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Morphological Variation Among Populations of *Uca rapax* in the Gulf of Mexico and Northern Caribbean

Amanda L. Brase
University of Northern Iowa

MORPHOLOGICAL VARIATION AMONG POPULATIONS OF *UCA RAPAX* IN THE GULF OF MEXICO AND NORTHERN CARIBBEAN

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Amanda L. Brase

University of Northern Iowa

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Date — Carl Thurman, Honors Thesis Advisor, Biology

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INTRODUCTION

Fiddler crabs are crustacean invertebrates inhabiting many coastal habitats in temperate and tropical regions around the world (Crane, 1975). The adult crab lives on sand or mud in or above the tide marks. The larval stages (zoea and megalopae), on the other hand, are planktonic living temporarily in bays and the coastal oceans (Hyman, 1922). In the Atlantic Ocean there are 21 known species of *Uca* (Bienlich & von Hagen, 2006). Among the 20 species found along the western shores of the Atlantic Ocean, *Uca rapax* (Smith, 1870) has the largest geographic range. It is distributed from Volousia County, Florida to Cananeria, Brazil (Vernberg & Vernberg, 1967, Tashian & Vernberg, 1958). Across the latitudes, populations of *U. rapax* live in many different environments. Being so widely-spread, various populations most likely experience different selection pressures. Also, it is likely that there are physical barriers serving as obstacles blocking the oceanic dispersal and transport of larvae. The dissemination of larvae allows for gene flow among populations which should promote uniformity of phenotype (Grantham et al., 2003, Kelly & Palumbi, 2010). If populations are reproductively disconnected, local inbreeding and selection would promote the appearance of morphological and genetically distinct populations. Significant morphological variation among populations would support the notion that this species is a complex of geographically distinct subspecies. Should sufficient morphological variation occur among geographically isolated or remote populations, a new distinct species could be forming.

This study explores the relationship between phenotypic variation in the carapace morphology and the geographic distribution of *Uca rapax* population in the Gulf of Mexico, Florida, and the Northern Caribbean Sea. Carapace or body shape in 37 populations is analyzed quantitatively and correlated with two habitat variables: salinity and location. Although there is

morphological overlap among populations grouped by habitat, they are significantly different when sorted by geographic region. Populations from the northern and western Gulf of Mexico diverge from those in Florida, the Yucatan and the northern Caribbean implying restricted larval dispersal and/or differential habitat selection.

LITERATURE REVIEW

THE GENUS (*UCA*)

Fiddler crabs (genus *Uca*) are abundant, semi-terrestrial crustaceans. They feed by ingesting sediment and water from the tidal zone, and using their feeding appendages, known as setae, to clean food off the particles. This frees bacteria, microalgae, and detritus (organic particulate matter) for them to swallow, and the sediment is then spat out (Crane, 1975, Koch, 1999, Miller, 1961) By feeding on dead and decaying biological material fiddler crabs help to maintain the carbon cycle within the ecosystem. By creating burrows for protection, mating, and sleeping, they help to aerate the soil and help the local plants to thrive (Montague, 1980, Texas Parks & Wildlife, 2012).

Fiddler crabs start out as eggs attached to their mother's abdomen. They are released into the ocean at high tide as early stage larvae (zoea) and are capable of swimming on their own. They develop into late stage larvae (megalopa) while living in the ocean and their movement is dependent on the direction of the ocean currents. The megalopae return to land via the tides, and metamorphose to juveniles, which then develop terrestrially into adult crabs whose secondary-sexual characteristics distinguish males from females (Crane, 1975). Along with their life cycle, the behaviors of fiddler crabs are directly linked to tidal patterns (Crane, 1975, Caravello & Cameron 1987, Backwell et al., 1999). Tidal cycles can differ between locations due to the shape of the ocean basin, and fiddler crabs have been shown to adapt their daily activity to the local tidal rhythms. The Gulf of Mexico experiences primarily diurnal tidal rhythms (one high and one low each day), while in the Caribbean, Florida, and Belize, semidiurnal patterns (two highs and two lows each day) are more common (Stillman & Barnwell, 2004).

The distribution of fiddler crab larvae by ocean currents is important to the relationship among different populations of the same species. Figure 1 shows the ocean currents relevant to

the populations in this study. The dispersal of crabs from one population to a new location can introduce their genes into the new population and create gene flow among geographically distant populations. This gene flow promotes phenotypic uniformity. If gene flow was the only contribution to fiddler crab phenotype, then all the populations connected by larval dispersal via ocean currents would be physically similar, and populations disconnected from others would exhibit divergence (Cowen et al., 2006). This is not the case however, as many other variables influence phenotype (Hellberg, 2009).

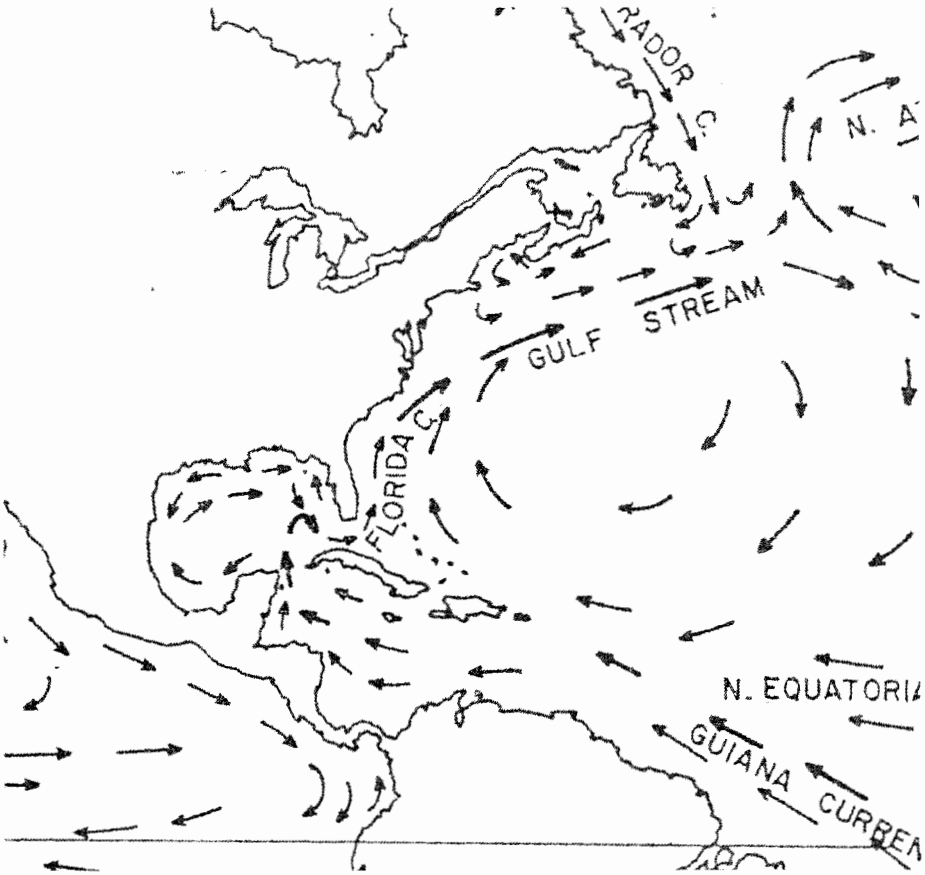

Figure 1. Direction of ocean currents in the western Atlantic. Currents move through the Caribbean from south and enter the Gulf of Mexico, then exit around Florida and to the north (Created by Dr. Carl Thurman and Dr. Peter Berendzen).

TAXONOMIC HISTORY OF *U. RAPAX*

Gelasimus rapax was first described in 1870 by Sidney I. Smith, an American zoologist. Smith detailed the anatomy of the one specimen he had encountered, and indicated that it is quite distinct from other fiddler crabs species. According to his work, it most closely resembled *G. pugnax*, and he suggested that these two species could be related (Smith, 1870). The older genus name, *Uca* (Leach), was revived in 1897 (Rathbun), and *G. rapax* was classified as a subspecies of *U. pugnax* (Rathbun, 1902, 1918). By the 1920's the distribution of *U. pugnax rapax* was known to extend from the Atlantic coast of Florida and Gulf coast of Texas, through the Caribbean to the state of Bahia in Brazil (Rathbun 1918; Crane 1943). In 1939, de Oliveira described another subspecies *Uca pugnax brasiliensis*, endemic to Brazil. Consequently, *Uca pugnax* was considered a complex of three subspecies: *U. p. pugnax* (Massachusetts to Florida), *U. p. rapax* (Florida/Texas to equatorial Brazil) and *U. p. brasiliensis* (Rio de Janeiro to Sao Paulo. Brazil). Tashian and Vernberg (1958) examined the morphology, behavior, and ecology of the *pugnax* and *rapax* subspecies from their overlap region in northeast Florida and found them to be distinct. This resulted in restoring each to a full-species status (i.e. *Uca rapax* and *Uca pugnax*). After collecting in Brazil Crane (1975) considered the South American subspecies *brasiliensis* to be identical with other *Uca rapax.* Recently, this has been confirmed by Tavares and Mendonça (2003). Consequently, the range for *U. rapax* extends from the southeastern United States to the south Atlantic coast of Brazil (Vernberg & Vernberg 1967).

In 1968, Salmon and Atsaides described two new species of fiddler crabs in the northern and western Gulf of Mexico, *Uca longisignalis* and *Uca virens.* These were tentatively accepted by Crane (1975) as subspecies of *Uca rapax* and *U. pugnax*, respectively. However, both species were deemed unacceptable by von Hagen (1980). Later Thurman (1982) demonstrated the distinctness of *Uca longisignalis* among *U. pugnax, U. rapax and U. minax.* In 1984, *U. virens*

was regarded as morphologically, behaviorally, and ecologically identical to *U. rapax* (Barnwell & Thurman). Although Salmon and Kettler (1987) argued to revive its status, current opinion has not recognized *U. virens* as a viable species (Beinlich and von Hagen 2006; Ng et al. 2008).

