Pectoral Spine with Posterior Projecting Teeth (TeL). Two meristic measurements were taken on the Pectoral Spine: (vii) Number of Complete Posterior Projecting Teeth (SpT), and (viii) Number of Partial Posterior Projecting Teeth (PaT) (Martin and Bermingham, 2000). The proportion of the pectoral spine with posterior projecting teeth (complete and partial teeth counted together) was calculated by dividing TeL/SpL. The measurements and counts were made on the left side of the specimen whenever possible to further reduce the bias of the research (Hee Ng and Sparks, 2002), and the measuring was done blind, i.e. without knowing the lineage of the fish.

<b>Measurement</b>	<b>Abbreviation</b>
<i>Standard length</i>	SL
<i>Caudal peduncle length</i>	PeL
<i>Caudal peduncle depth</i>	CPD
<i>Length between adipose fin and end of back bone</i>	A-HP
<i>Pectoral spine length</i>	SpL
<i>Length of pectoral spine with posterior projecting teeth (both complete and partial)</i>	TeL
<i>Proportion of pectoral spine with posterior projecting teeth (both complete and partial)</i>	TeL/SpL
<i>Number of complete posterior projecting teeth on pectoral spine</i>	SpT
<i>Number of partial posterior projecting teeth on pectoral spine</i>	PaT

**Table 1. List of abbreviations for measurements used in this study.**

Additionally, one specimen was re-measured 20 times over a 1 month period, to assess the intra-observer measurement error for each trait. Measurements were performed at different times during the day, the repeats being separated by periods of at least 2 hours to avoid effects of learning or memory (Hayek, Heyer and Gascon, 2001). Finally, the measurements were done alternating the origin of the specimens, to avoid any effects due to variation in measurement habits or ability over time.

### *Data analysis*

Morphometric and meristic measurements were first standardized using the following formula for allometric adjustment (Hendry, Taylor and McPhail, 2002):

$$M_{std} = M_o (X/L_o)^b$$

Where  $M_{std}$  is the Standardized measurement  
 $M_o$  is the Observed measurement on the fish  
 $X$  is the Average of the Standard Length for all fish  
 $L_o$  is the Observed Length of the fish  
 $b$  is the Correlation Coefficient (ANCOVA result)

Basic statistics were calculated for the standardized measurements using SYSTAT (version 10.2) in order to calculate, for every trait, the norm of reaction for lineages A and B across watersheds. The standardized measurements were then log transformed ( $X' = \log(X+1)$ ), except for the proportion of TeL/SpL, which was arcsine transformed ( $X' = \sqrt{\arcsine X}$ ). The resulting data was analyzed to look at the differences in morphological traits between individuals over the three watersheds, as well as between lineages. Multivariate General Linear Models (SYSTAT 10.2) were used to analyze the variance of the sample. Three effects were included in the model (Lineage, Drainage Basin, and Drainage Basin-Lineage Interaction), while the variables were the 8 traits measured on the fish.

In order to determine what proportion of the variance was due to genetic factors, and what proportion was due to phenotypic plasticity, I calculated the percentage of variance for every effect included in the multivariate model, using the following formulas, designed for data sets with widely varying number of cases (Winer, 1971):

$$\% \text{ Variance Lineage} = \frac{MS_{lineage} - MS_{interaction}}{np}$$

Where  $MS_{lineage}$  is the Mean Square of trait, under lineage effect (Univariate Model)

$MS_{interaction}$  is the Mean Square of trait, under interaction effect (Univariate Model)  
 $n$  is the average number of cases for which effect calculated  
 $p$  is the number of categories in the watershed effect

$$\% \text{ Variance Watershed} = \frac{MS_{watershed} - MS_{interaction}}{nq}$$

Where  $MS_{lineage}$  is the Mean Square of trait, under watershed effect (Univariate Model)

$MS_{interaction}$  is the Mean Square of trait, under interaction effect (Univariate Model)  
 $n$  is the average number of cases for which effect calculated  
 $q$  is the number of categories in the lineage effect

$$\% \text{ Variance Interaction} = \frac{MS_{interaction} - MS_{error}}{n}$$

Where  $MS_{interaction}$  is the Mean Square of trait, under interaction effect (Univariate Model)

