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Source: Tropical Conservation Science, 10(1)

Published By: SAGE Publishing

URL: https://doi.org/10.1177/1940082917710388

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Research Article

Tropical Conservation Science
Volume 10: 1–9
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DOI: 10.1177/1940082917710388
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Genetic Diversity of the Atlantic Ghost Crab *Ocypode quadrata* (Decapoda: Ocypodidae) in Two Beaches With Different Anthropogenic Disturbance in the North Coast of Veracruz, Mexico

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Abstract

The constant demographic expansion of human population is now recognized as a stressing factor for ecological communities on beaches, and their effects have been barely explored in developing countries such as Mexico, where heavy coastline industrialization is currently undergoing. In this work, we study how anthropogenic factors have affected the Atlantic Ghost Crab (*Ocypode quadrata*) in two beaches with different anthropogenic disturbance in northern Veracruz (near Tuxpan and Tamiahua), Mexico. To this end, we evaluated the species genetic diversity using a fragment of the Cytochrome Oxidase I gene, along with several measurements (number of haplotypes, haplotype diversity, and nucleotide diversity, etc.), estimations of genetic relationship (haplotype network, phylogenetic analysis, gene flow), and statistical tests on average genetic distances (Student's *t* test). We found 32 haplotypes, 22 from Tuxpan and 15 from Tamiahua. Despite the occurrence of almost 50% more haplotypes in Tuxpan than in Tamiahua, the correction for differences in sample size indicated that such a difference is statistically nonsignificant. A similar pattern was found with other genetic measurements. Similarly, the haplotype network and the phylogenetic reconstruction failed to recover haplotype clusters or haplogroups associated exclusively to one or another beach, whereas gene flow between localities was of the same order of magnitude in both directions. The Student's *t* test showed that differences in genetic distances between localities (estimated using *p*-distances and Jukes-Cantor 69) were not statistically significant. Finally, although the anthropogenic effects between beaches in Tuxpan and Tamiahua are remarkable different, this has not been reflected in the genetic diversity of *O. quadrata*.

Keywords

bioindicator, *Ocypode quadrata*, anthropogenic disturbance, genetic diversity, Mexico

Introduction

Beaches are one of the most important coastline resources, as they are a transitional ecotone between continental land and sea with large biological richness (Yepes, 1999). Worldwide, the increase of human population in coastlines has become a stressing factor on beaches at an unprecedented scale, resulting in significant alterations of ecological communities (Ocaña, Vega, & Córdova, 2012). In many of the beaches in urban areas worldwide, most of the dunes have been modified by a wide range of anthropogenic impacts, such as the

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Received 20 February 2017; Revised 27 March 2017; Accepted 28 March 2017

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construction of roads and buildings, pollution, damage to dune vegetation, and the impact caused by recreation and tourism (e.g., Bezuidenhout, Nel, & Hauser, 2014; Monteiro & Bemvenuti, 2006).

In this context, several studies have determined the effect of anthropogenic activities on invertebrate community of sandy beaches, evaluating the effectiveness of indicator species for the assessment of environmental impact. For example, there is the evaluation of the relationship between the use of recreational vehicles and the mortality of the pipi clam (*Donax deltoides*) in eastern Australia (Schlacher & Thompson, 2008); the relationship between the number of swimmers and population density of a sand hopper (*Talitrus saltator*) on the coast of Tuscany, Italy (Ugolini et al., 2008); and the correlation between the population density of a marine isopod (*Excirolana braziliensis*) and the urbanization in three beaches in Barra da Tijuca, Brazil (Gomes, Neves, & Almeida, 2011). These studies showed that invertebrates have been identified as efficient indicators of anthropogenic activities and generally respond to finer spatial disturbances than vertebrates. In addition, their distribution and population sizes often correlate well with environmental conditions (Lucrezi, Schlacher, & Robinson, 2009a).