HABITATS OF *U. RAPAX*

Uca rapax has the largest distribution of any fiddler crab species in the western Atlantic (Figure 2). This requires it to live in a variety of habitats in both tropical and temperate regions. Figure 3 is a two-dimensional ecological model comparing the substrates and ocean water salinities of *U. rapax* with other fiddler crab species, and shows that it has an unusually wide range of habitat salinities (Thurman et al., 2010).

Figure 2. The distribution of *Uca rapax*, extending from the Gulf of Mexico and Florida to Brazil (Rosenberg, 2007).

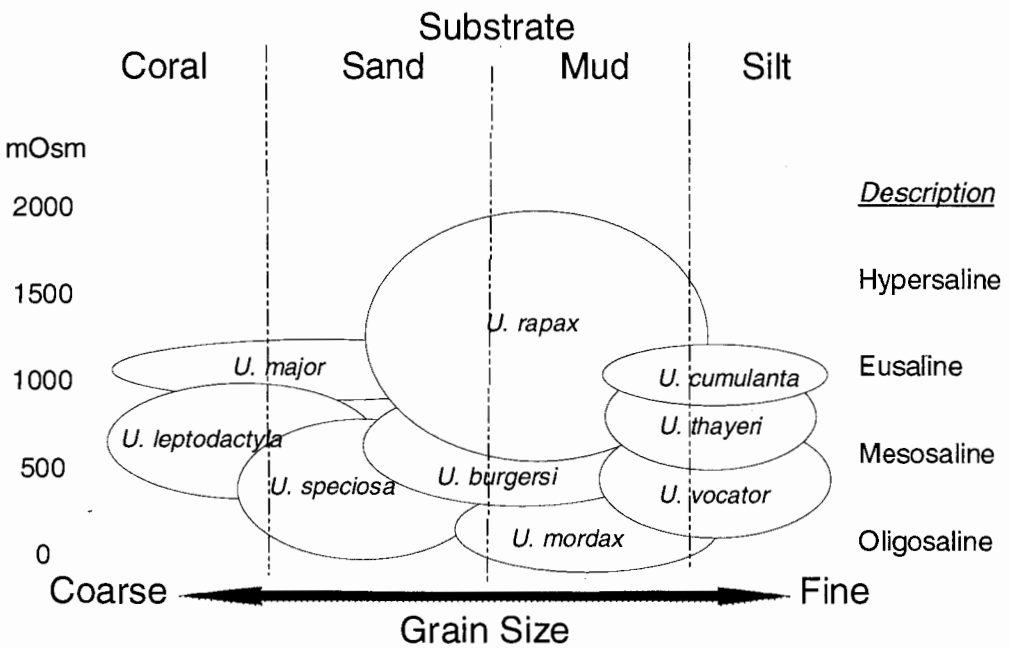

Figure 3. Two-dimensional ecological model of fiddler crab habitats. The y-axis represents water salinity, and the x-axis represents substrate type and size (Thurman et al., 2010).

GENETIC VARIATION

Genetic variation may be closely associated with physiological and morphological variation. Morphological or physiological variation that is present without a similar pattern of genetic variation may indicate epigenetic roles in crabs' adaptation to different environments. Previous studies of genetic variation among fiddler crabs have been performed at UNI through past Honors Theses. Anna Wieman (2011) analyzed mitochondrial cytochrome oxidase subunit 1 (CO1) in another species of fiddler crabs, *Uca maracoani.* She found that there was very little genetic differentiation with respect to the population structure. Another study used the species, *Uca minax*, to analyze CO1 and nuclear internal spacer (ITS) sequences. Within the USA, *Uca minax* has a disconnected distribution between Gulf and Atlantic populations which could promote genetic diversity by inhibiting gene flow. However, the Gulf and Atlantic groups were found to have no significant genetic distinction (Warwick, 2009).

Genetic differentiation within *Uca minax* was also studied by analyzing analogous enzymes (allozymes) separated out by electrophoresis. Very little allozyme divergence was found among populations of *Uca minax*, and the variation that did occur was not related to geographic distance (Felder & Staton, 1994). The composition of hemocyanin (a blood protein of crabs) was analyzed in many species of fiddler crabs, including *Uca rapax.* There was found to be low genetic divergence within the species of *Uca rapax*, especially when compared to divergence among different species (Mangum, 1996). Another study compared *Uca rapax* and *Uca virens* using biochemical differences at 21 loci. The results of the two species were nearly identical, which implies that the differences within these two groups of fiddler crabs are not genetic (Salmon & Kettler, 1987). Silva et al. (2010) analyzed mitochondrial DNA cytochrome oxidase I sequences in eastern African populations of *Uca annulipes*, which revealed no relationship between geographical location and genetic variation. Overall, these studies support

the idea that fiddler crabs exhibit very little intraspecific genetic variation. This indicates that phenotypic divergence occurring within a species is likely due to environmental factors or differences in epigenetics.

PHYSIOLOGY OF *U. RAPAX*

Exploring physiological variation as it relates to geographical distribution may help explain related morphological variation across the same distribution. Physiological abilities of a species can reflect survival requirements based on the habitats it lives in. *U. rapax* has a high desiccation tolerance, which is well suited for its dry, hyperosmotic terrestrial environments (Thurman, 1998). This characteristic is related to its osmoregulation. It is known to have exceptional osmoregulatory abilities, and tolerates both hyperosmotic and hyposmotic conditions (Thurman, 2003, Thurman, 2005). This may contribute to its large geographical distribution, which contains a broad range of habitat salinities. *U. rapax* has been shown to exhibit ecophenotypic variation in its osmoregulatory ability, which means it acclimates to the osmotic conditions of its particular habitat (Thurman et. al., 2010).

Uca rapax lives in both the tropics and subtropics. These regions differ in their environments, especially with respect to temperature. Many studies by Vernberg et al. (1959, 1967, 1968, 1969) have shown that fiddler crabs have adapted to their local thermal conditions through a variety of physiological means, including metabolic rate and oxygen consumption. Vernberg and Vernberg (1968) studied metabolic acclimation patterns and found that tropical and temperate populations of *Uca rapax* are physiological distinct in this respect.

Local adaptations are small adjustments in the characteristics of organisms to suit their environment through natural selection. Populations of *U. rapax* have been shown to exhibit differences in physiological abilities that are related to their local environmental conditions. This

supports the idea that local adaptation plays a part in divergence among populations of *U. rapax.* If local adaptations were the only contributors to phenotype, populations with similar environments would be phenotypically similar, while different environmental conditions between populations would create divergence with respect to that habitat variable. This is the opposite of the previously described gene flow model. Local adaptations and gene flow both play a part in phenotypic characteristics of many marine invertebrates, which causes a balance between uniformity and adaptive differentiation (Sanford & Kelly, 2011).

REGIONAL STRUCTURE OF VARIATION

Based on larvae from reef fish, Cowen et al. (2006) found typical dispersal distances are only 10 to 100 kilometers. Their results revealed regionalization corresponding to genetic and morphological clines across the range of the marine species. In the Caribbean, two regions are strongly recruitment limited: the Mexican Caribbean- Bahia de Campeche, and the Windward Islands. Consequently, the western and eastern Caribbean are relatively isolated. Populations from Belize, Yucatan and Cuba are strongly connected as are those in the Bahamas and Turks and Caicos. The open Caribbean south of Jamaica, Hispanola, and the Windward Islands forms another isolated subregion: the Panama-Colombia Gyre (Cowen et al., 2006). It would be reasonable to expect that populations within each of the four subregions would be homogenous. Alternatively, isolation among the subregions would promote regional differences.

GEOMETRIC MORPHOMETRICS

Geometric morphometrics is a technique used to quantitatively analyze shape. The data comes from the placement of landmarks on digital images of the specimens of study (Zeldich et al., 2004). Both the photographic method and the digitization of landmarks need to be

consistent in order to assure the variation being analyzed accurately represents the morphological variation between specimens (Rufino et al., 2006).

Morphological variation in claw shape across the fiddler crab genus has been performed using geometric morphometrics. The study by Rosenberg (2002) found that allometric growth (growth of different parts at different rates) of major claws contributed to consistent shape and size variation. Both claw and carapace shape of *Uca annulipes* were analyzed in a geometric morphometric study. No relationship between the geographical location of populations and shape differentiation was found (Silva et al., 2010). On the other hand, a study on the carapace morphological variation of eight species of fiddler crabs was performed to analyze intraspecific and interspecific morphological divergence across the eastern U.S. and Mexico (Hopkins & Thurman, 2010). Even though the dispersal potential of crab larvae is very high, morphological divergence was found to occur over very short distances. Accordingly, the geographic range size of a species did not seem to relate to greater intraspecific variation in the carapace. Some of the divergence found was due to allometric growth and differences in maximum body size. The remaining variation was attributed to local variables, including the patterns of ocean currents, and environmental factors.

MATERIALS AND METHODS

SPECIMENS

The specimens used in this study were collected by Carl Thurman between 1976 and 2008 and were consistently frozen and preserved. Female specimens were photographed by placing them on a level horizontal surface with their carapaces facing up, and were only photographed by one person (Melanie J. Hopkins). Only the females were used for this study in order to decrease the influence of sexual selection, since males have asymmetrical carapaces (Hopkins & Thurman, 2010). The digital images of these specimens were provided by Dr. Thurman and his colleague, Melanie Hopkins, Ph.D., to use for my data collection and morphological analysis.