$MS_{error}$  is the Mean Square of the error of that trait, under interaction effect (Univariate Model)  
 $n$  is the average number of cases for which effect calculated

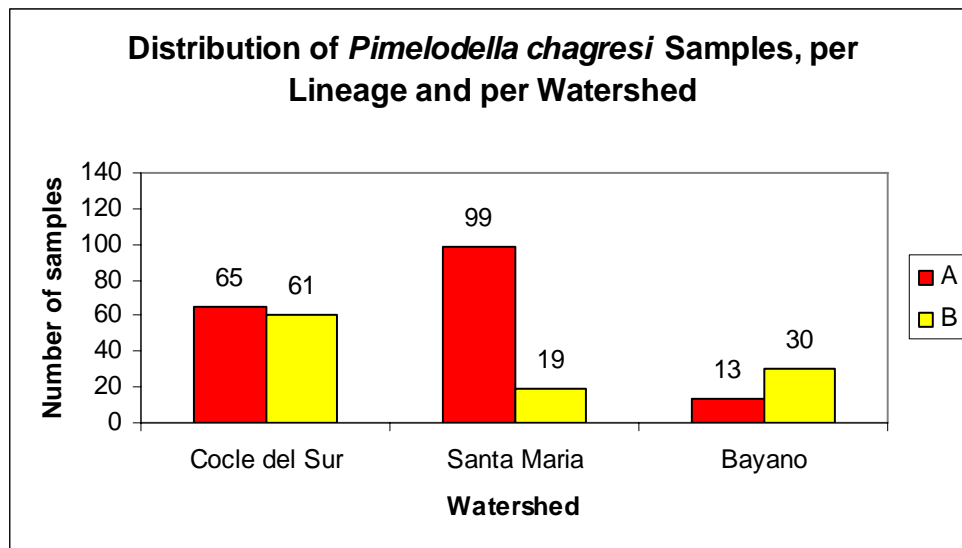
Standard descriptive statistics (mean, standard deviation, minimum, maximum, and coefficient of variation) were used to assess intra-observer measurement error for every morphometric and meristic measurement.



## Results

### *Distribution of individuals*

Sampling over the three watersheds, both in the 2004 sampling season and in previous years, resulted in the capture of 287 specimens of *Pimelodella chagresi*. However, the distribution of individuals of each lineage (A or B) varied greatly among the watersheds. Rio Cocle del Sur watershed exhibited very similar distributions of lineages ( 65 A individuals, 61 B individuals), while neighboring Rio Santa Maria watershed (99 A individuals, 19 B individuals) exhibited significant dominance by the Lineage A. Rio Bayano watershed sampling resulted in a majority of Lineage B samples (30 individuals) over Lineage A (13 individuals) (Figure 3).



**Figure 3. Distribution of *P. chagresi* samples, per lineages and per watershed.**

Within the watersheds, individuals of A and B lineages were also distributed unevenly between rivers and streams (Table 2). Six sites were sampled within the Cocle del Sur watershed. Out of those, 3 contained individuals of both lineage A and B, and 3 contained only lineage A individuals. Eight sites were sampled in the Santa Maria basin, out of which only 3 contained fish of both lineages, while the individuals caught at the other 5 sites were of lineage A only. In the case of the Bayano basin, all sampled sites contained

a single lineage, with lineage B individuals caught at 3 sites and lineage A individuals caught at 2 sites.

<b>Drainage Basin</b>	<b>Locale</b>	<b>Site Code</b>	<b>A Individuals</b>	<b>B Individuals</b>
<b>Cocle del Sur</b>	<i>Rio Chico</i>	CS1	10	36
	<i>Rio Cocle del Sur</i>	CS3	17	5
	<i>Rio Cocle del Sur</i>	CS4	10	-
	<i>Rio Cocle del Sur (at Interamericana)</i>	CSX	2	20
	<i>Rio El Harino</i>	CS5	18	-
	<i>Rio Zarati</i>	CS6	8	-
<b>Santa Maria</b>	<i>Quebrada El Nance</i>	SM1	11	-
	<i>Rio Conaca</i>	SMX	11	5
	<i>Rio Gatun</i>	SM2	18	-
	<i>Rio Lajas</i>	SM3	5	-
	<i>Rio Las Guías</i>	SM4	3	3
	<i>Rio Santa Maria (at bridge)</i>	SM6	26	-
	<i>Rio Santa Maria (at Tierra Hueca)</i>	SMY	8	11
	<i>Rio Santa Maria (Upper site)</i>	SM7	17	-
<b>Bayano</b>	<i>Rio Aguas Claras</i>	BY1	10	-
	<i>Rio Bayano</i>	BY2	-	5
	<i>Rio Chichebre</i>	BY3	-	20
	<i>Rio Ipeti</i>	BY4	-	5
	<i>Quebrada Upper Bayano</i>	BY5	3	-