Among the most studied bioindicator taxa of human disturbance are the species of genus *Ocypode*, as a direct correlation between their population density and the impact of human activities on the beaches has been observed (Correa et al., 2014). These studies have evaluated the impact of anthropogenic activities such as beach urbanization (Barros, 2001), vehicle traffic (e.g., Hobbs, Landry, & Perry, 2008; Moss & McPhee, 2006), and other human activities (e.g., Lucrezi et al., 2009a; Lucrezi, Schlacher, & Walker 2009b; Noriega, Schlacher, & Smeuninx, 2012). For example, to estimate the impact of human activity in *O. cordimana*, Barros (2001) evaluated the difference in number of burrows on the beaches of New South Wales, Australia. He found fewer burrows on beaches with high levels of urbanization and conversely, higher where urbanization was less developed. To evaluate the effect of passing vehicles, Hobbs et al. (2008) investigated the impact of off-road vehicles in *O. quadrata* population density in two beaches in North Carolina, USA. They designated open access and restricted areas to off-road vehicles for 24 h, finding a statistically significant increase in the density of crab burrows in restricted areas. In the case of the effect of human trampling, Lucrezi et al. (2009a) assessed this factor on the number and diameter of burrows of *O. cordimana* and *O. ceratophthalma* in Queensland, Australia. They recorded burrow number and diameter before and after disturbance, which involved the pacing of people for 5 h. The authors found that the number and diameter of burrows were higher before the disturbance, while trampling affected these values in the disturbed area. However, the usefulness of *Ocypode* species as a bioindicator is not consistent. For example, Ocaña et al. (2012) evaluated the population density of *O. quadrata* by directly counting the number of burrows in an area of 20 m^2 on beaches with different levels of human influence on the northeastern coast of Cuba; no direct relationship was found between the tourism development and the density of crab burrows.

Most studies have evaluated the effect of human activity on *O. quadrata* using burrows as a proxy of population size. However, this estimator is a direct consequence of behavior and it is not a measurement of a real, intrinsic propriety of the individual, which may range from population to molecular level. Moreover, crab burrow counting may be highly biased, as a crab may build several burrows (Silva & Calado, 2013). On the other hand, genetic variation of this species (either nuclear or mitochondrial) has not been determined, and consequently, its value as an actual, measurable *O. quadrata* propriety is completely unexplored. In this sense, the estimation of connectivity among populations through genetic techniques could be a method to assess the extent of fragmentation that may occur in an originally continuous distribution area. As such, this would involve the reduction in the exchange of individuals between locations and, thus, gene flow is expected to decrease (Bezuidenhout et al., 2014). Other consequences are also possible, such as the alteration of processes that allow the maintenance of genetic variability and an effect on biological adaptation and fitness of individuals (Freeland, 2005).

The increasing human activity in the beaches and ports of Mexico is causing modifications that could negatively impact species inhabiting ecosystems in coastal zones. Here, we propose the evaluation of *O. quadrata* genetic diversity as a bioindicator of anthropogenic impact. Its abundance, easiness to be identified in the field, and unexplored impact on their populations by anthropogenic disturbance make this species ideal as a study model. Specifically, we searched for distinguishable differences in the genetic diversity of *O. quadrata* in two beaches located in the Gulf of Mexico, with different levels of human disturbance.

Methods

Study Area

The study was conducted in two sandy beaches in the localities of Playa Azul, Tuxpan (20° 58' 28.6'' N, 97° 18' 26.1'' W), and Playa Norte, Tamiahua (21° 17' 24.7'' N, 97° 25' 14.1'' W), located north of the State of Veracruz, Mexico (Figure 1). Playa Azul is in the north side of the Tuxpan River mouth. The area and surroundings present recreational, commercial, and industrial activities. There are operating oil facilities (dating from

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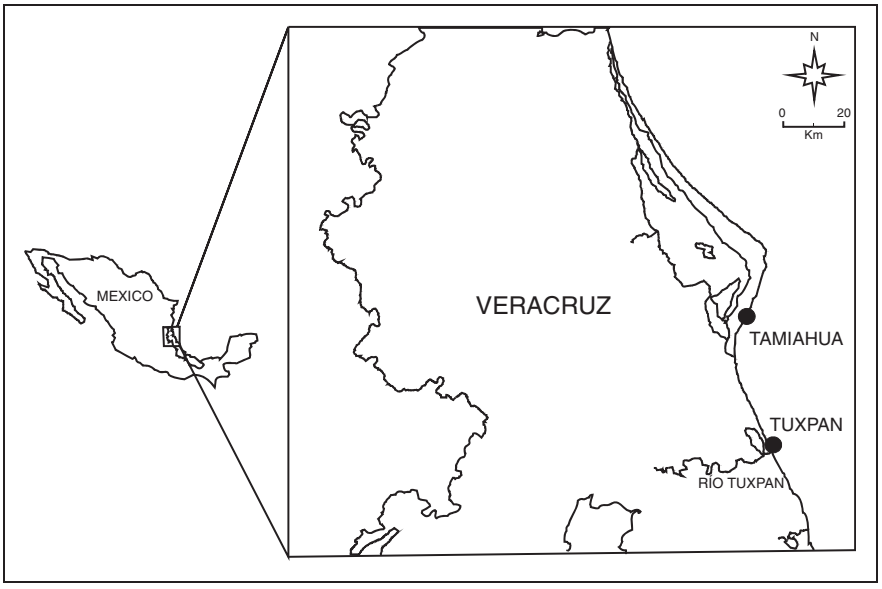