DATA COLLECTION

Using the program tpsDig, (Rohlf, 2010) 25 landmarks on photographs of the specimens were digitized. The first 23 landmarks were chosen because they summarize the overall shape of the carapace and internal features of the crab. Figure 4 shows the landmark positions and an example of a superimposition plot of data (Hopkins & Thurman, 2010). Landmarks 24 and 25 were placed one centimeter apart (using a ruler in each photograph) in order to scale each landmark configuration.

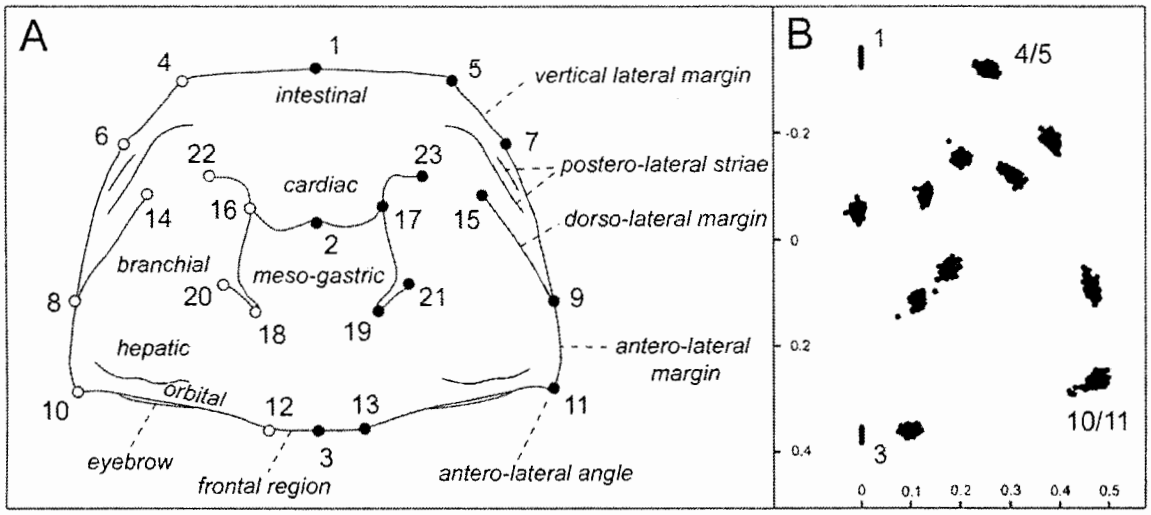

Figure 4. (A) Anatomical regions and location of 23 landmarks on carapace. Closed circles show configuration of landmarks after averaging. (B) Superimposition plot of *Uca (Minuca) minax* (N = 141) landmark configurations after averaging. The superimposition method shown is sliding baseline, appropriate for visual assessment of landmark variation in bilaterally symmetric organisms. This aligns all landmark configurations to two axial landmarks (1 and 3), allowing variation only along the sagittal axis for these two landmarks (Webster, Sheets & Hughes, 2001; Kim et al., 2002, Hopkins & Thurman, 2010).

Population	# of Specimens	Country	State	County	Latitude	Longitude	Date collected
Tobacco Range Cay	4	Belize			16.892	-88.086	1/3/2006
Sittee Belize	4	Belize			16.809	-88.255	1/3/2006
Dawkins	2	Jamaica			17.958	-76.866	3/12/2005
Kingston Bay	9	Jamaica			17.964339	-76.854469	3/12/2005
North Champoton	2	Mexico		Campeche	19.461814	-90.705894	1/1/1976
Rio Champoton	11	Mexico		Campeche	19.358928	-90.671161	9/1/1976
Isla Carmen	17	Mexico		Campeche	18.765386	-91.523781	8/31/1976
Puerto Ceiba	12	Mexico		Tabasco	18.422383	-93.169614	8/29/1976
La Pesca	1	Mexico		Tamauplas	23.792606	-97.803903	9/1974
Veracruz	1	Mexico		Veracruz	20.239094	-96.784211	9/14/1976
Progresso	1	Mexico		Yucatan	21.275394	-89.663217	9/4/1976
Nuo Progresso	1	Mexico		Yucatan	21.259733	-89.699567	9/4/1976
Boca Lagartos	1	Mexico		Yucatan	21.594067	-88.161781	9/5/1976
Rio Lagartos	2	Mexico		Yucatan	21.596228	-88.145944	9/5/1976
Werner Salt	12	US	FL	Pasco	28.28833	-82.72278	6/2003
Oscar Sarasota	4	US	FL	Sarasota	27.16917	-82.47639	6/2003
Flamingo	3	US	FL	Monroe	25.13833	-80.93083	6/2003
Oleta Mangrove	3	US	FL	Dade	25.92667	-80.13667	6/2003
Oleta River	4	US	FL	Dade	25.92667	-80.13667	6/2003
Desoto	5	US	FL	Manatee	27.52361	-82.645	6/2003
Boynton Beach	5	US	FL	Palm Beach	26.51361	-80.05528	6/2003
Ft. Pierce	1	US	FL	St Lucie	27.47667	-80.31528	6/2003
Dauphin	10	US	AL	Mobile	30.25	-88.06667	6/20/2002
Grand Isle	18	US	LA	Jefferson Parish	29.2	-90.05	6/18/2002 & 2006
GCRL	8	US	MS	Jackson	30.4	-88.85	6/19/2002 & 2006
Boca Chica	2	US	TX	Cameron	25.953711	-97.150047	8/25/1976
S Padre Island	13	US	TX	Cameron	26.075422	-97.166369	1994
Ingleside	25	US	TX	San Patrico	27.837739	-97.220053	7/8/2000

Princess	6	US	USVI		18.357	-64.691	2007
Enighed Pond	1	US	USVI		18.327	-64.799	2007
Elk	2	US	USVI		18.349	-64.681	2007
Coral Bay	3	US	USVI		18.346	-64.708	2007
Compass	2	US	USVI		18.319	-64.865	2007
Jaguey	18	US	Puerto Rico		17.936	-67.192	11/26/2008
Jobos	3	US	Puerto Rico		17.952	-66.184	11/25/2008
Playa Santa	3	US	Puerto Rico		17.962	-66.931	11/25/2008
Laguna Joyda	1	US	Puerto Rico		18.121	-67.18	11/26/2008

Table 1. A list of the populations, how many specimens from each were used, and the locations and dates of collection. Date and latitude and longitude information were taken from the notes of Dr. Carl Thurman.

All subsequent statistical analyses was performed using the Integrated Morphometrics Package (IMP) software by Sheets (Sheets, 2003). Using the BigFix program, the landmarks were paired across the 1-3 center line and averaged, because the right and left sides are not independent of one another. This superimposition lowers the number of landmarks to 13, as is present in Figure 4B. The data from all populations was compiled into a single file and run through the PCAgen6 program to check for outliers. The data was standardized by size, using the program Standard and landmarks 24 and 25 as a ruler.

IDENTIFICATION OF GROUPS

Two group files were created in order to help the software identify chosen categories of populations based on location and habitat (water osmolality). The location group analyses allowed comparison among groups with high vs. low potential for gene flow based on distance and ocean currents. The habitat group analyses allowed for determination of salinity influence on morphology. Tables 2 and 3 show which populations are represented by each group.

Groups	Habitat Type (Salinity)	Populations
1	Oligohaline (<300 mOsm)	Boynton Beach Elk Flamingo Grand Isle Isla Carmen Oleta River Oscar Sarasota
2	Mesohaline (300-630 mOsm)	Dauphin GCRL North Champoton Oleta Mangrove Playa Santa Puerto Ceiba Rio Champoton Sittee S. Padre Island Werner Salt
3	Euhaline (630-1050 mOsm)	Boca Chica Boca Lagartos Dawkins Desoto Enighed Pond Ft. Pierce Jobos Kingston Bay Laguna Joyda Nuo Progresso Princess Progresso Rio Lagartos Tabacco Veracruz
4	Hypersaline (>1060 mOsm)	Compass Coral Bay Ingleside Jaguey La Pesca

Table 2. Salinity groups are based on salinity categories defined by Hedgpeth (1957).

Group	Locality	Populations
1	US Virgin Islands	Compass Coral Bay Enighed Pond Elk Princess
2	Puerto Rico	Laguna Joyda Jaguey Jobos Playa Santa
3	Northern Mexico/Texas	Boca Chica Ingleside La Pesca S. Padre Island
4	Louisiana/Mississippi/Alabama	Dauphin GCRL Grand Isle
5	Western Florida	Desoto Oscar Sarasota Werner Salt
6	Southeastern Florida	Boynton Beach Flamingo Ft. Pierce Oleta Mangrove Oleta River
7	Jamaica	Dawkins Kingston Bay
8	Belize	Sittee Tabacco
9	Southern Mexico	Boca Lagartos Isla Carmen North Champoton Nuo Progresso Puerto Ceiba Rio Champoton Rio Lagartos Progresso Veracruz

Table 3. Regional groups based on location of collection site of adult crabs.

Figure 5. Map of the Gulf and Caribbean showing all the populations. The box shows a close up of the Puerto Rico and U.S. Virgin Islands populations.