**Table 2. Distribution of *P. chagresi* individuals among lineages, for all sites within three watersheds.**

#### *Intra-observer Measurement Error*

Standard descriptive statistics were calculated for the twenty repeated measurements on each of the characters measured on one specimen of *P. chagresi* (Identification number: STRI-17871) (Table 3). The lowest coefficient of variation was measured for standard length (CV= 0.002951), indicating a high degree of precision. Precision was generally high for 5 of the remaining 7 characters, but was low for the A-HP (CV= 0.06788), and very low for the caudal peduncle length (CV= 0.219671).

<i>Character</i>	<b>Mean</b>	<b>SD</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Coefficient of variation</b>
<i>SL (mm)</i>	54.395	0.1605	54.10	54.70	0.002951
<i>PeL (mm)</i>	1.925	0.4428	1.35	2.55	0.2197
<i>CPD (mm)</i>	4.5425	0.1498	4.30	4.85	0.03298
<i>SpL (mm)</i>	7.8825	0.1320	7.60	8.10	0.01675
<i>TeL (mm)</i>	6.0275	0.1418	5.80	6.30	0.02354
<i>A-HP (mm)</i>	7.1825	0.4875	6.20	7.95	0.06788
<i>SpT</i>	6	0	6	6	0
<i>PaT</i>	1	0	1	1	0

**Table 3. Descriptive statistics for 20 repeated measurements of a single specimen of *Pimelodella chagresi*.**

#### *Morphometric and Meristic Measurements*

Both meristic and morphometric measurements showed important variation in mean, the variation being apparent not only between lineages but between watersheds as well (Table 4). In view of these results, analysis of variance was carried out, using both univariate and multivariate General Linear Models (GLM), to calculate if the variation was significant between lineages and watersheds. Tukey tests were also performed to compare the variation in measured traits between watersheds (Table 5).

The MANOVA showed highly statistically significant results for all three effects: Lineages (Pillai's Trace=0.463,  $F=29.484$ ,  $df=8.274$ ,  $P<0.000$ ); Watersheds (Pillai's Trace=0.373,  $F=7.880$ ,  $df=16.550$ ,  $P<0.000$ ); Lineage-watershed interaction (Pillai's Trace=0.296,  $F=5.960$ ,  $df=16.550$ ,  $P<0.000$ ). Pairwise comparisons between watersheds revealed a statistically significant ( $P<0.05$ ) difference between the Bayano and Cocle del Sur specimens for 7 out of 8 measured traits, with the Caudal peduncle length value being marginally significant at  $P=0.061$ . The difference between Bayano and Santa Maria specimens was highly significant ( $P<0.000$ ) for all 8 measured traits. The comparison between the Cocle del Sur and the Santa Maria watersheds revealed that the difference between specimens was not significant for 6 of the 8 measured traits, being only significant for the Caudal peduncle length ( $P<0.000$ ) and the Caudal peduncle depth ( $P=0.032$ ).