Figure 1. Map showing the two localities where *Ocypode quadrata* individuals were collected (Tamiahua and Tuxpan, black dots) in the State of Veracruz, Mexico.

the beginning of the last century), oil storage tanks, and docks (Bravo, 2010). In addition, the on setting and operation in 1991 of the Thermoelectric Central Adolfo Lopez Mateos has had negative consequences in the region. For example, it has changed the profile of the beach due to various constructions (plants and chimneys, storage tanks and fuel pipelines, jetties, etc.), it has many industrial activities (intake and discharge of seawater, fuel transportation, greenhouse gases emission, etc.), and it has modified the distribution of coastal currents and the natural sediment movement (Bravo, 2010). Conversely, Playa Norte is located at 37 km north of Tuxpan, in the municipality of Tamiahua (Figure 1). Here, fishing is the most important socioeconomic activity, and, in contrast with Tuxpan, there is no oil or industrial facility. Touristic and commercial activities are scarce, and inhabited area is very small.

Changes in the beach surface is a critical element in the biology and life history of *O. quadrata*. Therefore, anthropogenic activities may have negative consequences in its behavior, by interfering with the reproductive circle or altering their habitat (Schlacher & Lucrezi, 2010; Steiner & Leatherman, 1981). Although the differences in the level of anthropogenic disturbance in Tuxpan and Tamiahua were not directly estimated in our study (see Schlacher et al., 2016), there was evidence leading to consider the beach of Tuxpan as much more disturbed than the beach of Tamiahua. For example, the occurrence of motorized vehicles in the beach of Tuxpan has led to intense traffic, causing in turn a higher mortality of *O. quadrata* individuals by direct crushing, mainly at night; although mortality by crushing also occurred at daytime, when a higher anthropogenic activity takes place (EAR, personal observation). Tuxpan showed activities involving the use of different vehicles (four-wheel motorcycles, cars, cargo transportation), which in turn had a significant impact on *O. quadrata* mortality by burying or crushing them when driving over their burrows. The most affected were juveniles, as their burrows are only a few centimeters deep (Schlacher & Thompson, 2008). Conversely, the effect of these factors in Tamiahua were almost nonexistent. Further, presence of persons in the beach can also have negative effect, for the entrance to the burrows may collapse under trampling. For example, in Tuxpan, this factor had a higher negative impact due to the presence of workers, street vendors, and mainly tourists performing recreational activities such as swimming, sports, and sand manipulation. In Tamiahua, most of the inhabitants were fishermen whose presence in the beach was not very frequent. The burrows of the juvenile were also affected by the presence of persons on the beach by disturbing them while crabs were feeding during daytime to avoid competence and predation from larger crabs at night (Fisher & Tevesz, 1979). Conversely, since adult crabs in Tuxpan built deeper burrows (approximately 1 m deep, EAR personal observation), these crabs remain inside until the trampling stops at dusk, and then clear the entrance to exit (Lucrezi et al., 2009b). Adult crabs have a higher resistance for dehydration and heat than juvenile crabs (Chan, Chan, & Leung, 2006).