Dallas
Austin
San Antonio
Houston
Atlanta
Charlotte
GCRL
Dauphin
Grand Isle
Ingleside
S Padre Island
Boca Chica
Gulf of Mexico
Werner Salt
Orlando
Tampa
Ft Pierce
Desoto
Oscar Sarasota
Boynton Beach
Miami
Oleta Mangrove
Oleta River
Flamingo
La Pesca
Havana
CUBA
Boca Lagartos
Progresso
Rio Lagartos
Nuo Progresso
Veracruz
Mexico City
North Champoton
Rio Champotori
Puerto Ceiba
Isla Carmen
Tabacco
Sittee Belize
GUATEMALA
HONDURAS
Guatemala
Tegucigalpa
San Salvador
NICARAGUA
EL SALVADOR
Managua
COSTA RICA
San Jose
Panama
PANAMA
Caribbean Sea
JAMAICA
Kingston Bay
Kingston
Downins
HAITI
Port-au-Prince
Santo Domingo
DOMINICAN REPUBLIC
PUERTO RICO
Barranquilla
Maracaibo
Caracas
Valencia
San Juan
Coral Bay
Road Town
Princess
Charlotte Amalie
Enighed Pond
Compass
Laguna Joyuda
Ponce
Jobos
Playa Santa

DATA ANALYSIS

Principal components analysis (PCA) and canonical variates analysis (CVA) of the partial warp scores were used to create visualizations of the morphological variation. PCA takes the superimposed and standardized data of all the specimens (Figure 4B) and rotates them in multivariate space in order to express the most variation on only a few axes. It does this without any respect for the defined groups. CVA is similar, except that it transforms the data in order to maximize the variation between groups. Each specimen is given a score that shows how its morphological data compared to the other specimens after the rotation. The scores of the first axis of each program (i.e. PC 1 and CV 1) explain the most variance, and each subsequent axis explains successively less variance. Two (or more) of these axes together create a plot that show the specimens scores in what is known as morphospace. Each point on these plots represents a single specimen, and the location of each is defined by its scores from both axes.

These programs also show how the morphology between a low score and a high score vary, using a graph that contains the thirteen landmarks of the superimposed data. These graphs are called deformation plots, and they use a grid and vectors to show the extent of variance, and where on the carapace it is located. In order to determine if there were significant differences among two groups, a resampled F-test using Procrustes coordinates in the program TwoGroup was used. The test gives two values used in this study. The distance between means indicates how far apart pairs of samples are in morphospace. This helps by showing which groups have more in common morphologically, and which are more different. The p-value is the probability that the differences between the two groups could occur by chance. P-values less than 0.01 were defined to be significant for this study.

ERROR ANALYSIS

Digitization error was evaluated in order to determine if inconsistent digitization was responsible for any significant variation among specimens. This was done by digitizing a randomly chosen photograph twenty times, and then comparing this variation to the variation in the entire sample using the program DisparityBox. The variation due to inconsistent digitization was at least one order of magnitude less than the intraspecific morphological variation, so digitization error is considered insignificant.

RESULTS

The plots of the data that were entered into the principal components analysis and canonical variates analysis show cohesive landmarks among all the specimens. This supports the classification of all the different populations as a single species- *U. rapax*. Figures 6 and 7 show these landmarks grouped based on the variables of salinity and location, respectively.

IMPACT OF HABITAT SALINITY

Principal components analysis show that the specimens form a cohesive group in morphospace (Figure 8, 10). Again, this supports the idea that the specimens within the populations used are of a single species. 35% of the variation in the dataset is represented along PC 1 and 2 (Table 4).

The PCA plots showing the specimens grouped by habitat salinity show a lot of overlap between groups, with none of the groups separating out (Figure 8). Using the canonical variates analysis, CV 1 shows some separation of the oligohaline group from the others, while CV 2 shows some separation of the euhaline groups from the others (Figure 9). However, this plot still shows considerable overlap among the different salinity groups. Resampled F-tests (Table 5) reveal two significant differences: among mesohaline and euhaline groups, and among euhaline and hypersaline groups.

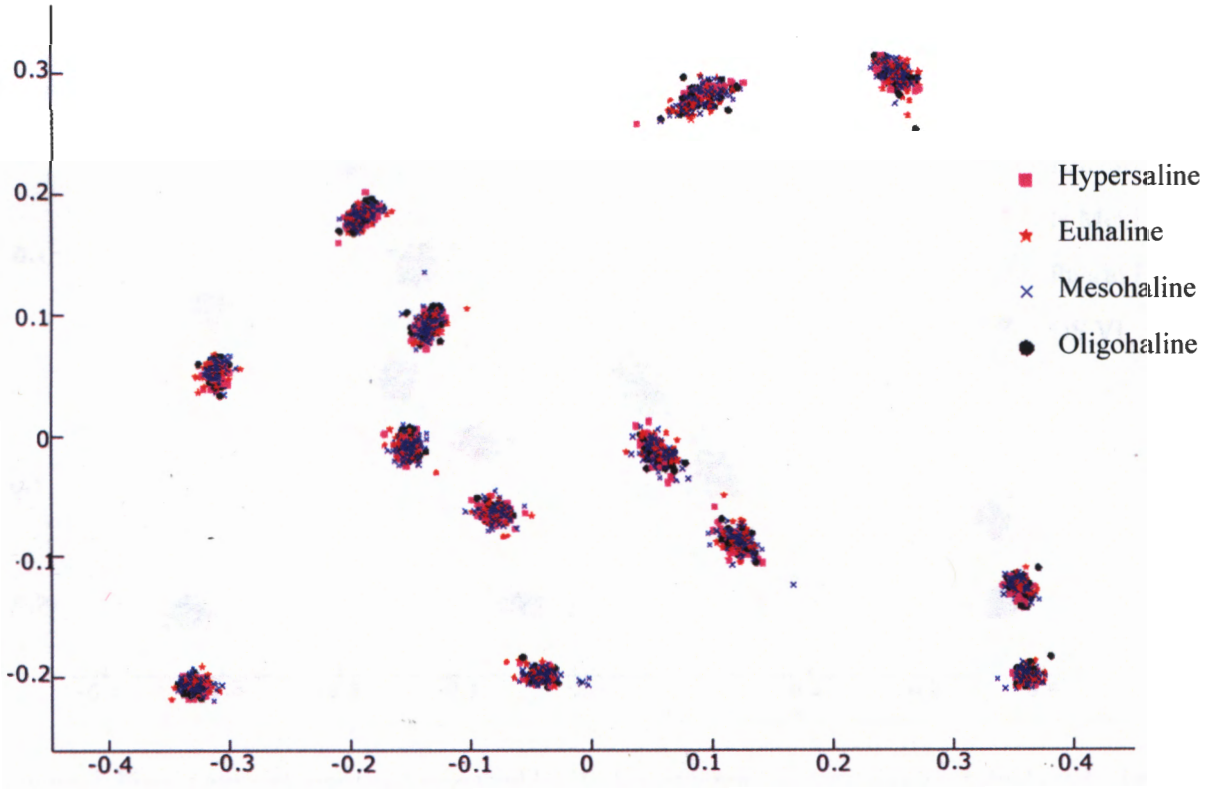

Figure 6. Fixed (superimposed and averaged by BigFix program) and standardized (by Standard program) landmark data of all 220 specimens grouped by habitat salinity.

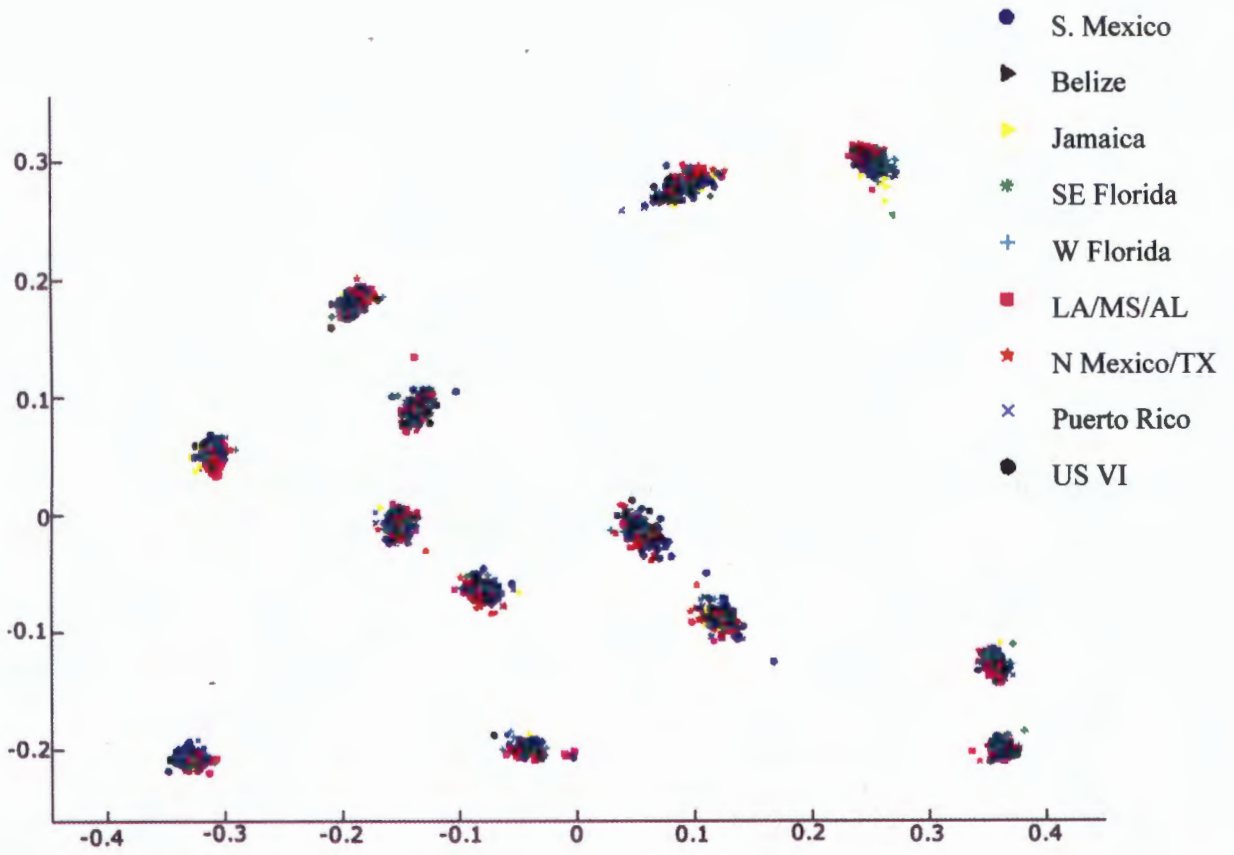

Figure 7. Fixed (superimposed and averaged by BigFix program) and standardized (by Standard program) landmark data of all 220 specimens grouped by habitat location.