Characteristic	Cocle del Sur A (n = 65)		Cocle del Sur B (n = 61)		Santa Maria A (n = 99)		Santa Maria B (n = 19)		Bayano A (n = 13)		Bayano B (n = 30)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>Morphometric</i>												
SL	53.446	15.104	46.080	9.459	45.945	13.830	44.905	10.590	71.669	8.510	78.057	11.912
PeL Std	1.513	0.703	2.326	0.959	1.324	0.752	1.541	0.659	1.977	0.582	2.390	0.656
CPD Std	4.935	1.471	3.563	0.833	4.100	1.211	3.488	0.893	6.012	0.618	6.506	1.045
SpL Std	7.356	1.825	6.815	1.648	6.573	2.152	7.074	1.379	10.454	1.495	11.216	1.406
TeL Std	4.202	1.593	4.773	1.297	3.875	1.455	4.929	1.342	7.428	1.383	8.550	1.364
A-HP Std	5.717	1.532	5.321	1.320	4.909	1.469	5.697	1.052	7.003	1.032	9.354	1.298
TeL/SpL Std	0.535	0.152	0.912	1.803	0.510	0.178	0.691	0.68	0.710	0.076	0.761	0.051
<i>Meristic</i>												
SpT Std	4.463	1.669	4.976	0.955	4.001	1.677	5.371	0.814	6.682	1.572	7.141	1.283
PaT Std	2.368	1.535	1.809	0.853	2.105	1.436	1.267	0.805	3.582	1.590	2.142	1.162

**Table 4. Mean and standard deviation of the characters examined in each sampling area. (Note: All measurements, except for SL, were standardized using the allometric adjustment formula)**

Watershed Pair	SL	PeL	CPD	SpL	A-HP	SpT	PaT	TeL/SpL
Bayano-Cocle	0.000	NS	0.000	0.000	0.000	0.000	0.028	0.000
Bayano-Santa Maria	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cocle-Santa Maria	NS	0.000	0.032	NS	NS	NS	NS	NS

**Table 5. P-value results of Tukey tests among watersheds, performed on log transformed, standardized measurements.**

It thus appears that *Pimelodella chagresi* individuals differ both among lineages, and among watersheds. In order to determine what proportion of the variance in the sample means is due purely to genetics (lineage effect) and what proportion is due to phenotypic plasticity (watershed and lineage-watershed interaction effects), the percentage of variance for each effect was calculated, using results from the univariate GLMs (Table 6).

<b>Characteristic</b>	<b>% Variance Lineage</b>	<b>% Variance Watershed</b>	<b>% Variance Lineage-Watershed Interaction</b>
<i>Morphometric</i>			
<b>SL</b>	-4.4018	101.8024	12.4785
<b>PeL</b>	0.04354	0.04651	0.03592
<b>CPD</b>	0.008613	0.6065	0.2468
<b>A-HP</b>	0.007352	0.8416	0.5580
<b>SpL</b>	-0.05302	2.1131	0.1032
<b>TeL/SpL</b>	0.006683	0.002038	-0.005875
<i>Meristic</i>			
<b>SpT</b>	0.4827	0.7294	0.02079
<b>PaT</b>	0.2555	0.1310	0.02517

**Table 6. Distribution of variance of the mean for eight measured characteristics of *P. chagresi*.**

For all but one of the measured characteristics, the percentage of variance attributed to the watershed effect, in other words to the environment, was higher than the percentage attributed to the genetic effect (lineage). In order to visualize how the traits change across watersheds, for both lineages, and in order to relate it to the relatively higher phenotypic plasticity terms (environment and interaction effects), the norm of reaction was plotted for every measured trait, across the 3 watersheds (Appendix 1). In general, lineage B individuals present larger means than lineage A individuals (Table 4), and the pattern of change is relatively constant across watersheds, that is, a decrease in lineage A value is accompanied by a decrease in lineage B value, leading me to believe that morphological characters are very dependent on environmental conditions that apply to both lineages.

## Discussion

The first objective of this study was to describe the morphological variation that exists among *Pimelodella chagresi* individuals of the Rio Cocle del Sur, Rio Santa Maria, and Rio Bayano watersheds. Looking at the mean and standard deviation values for the standardized morphometric and meristic measurements, a first pattern is clearly discernible, consisting in the quasi invariable larger size of the measurements made on the Bayano individuals, and the great similarity that exists between the values of the measurements for Cocle del Sur and Santa Maria. This apparent division of the data into two geographic groups, with Bayano watershed on one side and Cocle del Sur and Santa Maria watersheds on the other, is corroborated by the values resulting from the Tukey pairwise comparisons, which report highly significant differences for 7 out of 8 traits between the Cocle del Sur and Santa Maria versus the Bayano watershed. They also report non-significant differences for 5 traits out of 8, and only marginally significant differences for 2 of the traits, between Cocle del Sur and Santa Maria watersheds.