Collection of Specimens and Molecular Analyses

Ocypode quadrata adult individuals (>25 mm, regardless of sex) from the two sites were collected at night due to

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their nocturnal habits (25 and 35 from beaches at Tamiahua and Tuxpan, respectively), and muscle samples were obtained from pereipods. These tissues were preserved in absolute ethanol for their transportation to the laboratory (Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional) and were preserved at −20°C. To perform the DNA extraction, the QIAGEN extraction protocol included in DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) was followed, with a previous mechanic lysis with dry ice. Later, a 700-pb fragment for the Cytrochrome Oxidase I gene was amplified using the primers: LCO1490 (5'-GGT CAA ATC ATA AAG ATA TTG G-3') and HC02198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'; Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994). All samples were amplified by polymerase chain reaction (PCR) in a Veriti 96 Well Thermal Cycler (Life Technologies, Carlsbad, California), with a 25 μL reaction volume with the following concentrations: Buffer (1×), $MgCl_2$ (1.5 mM), dNTPs (0.2 mM), primers (0.8 μM), and Taq 1 U/μL (Kapa Biosystems, Wilmington, Massachusetts). The reaction conditions were as follows: initial denaturalization at 95°C for 20 s, alignment at 48°C for 30 s, extension at 95°C for 90 s, and a final extension at 72°C for 2 min. The PCR products were purified following the purification protocol of GeneJET PCR Purification Kit (Thermo Fisher Scientific Inc, Waltham, Massachusetts) and were sent to Macrogen Laboratories (Seoul, Korea) for sequencing. Only the forward primer was used, leading to one direction sequences.

Sequence Analysis

Once the sequences were obtained and edited, they were deposited in GenBank (accession nos. KY568729-KY568760). Edition and alignment was computed with Seaview 4.5.3 (Gouy, Guindon, & Gascuel, 2010) and Clustal X 1.83 software (Thompson, Higgins, & Gibson, 1994), respectively. To describe the genetic variability of sampled populations, we estimated: the haplotype diversity (*Hd*) and nucleotide diversity (π; Nei, 1987; Tajima, 1983); number of haplotypes (*h*), segregating sites (*S*), and average number of nucleotide differences (*k*). These estimates were computed using Arlequin 3.11 software (Excoffier, Laval, & Schneider, 2005). Due to the differences in sample size taken at the two sites, haplotypes richness was estimated through a rarefaction analysis with CONTRIB 1.02 software (Petit, Mousadik, & Pons, 1998). To evaluate the relation between *O. quadrata* haplotypes from the two collecting sites, two methods were used: (a) based in the method by Templeton, Crandall, and Sing (1992), a haplotypes network was constructed using the statistical parsimony algorithm implemented in TCS 1.21 software (Clement, Posada, & Crandall, 2000); (b) using the Maximum Likelihood method, a phylogenetic tree was constructed, with the Jukes-Cantor 69 (JC69) model of nucleotide substitution in PHYML 3.0 software (Guindon & Gascuel, 2003). This model was chosen by means of the Akaike Likelihood Ratio Test, implemented in jModelTest 2.0 software (Darriba, Taboada, Doallo, & Posada, 2012). The phylogenetic analysis was performed using *Uca pugilator* (GenBank accession number: AB813678) as outgroup.

To evaluate the magnitude of gene flow between *O. quadrata* individuals from both sites, migration rates were estimated with a maximum likelihood analysis using the Monte Carlo Markov Chain algorithm and the coalescence theory, implemented in Migrate 2.4 software (Beerli, 2008). This was performed using 10 short and 3 long chains with 10,000 and 100,000 genealogies respectively, eliminating the first 10,000 genealogies as burn-in. To measure the average genetic distances between individuals from both sites, distance matrices were calculated using *p-distance* and Jukes-Cantor 69 (JC69) distances, implemented in MEGA 6 software (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). From these matrices, average distances (*d*) and standard deviations (*SD*) were estimated. The resulting datasets were analyzed (via student's *t* tests) to test for differences between the two sites.

Results

Fragments of 700 pb for the COI region were obtained from 51 tissue samples (Tamiahua, 21; Tuxpan, 30). After alignment and edition, sequences length was 628 pb, with 19 gaps and 162 polymorphic sites. From these, 89 were singletons and 54 parsimony informative sites. The sequences corresponded to 32 haplotypes, 22 from Tuxpan and 15 from Tamiahua. Only five haplotypes (Hap_3, Hap_4, Hap_9, Hap_22 and Hap_23) were shared between the two localities, and two were the most common: Hap_3 (10 individuals) and Hap_9 (6 individuals).