Axis	Variance explained
PC 1	18.75%
PC 2	16.41%
PC 3	12.83%

Table 4. Variance explained by the three most significant axes of the Principal components analysis for all 220 specimens.

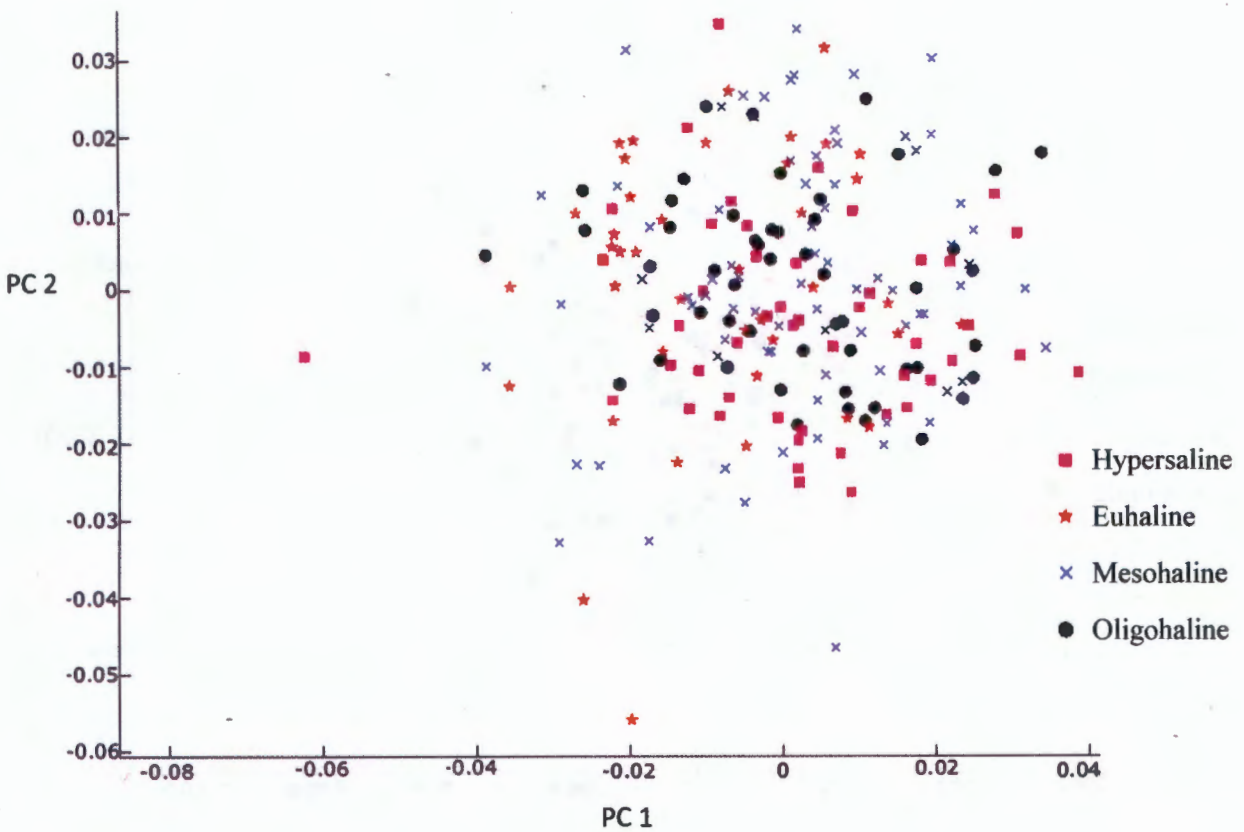

Figure 8. Principal components analysis plot (PC 1 vs. PC 2) of all 220 specimens. Symbol colors and shapes denote salinity groups. PC 1 and PC 2 scores of all specimens lie in a cohesive area. The different salinity groups almost entirely overlap each other except for a few outliers.

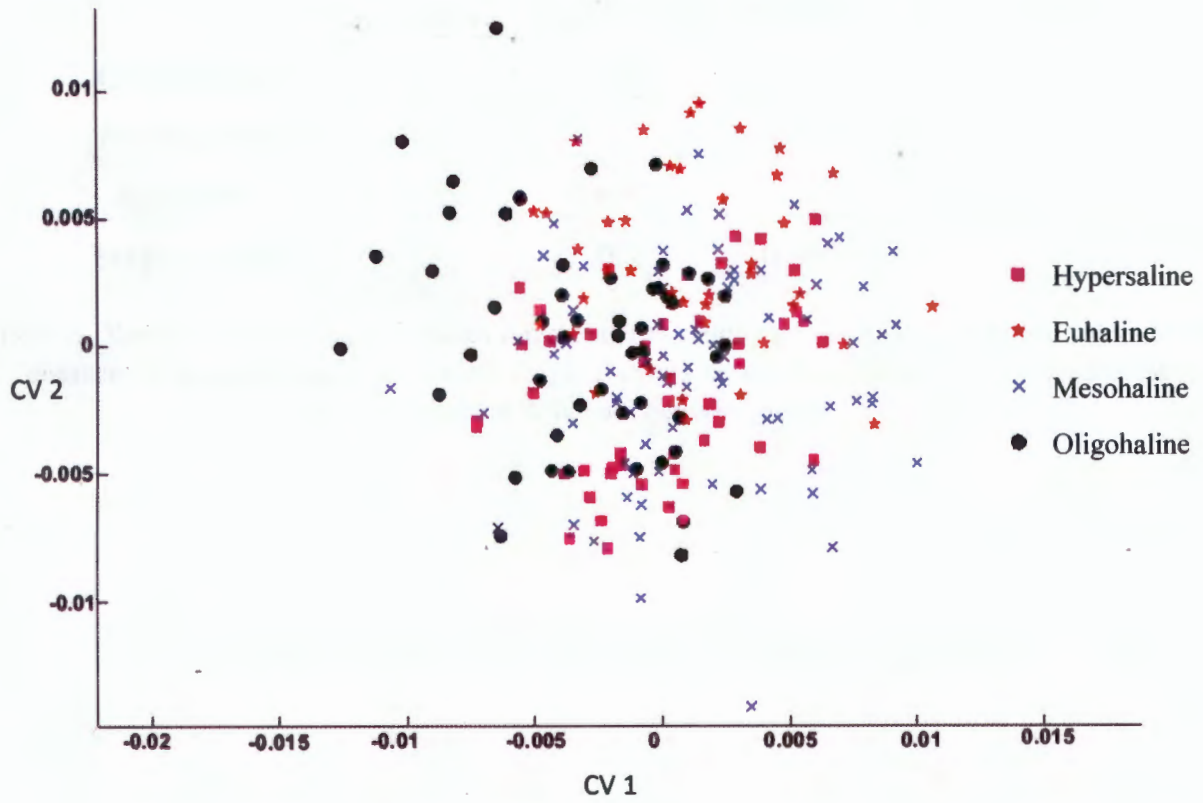

Figure 9. Canonical variates analysis plot (CV 1 vs. CV 2) of all 220 specimens. Symbol colors and shapes denote salinity groups. The CV scores of all the specimens again lie in a cohesive area, but some of the salinity groups do not overlap as much as they did in the PCA plot. The Oligohaline group is slightly distinguished from the remaining groups because of its low CV 1 scores. The Euhaline group separates from the others based on its high CV 2 scores.

	Oligohaline	Mesohaline	Euhaline	Hypersaline
Oligohaline		0.0065	0.0128	0.0083
Mesohaline	0.46		0.0123	0.0076
Euhaline	0.0175	**0.0075**		0.0146
Hypersaline	0.1775	0.2	**0.0025**	

Table 5. Results of resampling Procrustes F-test between salinity groups. Above-diagonal values are the distances between the means, and below-diagonal values are p-values. Bold, red p-values indicate a significant difference between groups.

ANALYSIS BY HABITAT LOCATION

The axes of the principal components analysis and the PC scores of each specimen are the same as in the salinity data, because the program rotates the data without respect for the defined groups. PC 1 and PC 2 show consistent overlap among the northwestern Gulf groups (northern Mexico/Texas and Louisiana/Mississipi/Alabama) with separation from the remaining groups (Figure 10). All of the remaining groups have considerable overlap, although Puerto Rico is somewhat isolated in the low scores of both axes. The canonical variates analysis shows a plot that is similar to PCA with the separations more exaggerated, and two additional groups appear distinct. Figure 11 shows that CV 1 separates Jamaica (on the low end) and the northwestern Gulf groups (on the high end) from the remaining groups. Along CV 2, Puerto Rico is again isolated.

Resampled F-test between locality groups (Tables 6 and 7) shows that the northwestern Gulf groups are significantly different from the remaining groups, but are not significantly different from each other. The F-test also reveals some results that could not be seen on the plots. For instance, the Virgin Islands are significantly different from the other Caribbean groups (Puerto Rico and Jamaica), but are not significantly different from Belize and southern Mexico. The Florida groups are only significantly different from the Virgin Islands and southern Mexico. These results do not seem to follow a geographical pattern.