The second objective was to examine the relationship between the genetic and morphological variation, hypothesizing that the two lineages, A and B, would have significantly different morphologies. The MANOVA result agrees with this hypothesis, as it calculated a highly significant relationship between lineage and the means of the measurements. The univariate GLM results showed a significant relationship between lineage and 6 of the 8 traits, while standard length (SL) and pectoral spine length (SpL) showed no significant differences. In other words, there are measurable differences in most traits between lineages A and B, differences that could potentially be used to identify the lineage of the fish in the field, provided that the mathematical relationships between the variables are well understood. More work needs to be done on understanding how the traits relate to each other mathematically, and for this the use of digitally-assisted geomorphometrics will be a must, as it will allow geometric relationships between traits to be included in the model (Rohlf, 1990).

The MANOVA results show that the environmental effect represented by the watershed is highly significant over all the traits, thus agreeing with the hypothesis that there is a significant relationship between geographic location and morphological traits, the third objective of this study. This high significance of the environmental effect (in this case, using watershed as a proxy) represents evidence of phenotypic plasticity (Schlichting, 1986), and in order to verify this, further investigation into the environments inhabited by *P. chagresi* within the sampled watersheds would be necessary. Many environmental factors could have created selective pressures, such as changes in river flow regimes and freshwater inputs to the sea, both of which affect dispersion patterns, as well as isolation and extinctions (Vidalis, Markakis and Tsimenides, 1996). The type of substrate as well as turbidity could impact on the type of food available to the catfish, and partly determine the availability of hiding and breeding grounds. The structure and composition of the fish community could also have an impact on the morphology, through inter-specific interaction pressures (competition, predation, and mutualism). It would also be interesting to investigate paleoclimate and paleogeography data, to compare the environmental conditions in which the two lineages evolved to the conditions available to them today, and try to decipher how those conditions might have led to current differences between the lineages.

The MANOVA also showed the existence of a highly significant lineage-watershed interaction across all the traits, which shows that the phenotypic plasticity varies in direction and amount across the two genotypes (lineages) (Schlichting, 1986). This would represent another interesting line of investigation, and would imply environmental conditions being investigated, as well as ecomorphological data collection.

Various limitations exist that restrict the validity of the presented results. A first issue is the reliability of the measurements, which I tried to estimate by calculating intra-observer error (Hayek, Heyer and Gascon, 2001). Measurements were highly precise in the case of standard length, which, being the largest morphometric trait, might also have been the easiest to repeatedly measure accurately. Measurements were also precise for 3 of the 6 morphometric characteristics, with coefficients of variation ranging between 0.016 and

0.032, in the acceptable range according to Hayek, Heyer and Gascon (2002). Two morphometric characteristics lacked precision (coefficients of variation of 0.06 and 0.21), which limits their use in this analysis, and forces me to question the significance of results for these two traits. In the case of the meristic characteristics, the coefficient of variation was 0.00, which shows that meristic characters might be easier to measure repeatedly with accuracy than morphometric characters. However, Schreck and Moyle (1990) mention how meristic characters are strongly influenced by environmental factors during development, which means that finer scale analysis needs to be performed in order to confidently use the meristic measurements to differentiate between lineages.

Other limitations related to the measurements are the possible presence of preservation distortion between specimens, and the so-called “researcher effect”. Preservation distortion can arise from the differential shrinkage of tissues during their fixation, and the extent of the effect varies according to species, the length of the preservation time, as well as on the type of chemical used in the fixation (Schreck and Moyle, 1990). This limitation might be particularly relevant in this study, which combined the use specimens at various stages of preservation. To increase the confidence in the results, the preservation distortion would have to be assessed between the specimens. Schreck and Moyle (1990) also mention the gradual changes in the overall technique used by an observer as a possible source of bias in a morphometric study, and mention the need for quantification of this effect, which can be done by statistically comparing sets of data collected early in the study with later sets of data.

A final limitation concerns the very unequal distributions of A and B individuals in the samples from Rio Santa Maria and Rio Bayano watersheds, which most probably affected the significance of the statistical results. This might represent the actual proportion of individuals from each lineage, but it might also be linked to the sampling method, which would be an undesired bias. Seining can only be performed in certain types of areas, and might have led to a certain bias in the collection of specimens, and in the proportion of individuals from each lineage that were collected. The fact that we only sampled during



the day could represent a further source of bias, in that the lineages could live sympatrically by occupying the space at different times in the day.