The number of haplotypes (*h*) in Tuxpan (22) was higher than in Tamiahua (15); however, after the rarefaction analysis correction, the corrected number of haplotypes (h_T) was only slightly different between localities (Tuxpan = 14.6, Tamiahua = 13.3, Total = 13.8). The same trend was observed in the number of segregating sites (*S*) and the average number of nucleotide differences (*k*; Table 1). The haplotype diversity (*Hd*) was high and nucleotide diversity (π) was low in each of the three datasets (Tuxpan, $Hd = 0.963$ and $\pi = 0.01883$; Tamiahua $Hd = 0.938$ and $\pi = 0.0164$; Total, $Hd = 0.948$ and $\pi = 0.01767$; Table 1). However, significant differences were only found in haplotype diversity ($t = 2.6$, $p = .015$).

The haplotype network showed a homogenous distribution of haplotypes, as no clusters belonging to one single beach were identified (Figure 2). Haplotypes Hap_20, Hap_30, Hap_31, and Hap_32 showed a higher number of mutations than the remaining, and

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Table 1. Estimates of Descriptive Genetic Diversity at Both Tuxpan and Tamiahua Beaches in the Mexican State of Veracruz.

Study site	*N*	*h*	h_T	*S*	*k*	Hd (*SD*)	π (*SD*)
Tuxpan	30	22	14.6	115	11.71	0.963 (0.022)	0.0188 (0.00834)
Tamiahua	21	15	13.3	94	10.16	0.938 (0.04)	0.0164 (0.00763)
Total	51	32	13.8	162	10.90	0.948 (0.02)	0.01767 (0.00603)

SD = Standard deviation; *N* = Number of organisms; *h* = number of haplotypes; h_T = number of corrected haplotypes; *S* = number of segregated sites; *k* = average number of paired nucleotide differences; H_d = haplotype diversity; π = nucleotide diversity (Nei, 1987; Tajima, 1983) for *Ocypode quadrata*, obtained from a 628 pb fragment from the mtDNA COI region.