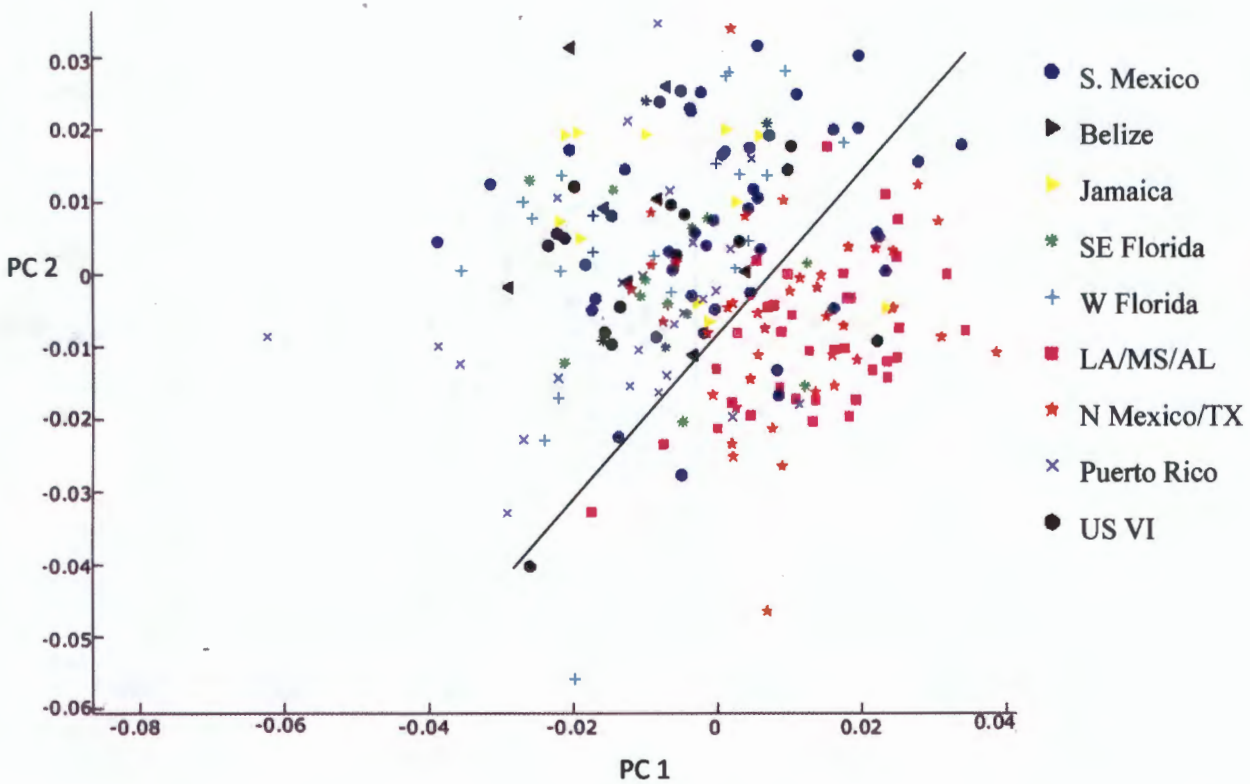

Figure 10. Principal components analysis plot (PC 1 vs. PC 2) of all 220 specimens. Symbol colors and shapes denote locality groups. Groups LA/MS/AL and N Mexico/TX separate from the remaining groups along both axes as indicated by the black line. The Puerto Rico group contains several specimens with low scores on both axes.

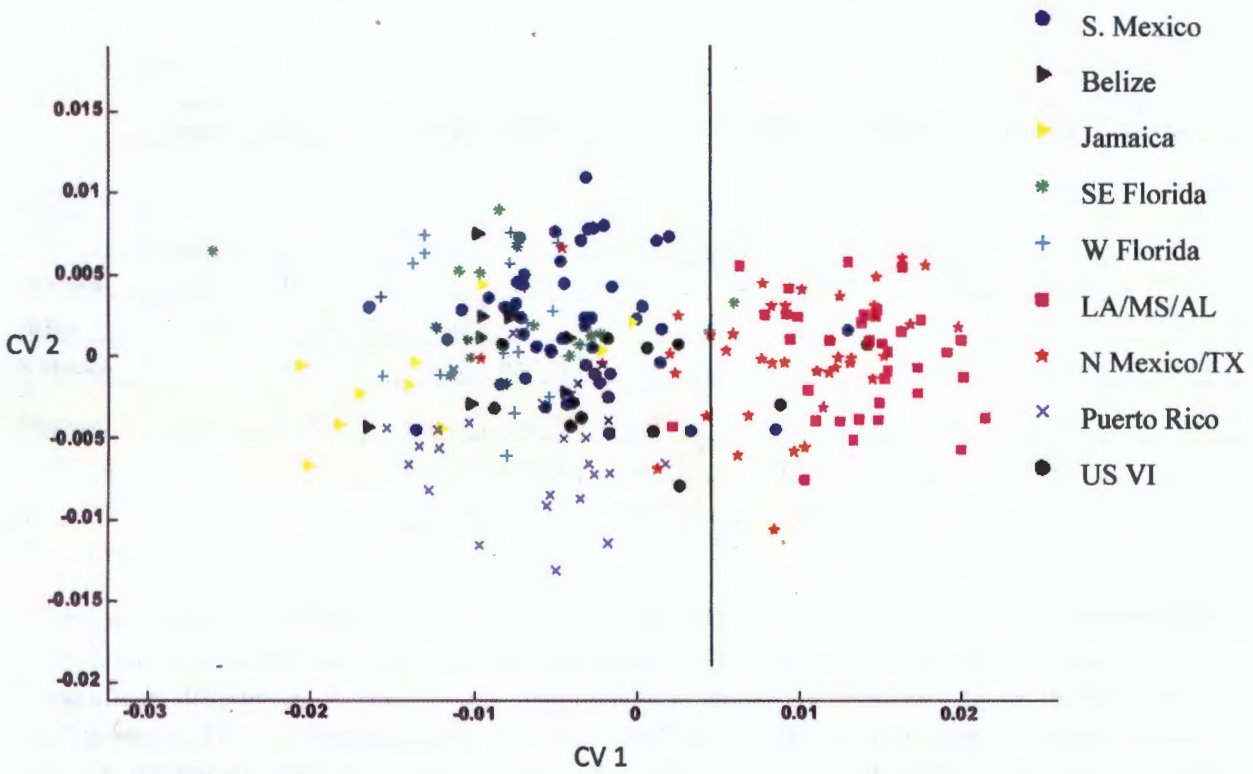

Figure 11. Canonical variates analysis plot (CV 1 vs. CV 2) for all 220 specimens. Symbol colors and shapes denote locality groups. Groups LA/MS/AL and N Mexico/TX are separate from the remaining groups along CV 1 as indicated by the black line. Among the other groups, CV 2 separates out the Puerto Rico group. The specimens from Jamaica distinguish themselves slightly along CV 1.

	Virgin Islands	Puerto Rico	Jamaica	Belize	S Mexico	N Mexico/TX	LA/MS/AL	W Florida	SE Florida
Virgin Islands		0.0205	0.0303	0.0175	0.0186	0.0217	0.0274	0.0202	0.0236
Puerto Rico	**0.0025**		0.0209	0.0202	0.0214	0.0267	0.0317	0.0156	0.017
Jamaica	**0.0025**	0.0125		0.024	0.0183	0.0313	0.0358	0.0198	0.0184
Belize	0.2075	0.055	0.02		0.0187	0.0292	0.034	0.0114	0.0162
S Mexico	0.0125	**0.0025**	0.02	0.0775		0.0201	0.0262	0.0167	0.0179
N Mexico/TX	**0.0025**	**0.0025**	**0.0025**	**0.0025**	**0.0025**		0.0098	0.0261	0.0255
LA/MS/AL	**0.0025**	**0.0025**	**0.0025**	**0.0025**	**0.0025**	0.0475		0.0318	0.0295
W Florida	**0.0025**	0.055	0.0325	0.7125	**0.0025**	**0.0025**	**0.0025**		0.0121
SE Florida	**0.0025**	0.05	0.0675	0.2625	**0.0025**	**0.0025**	**0.0025**	0.335	

Table 6. Results of a resampling Procrustes F-test between locality groups. Above-diagonal values are the distances between the means, and below-diagonal values are p-values. Bold, red p-values indicate significant differences between groups. Highlighted in yellow are p-values between the northwestern Gulf groups and the remaining groups. They are significantly different from each of the other groups, but are not significantly different from each other. The other significant differences do not seem to follow a predictable pattern.

	Closest	Furthest
Virgin Islands	Belize	Jamaica
Puerto Rico	W Florida	LA/MS/AL
Jamaica	S Mexico	LA/MS/AL
Belize	W Florida	LA/MS/AL
S Mexico	W Florida	LA/MS/AL
N Mexico/TX	LA/MS/AL	Jamaica
LA/MS/AL	N Mexico/TX	Jamaica
W Florida	Belize	LA/MS/AL
SE Florida	W Florida	LA/MS/AL

Table 7. Each locality group with both their closest and furthest groups using the distance between means. The means of the northwestern Gulf groups are closest to each other, and furthest from the mean of the Jamaica group. The Virgin Islands group is also furthest from Jamaica, whereas all the remaining groups are furthest from LA/MS/AL. This table shows that extent of morphological variation does not directly relate to geographical distance.

CARAPACE MORPHOLOGICAL VARIATION

The Procrustes deformation plot of PC 1 (Figure 12) shows that the most significant area of carapace morphological variation among all specimens is the anterolateral margin. Procrustes deformations from Canonical variates analysis with both groups (Figures 13 and 14) showed variation that is spread out between many regions of the carapace. These differences between the PCA and CVA deformations are due to the fact that these programs rotate the data in different ways.

The Procrustes deformation from the program TwoGroup shows the areas of carapace variation among the northwestern Gulf groups and the remaining groups, which is the most significant variation among populations of the entire sample (Figure 15). A large part of the variance is located in the anterolateral margin, similar to the variation within the entire group. Significant variance is also located in the posterolateral margin, with the remaining variation seen in the frontal and cardiac regions.