Despite its many limitations, and the need to further investigate their impact on the significance of the results, this study represents a first step in understanding the relationships that exist between the phenotypic expression, the genotypes, and the environments of *Pimelodella chagresi*.

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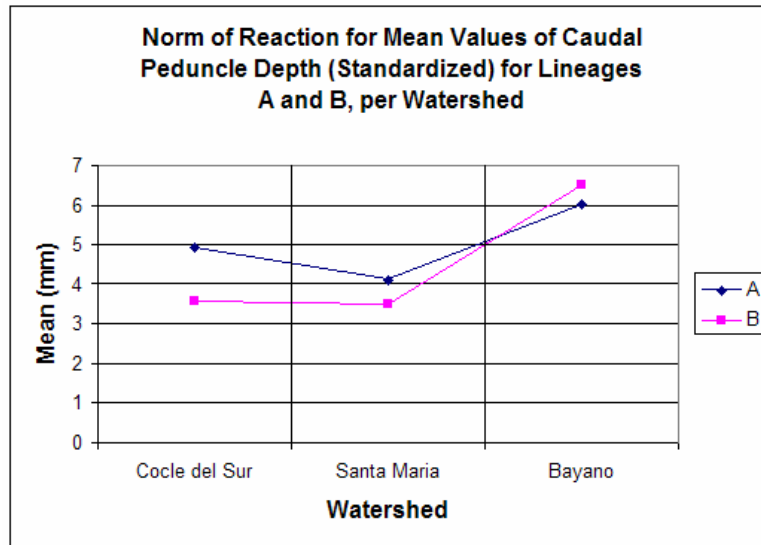
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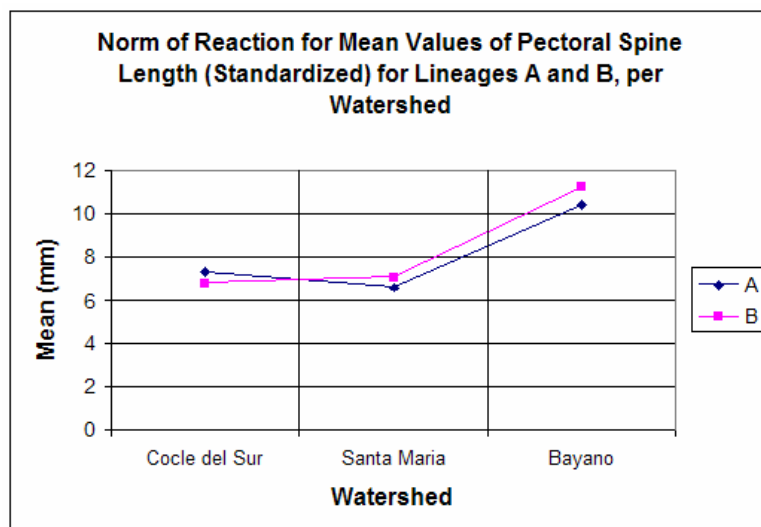
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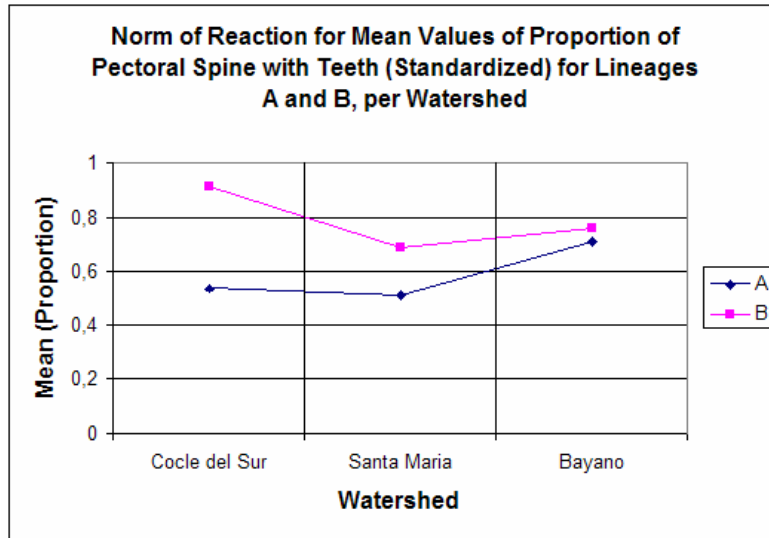
*Appendix 1*