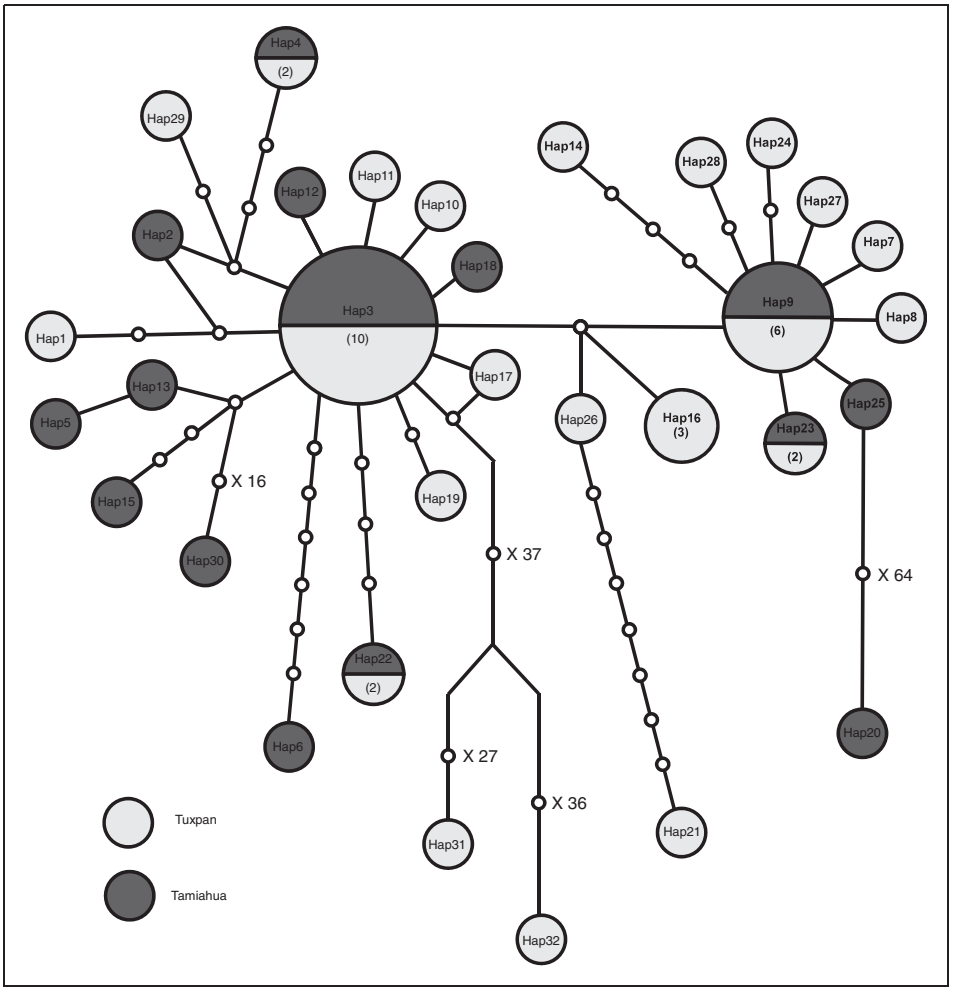


Figure 2. Haplotype network of *Ocypode quadrata* with 32 haplotypes obtained from a mtDNA COI fragment. Circle sizes are proportional to the number of individuals. Light gray are haplotypes from Tuxpan and dark gray haplotypes from Tamiahua. Empty circles represent inferred (but not sampled) haplotypes. Number of individuals sharing a haplotype is shown in parenthesis; when only one individual was found with the haplotype, no number is shown.

Hap_3 is ancestral while the rest of the haplotypes are derived. The phylogenetic reconstruction (except for haplotypes Hap_1 and Hap_21) formed one single clade, with haplotypes from both beaches distributed in subclades. No mutually exclusive groups (clades) of haplotypes were observed (Figure 3). The estimation of gene flow between beaches showed a number of migrants slightly higher from Tamiahua to Tuxpan ($M = 436$;

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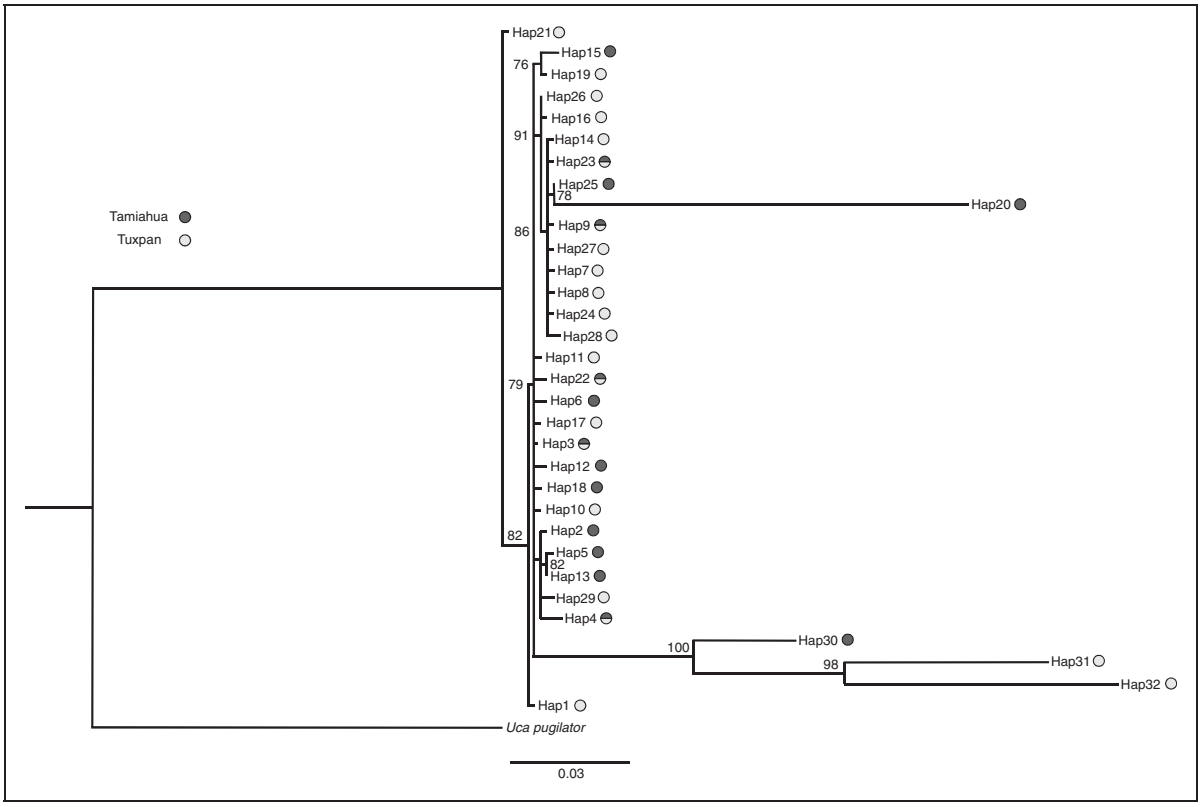