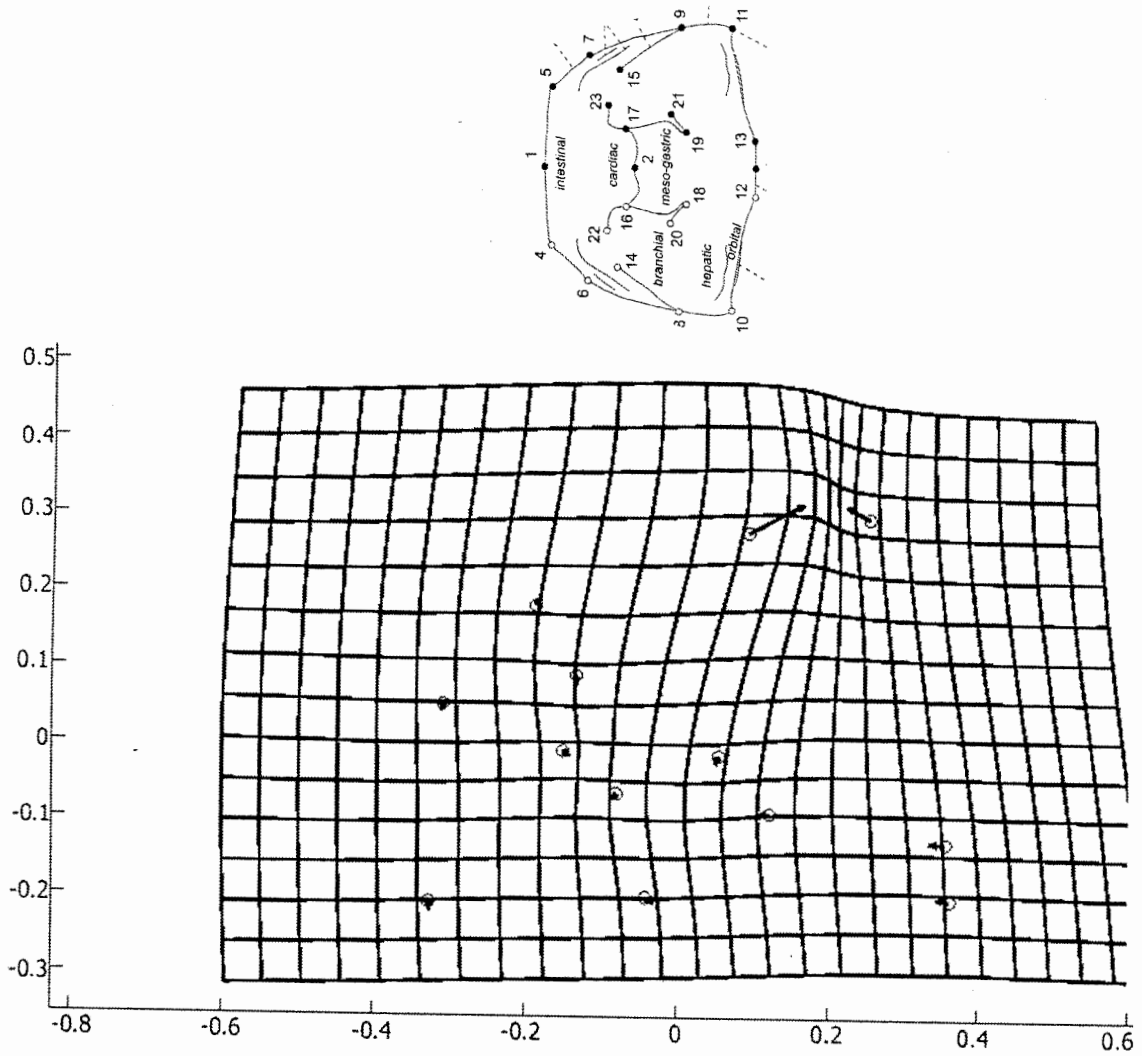

Figure 12. Procrustes deformation of PC 1. The vectors at the top right of the grid show that most of the variation is present in the Anterolateral margin of the carapace. The picture above the grid shows the carapace and landmark configuration in the same orientation as the deformation plot.

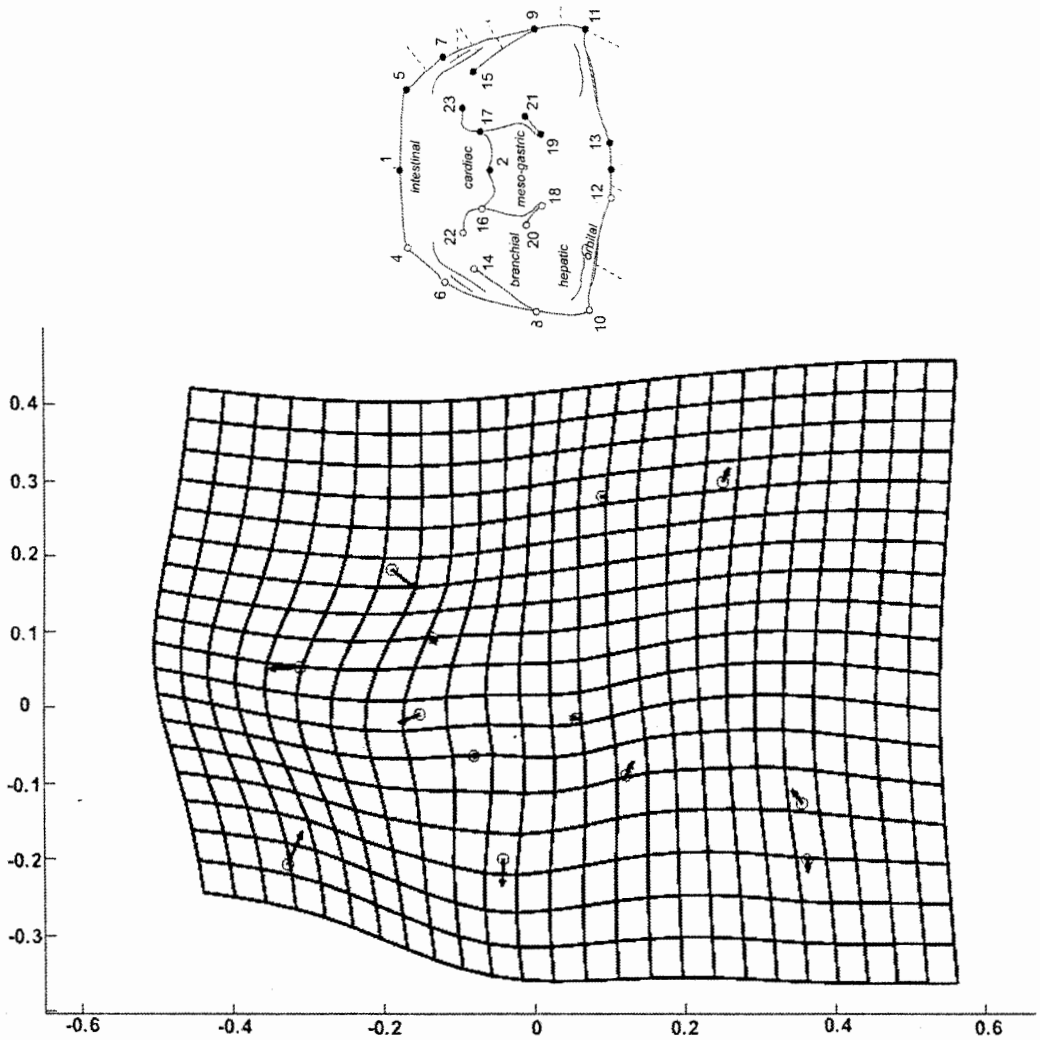

Figure 13. CV 1 Procrustes deformation with salinity groups. Most variation is seen in the intestinal region and the vertical lateral margin on the left side of the grid. The picture above the grid shows the carapace and landmark configuration in the same orientation as the deformation plot.