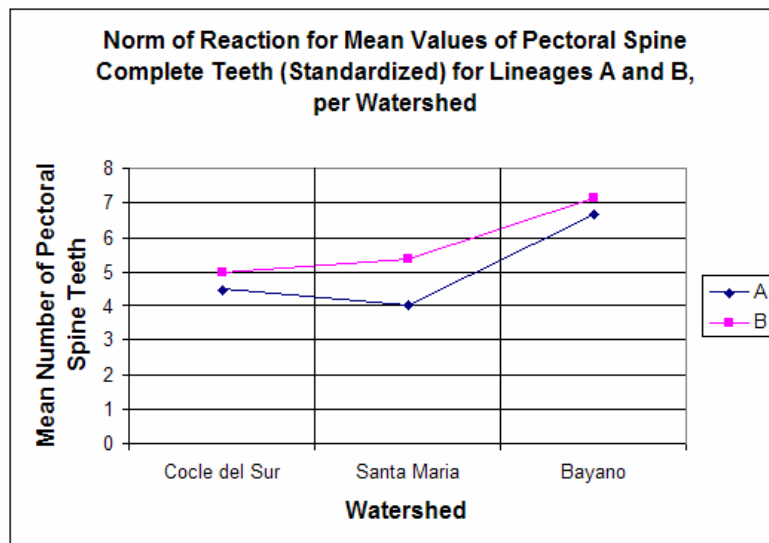
**Norm of reaction for the caudal peduncle depth measurements of *P. chagresi*, lineages A and B, across 3 watersheds.**



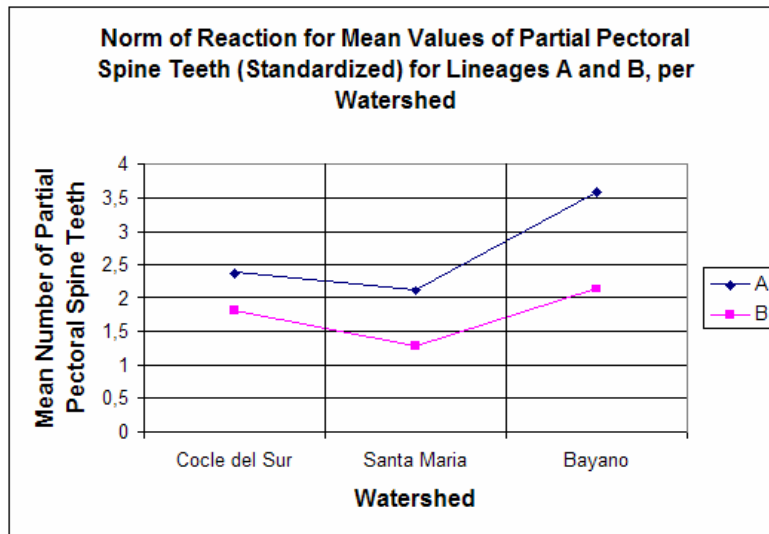
**Norm of reaction for the pectoral spine length measurements of *P. chagresi*, lineages A and B, across 3 watersheds.**