Figure 3. Phylogenetic tree of 32 haplotypes of *Ocypode quadrata*. The tree was constructed using the Maximum Likelihood method, under the Jukes-Cantor 69 model. Numbers correspond to branch support estimated with the Akaike Likelihood Ratio Test method. *Uca pugilator* was used as outgroup.

Table 2. Average Genetic Distances of *Ocypode quadrata* populations in Tuxpan and Tamiahua, respectively, Based in *p-Distance* and Jukes-Cantor 69 Models, as Well as *t* Values and Probabilities by Student's *t* test.

	Nucleotide distance	Tuxpan	Tamiahua	Student's *t*	Probability
With outlier haplotypes	*p*-distance (*SD*)	0.019 (0.034)	0.016 (0.027)	$t = 1.06$	.29
	Jukes-Cantor (*SD*)	0.020 (0.037)	0.017 (0.029)	$t = 0.97$	.33
Without outlier haplotypes	*p*-distance (*SD*)	0.006 (0.003)	0.006 (0.003)	$t = 0.43$	.67
	Jukes-Cantor (*SD*)	0.006 (0.003)	0.006 (0.003)	$t = 0.42$	.67

Note. No statistically significant differences were observed between both populations.

IC 95% 349–569) than from Tuxpan to Tamiahua ($M = 297$; IC 95% 222–412). However, both estimates are in the same order of magnitude; therefore, gene flow between *O. quadrata* populations from the two beaches is not limited.

Finally, the genetic distances between *O. quadrata* individuals within beaches were very similar. In Tuxpan, it was 0.019 ($SD = 0.034$) with *p-distance* and 0.020 ($SD = 0.037$) with Jukes-Cantor 69, while in Tamiahua, it was 0.016 ($SD = 0.027$) with *p-distance* and 0.017 ($SD = 0.029$) with Jukes-Cantor 69 (Table 2). The Student's *t* test showed no statistically significant differences in genetic distances between the two beaches, either with *p-distance* or Jukes-Cantor distances. When these distances were calculated eliminating the most divergent haplotypes (Hap_20, Hap_30, Hap_31, and Hap_32) from the analysis, given their higher number of mutations, genetic distances between both beaches were also nonsignificant (Table 2).

Discussion

Our study including genetic diversity, rarefaction analysis, number of migrants, haplotype network,

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phylogenetic tree, and genetic distances consistently showed no differences between *O. quadrata* individuals from both Tuxpan and Tamiahua beaches. For example, from the 32 detected haplotypes, 5 were shared among the individuals from both localities, even though the remaining individuals belonged to either beach. This result is explained by a large population size allowing the retention of haplotypes and favoring high levels of genetic connectivity (Liu et al., 2009). This trend of genetic connectivity is supported by the haplotype network and the phylogenetic tree, as these did not reveal genetic structure or genetic differentiation between both localities. These results are consistent with patterns expected for species that present long larval periods in the pelagic zone, and that do not cope with geographical barriers that hamper dispersion (Wieman et al., 2013). Further, the values of haplotype diversity and nucleotide diversity found in our study were similar (also using the COI gene) to the crab species *Pachygrapsus crassipes* (Cassone & Boulding, 2006), *Uca maracoani* (Wieman et al., 2013), and *Carcinus aestuarii* (Ragionieri & Schubart, 2013), showing high gene flow. When our results of migration rates are compared with other species, as *Carcinus aestuari* (Ragionieri & Schubart, 2013) from the European Mediterranean coast (with values ranging from 0.78 to 3.17 migrants), the migration rate between the *O. quadrata* individuals from Tuxpan and Tamiahua was extremely high. The results of genetic distances showed that there were no statistically significant differences between *O. quadrata* individuals from Tuxpan and Tamiahua, using either *p-distance* or Jukes-Cantor 69 distances, a result also found when the more divergent haplotypes were included. Overall, these results showed that *O. quadrata* individuals from both localities belong to a single population.