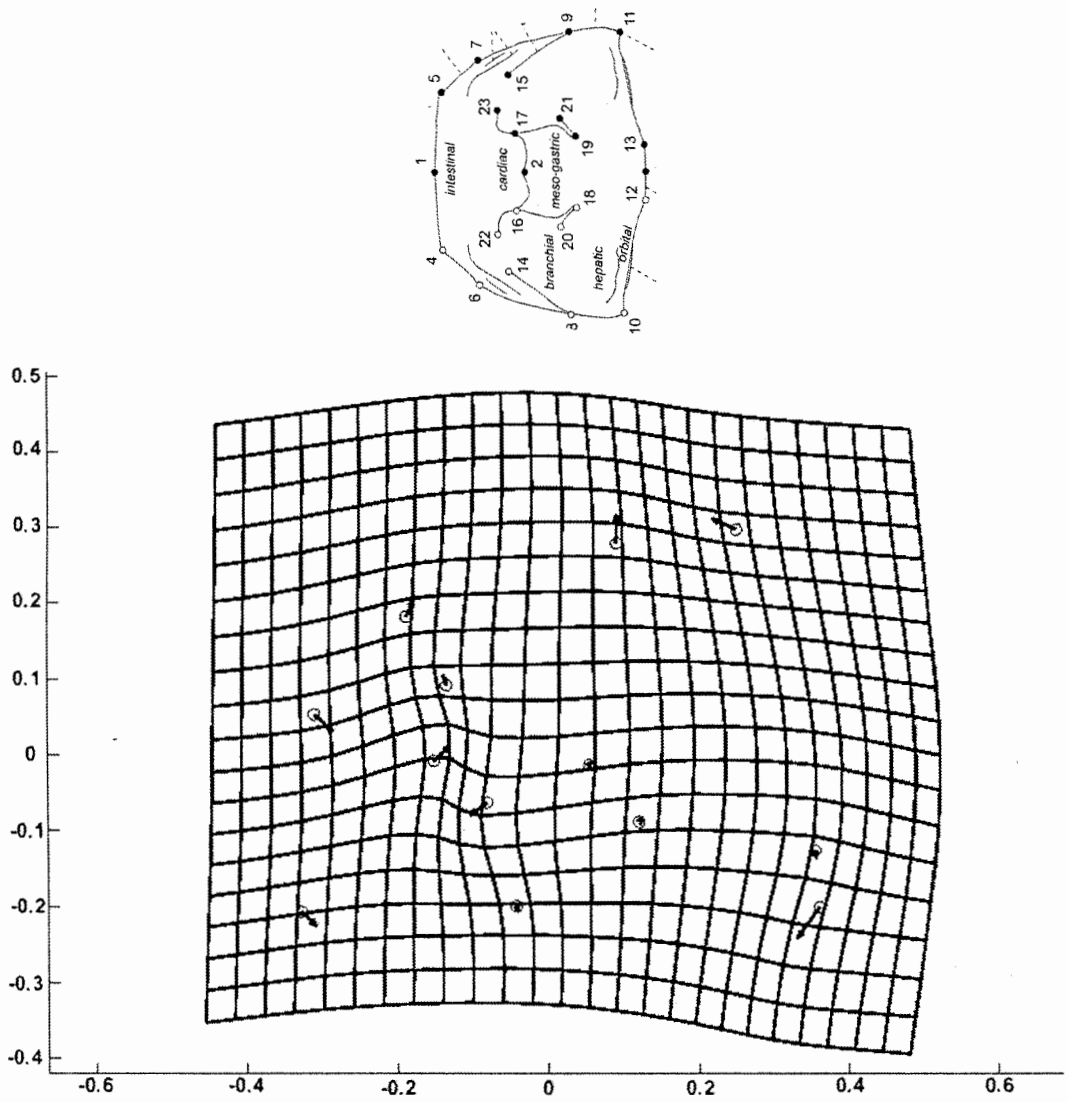

Figure 14. CV 1 Procrustes deformation with locality groups. Most variation is seen in the frontal region and the anterolateral margin in the bottom right and top right corners, respectively. The picture above the grid shows the carapace and landmark configuration in the same orientation as the deformation plot.

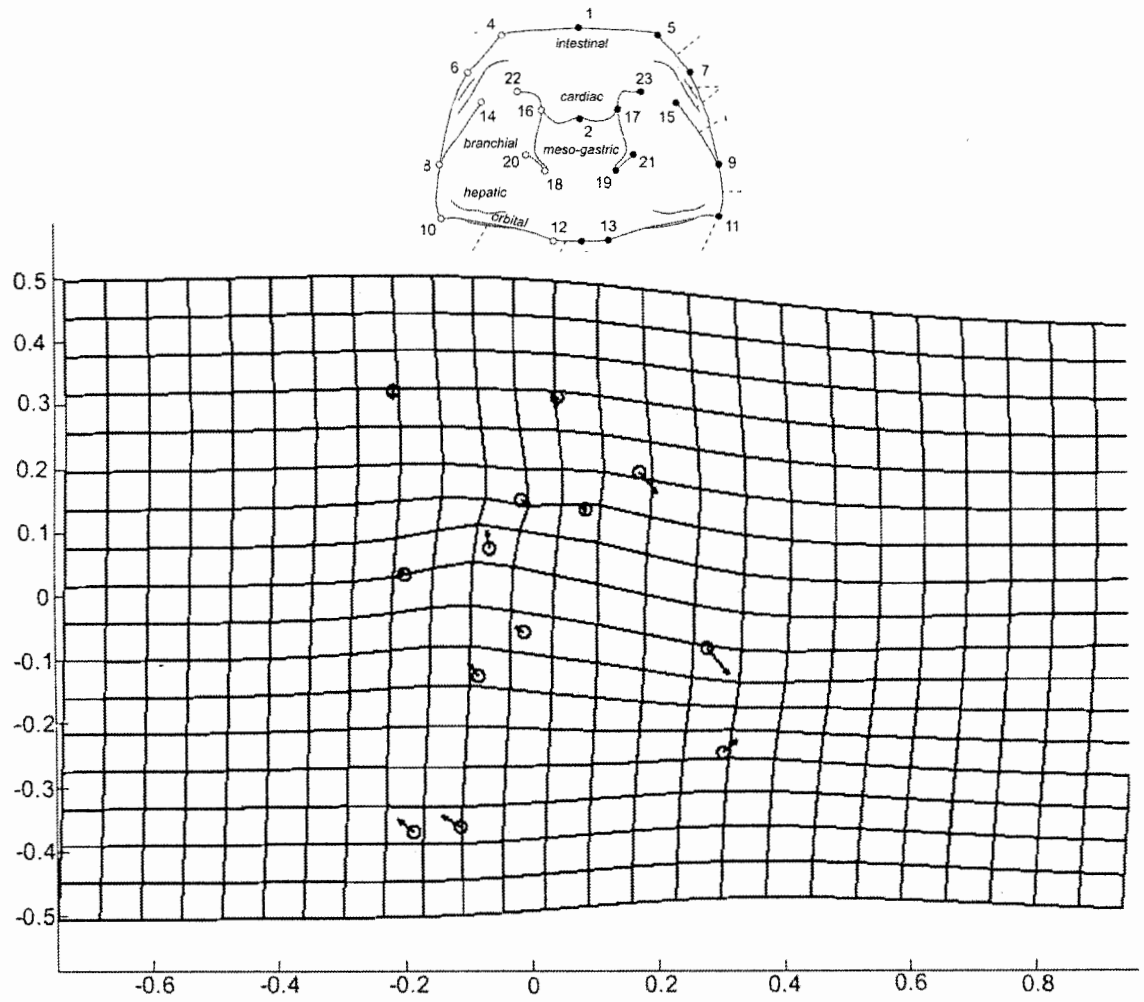

Figure 15. Procrustes deformation from TwoGroup showing the carapace morphological variation among specimens from the northwestern Gulf groups and specimens from the remaining locality groups. The majority of the variation is seen in the anterolateral margin, which is towards the bottom right in this grid. The picture above the grid shows the carapace and landmark configuration in the same orientation as the deformation plot.

DISCUSSION

The potential drivers of morphological variation used in this study were habitat salinity and geographical location. Differences in habitat salinity require crabs to physiologically adapt to their local conditions through osmoregulation in order to limit their water loss and resist desiccation (Thurman, 1998). Across the northern distribution of *Uca rapax* there also exist a range of tidal patterns. Together with the direction of ocean currents, these factors contribute to larval dispersal among populations, and explain why geographical location may relate to carapace morphology.

Salinity of habitat was found to explain very little of the morphological variation across the northern range of *U. rapax*. Fiddler crabs express ecophenotypic plasticity with respect to osmoregulation in order to adapt to their local salinity conditions, but this phenotypic divergence does not relate to any predictable pattern of morphological variation in the carapace (Thurman et al., 2010). However, there are many other environmental variables, such as temperature and substrate, that are potential drivers of carapace morphological variation in *U. rapax*.

Results show that there is a greater geographic influence on the intraspecific morphological variation. The most significant variation between populations occurred among the northwestern Gulf populations and the remaining populations. This indicates a barrier preventing larval dispersal, and correlates well to differences in the tidal patterns of the locations, as well as the direction of the ocean currents. The semidiurnal tidal pattern in the Caribbean, Florida, and the Mexican peninsula may promote more mixing of the larvae with the ocean than the diurnal rhythm in the Gulf of Mexico. This may increase the distance that the larvae travel in the ocean before returning to land. The unidirectional ocean currents outside the Gulf may also enhance the dispersal of larvae in comparison to the mixing currents present in the Gulf.

Consequently, both the tidal patterns and ocean currents in the northwestern Gulf promote a local retention of the larvae. This leads to inbreeding causing the within population variation to be low, which was shown by the similarities of specimens in the northwestern Gulf. This retention of larvae would also increase the variation among populations, which was shown by the significant differences when comparing specimens in the northwestern Gulf to the remaining specimens (Rasanen & Hendry, 2008).

Comparing the groups outside the Gulf reveals that morphological divergence does not directly correspond to geographical distance. Similar to the findings of Hopkins and Thurman (2010), significant morphological variation occurred over very short distances. There are likely other environmental factors driving morphological divergence that have not yet been identified. The epigenetic regulation of gene expression has not been studied with respect to carapace morphology, and may be an influential factor as well.

The habitat variable of location does not describe all the morphological variation seen in the northern range of *U. rapax*. However, the most apparent divergence among populations relates to geography, particularly the elements of tidal patterns and ocean currents. This supports the hypothesis that larval dispersion and geographic location are important factors in the phenotypic characteristic of carapace morphology for *U. rapax* (Cowen et al., 2006).

This study has demonstrated that there is carapace morphological variation among populations of *Uca rapax*. However, previous research has failed to discover intraspecific genetic variation in fiddler crabs. This could be due to the structure of the genetic analysis, and perhaps future studies with different techniques will demonstrate genetic variation.

LIMITATIONS

A limitation of this study was its inability to identify genotypic variation, which would tell us about gene flow. This study focused on phenotypic variation, which could be influenced by numerous factors including environmental conditions. A better understanding of what causes morphological variation will come in later studies involving genetics and physiology.

Limited female specimens were available for study from the Belize and Jamaica localities. The small sampling sizes may not accurately reflect the morphology of crabs in the Sittee River, Tobacco Range Cay, Kingston Bay, and Dawkins Lagoon populations. Larger samples may have been a better representation to use for comparison with the other locality groups.

FUTURE

In order to get a global picture of the morphological variation among populations of *U. rapax*, 70 Brazilian populations (120 specimens) will be added to this study. Morphological divergence of the entire range of *U. rapax* has never been studied before. The augmentation of the present study may help elucidate other variables contributing to variation in carapace morphology.

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