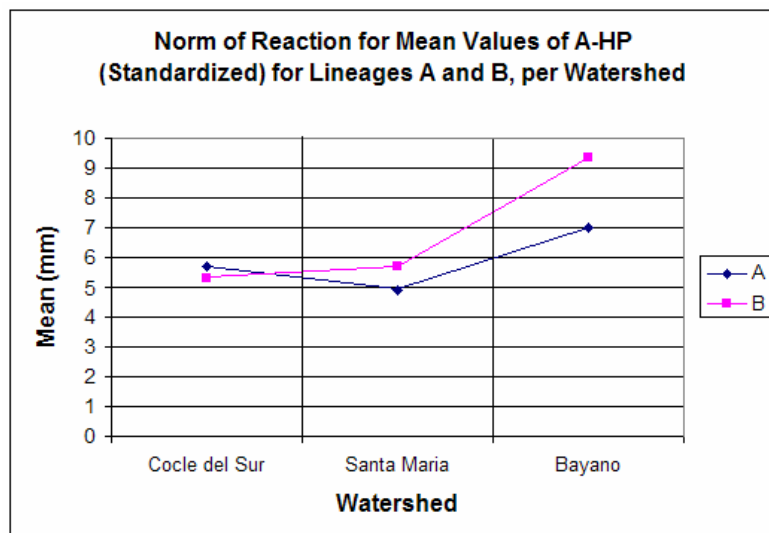
Norm of reaction for the proportion of pectoral spine with teeth measurements of *P. chagresi*, lineages A and B, across 3 watersheds.



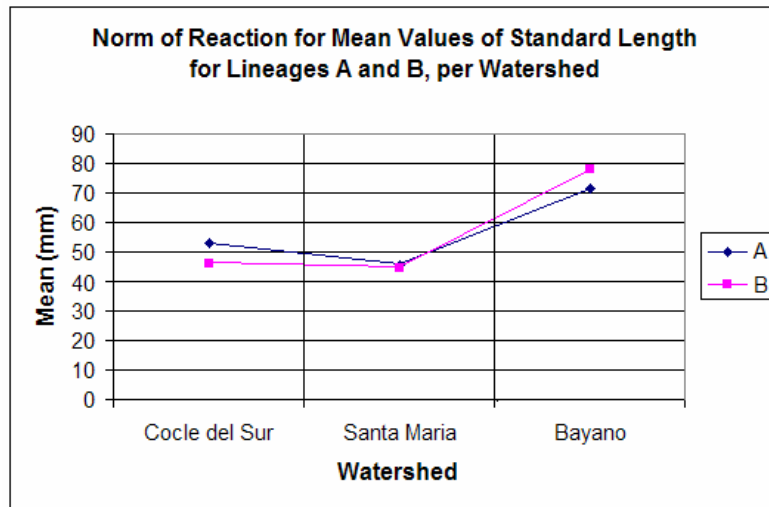
Norm of reaction for the number of complete posterior projecting teeth on the pectoral spine of *P. chagresi*, lineages A and B, across 3 watersheds.



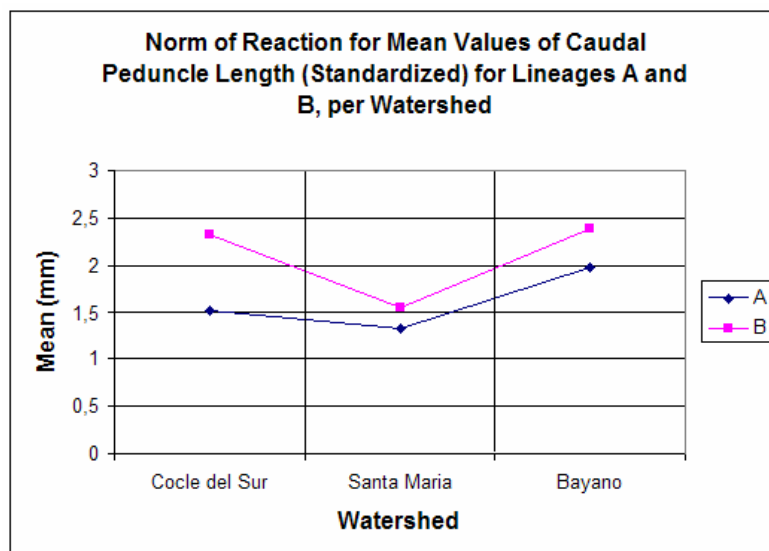
**Norm of reaction for the number of partial posterior projecting teeth on the pectoral spine of *P. chagresi*, lineages A and B, across 3 watersheds.**



**Norm of reaction for the length between the adipose fin and the end of the spine of *P. chagresi*, lineages A and B, across 3 watersheds.**



**Norm of reaction for the standard length of *P. chagresi*, lineages A and B, across 3 watersheds.**



**Norm of reaction for the caudal peduncle length of *P. chagresi*, lineages A and B, across 3 watersheds.**