Altogether, results could be explained by a set of biological and geographical factors or by the lack of quantitative estimation of human activity explored in this study. The high genetic diversity may be explained by the dispersion mechanisms, mainly during their larval phases. The larval stages of *O. quadrata* comprise five stages of zoea and one of megalopa, which take about 60 days in the water before returning to the beach (usually at low tide), when they spend the first crab stage (Diaz & Costlow, 1972; Hobbs et al., 2008). This period is sufficient for the passive dispersion of the larvae through ocean currents, allowing the genic flow between both localities. Further, the geographical distance between both localities (37 km) is probably not long enough as to detect significant differences, given that adult individuals show great vagility and can disperse very long distances reaching up to 300 m, while foraging at night (Hobbs et al., 2008). This is also true for *O. quadrata* zoea and megalopa stages, as vagility in this planktonic form is even greater, fostered by strong oceanic currents.

Implications for Conservation

Despite that the degree of anthropogenic disruption in Tuxpan and Tamiahua beaches were different, our study was designed for qualitatively testing human impacts. For example, it was assumed that Tuxpan economic activities have fragmented the beach continuity with constructions (such as the Adolfo López Mateos thermoelectric central). However, the genetic diversity in *O. quadrata* did not show strong evidence of genetic isolation. Therefore, no effective barrier to gene flow between both beaches was inferred, so human activities may have not exerted a selective pressure over *O. quadrata*, or this pressure has not been enough to be detected at the genetic diversity level. In the latter case, it may be a consequence of a short time since the start of human activity as to detect an impact on genetic diversity in this species. If these factors are further considered, the impact of human activity over *O. quadrata* could be explored in more detail. In this sense, more immediate physiological effects of anthropogenic disruption on *O. quadrata* population could be assessed, for example, by addressing the associated effects of human-related waste materials, thermal discharges, chemical industrial residues, and oil. To this end, the development of specific biomarkers would lead to the quantification of physiological stress to which this species is subject in highly anthropogenic disrupted environments.

Acknowledgments

Authors are grateful to Jacqueline Flores-Galíndo and Odin Erik Romero-Fernández for their valuable collaboration for collecting the studied organisms and for their field support. Flor Nohemi Rivera-Orduña supported APT in the lab and with some analyses.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interests with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by projects IPN-SIP-20140812, IP-SIP-20150215, and IPN-SIP-20151557. Partial support for fieldwork was provided by Instituto de Biología-UNAM.

References

Barros, F. (2001). Ghost crabs as a tool for rapid assessment of human impacts on exposed sandy beaches. *Biological Conservation*, *97*, 399–404.

Beerli, P. (2008). Migrate version 2.4. Retrieved from http://popgen.sc.fsu.edu/Migrate/Migrate-n.html

Bezuidenhout, K., Nel, R., & Hauser, L. (2014). Demographic history, marker variability and genetic differentiation in sandy

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beach fauna: What is the meaning of low FST's? *Estuarine, Coastal and Shelf Science*, *30*, 1–5.

Bravo, R. S. (2010). *Caracterización de las playas de Tuxpan, Veracruz mediante criterios de certificación (Characterization of the beaches of Tuxpan, Veracruz according to criteria of certification)* (Tesis de maestría). Universidad Veracruzana, Tuxpan, Veracruz.

Cassone, B. J., & Boulding, E. G. (2006). Genetic structure and phylogeography of the lined shore crab, *Pachygrapsus crassipes*, along the northeastern and western Pacific coasts. *Marine Biology*, *149*, 213–226.

Chan, B. K. K., Chan, K. K. Y., & Leung, P. C. M. (2006). Burrow architecture of the ghost crab *Ocypode ceratophthalma* on a sandy shore in Hong Kong. *Hydrobiologia*, *560*, 43–49.

Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: A computer program to estimate gene genealogies. *Molecular Ecology*, *9*, 1657–1659.

Correa, M. O. D. A., Andrade, L. S., Costa, A. L., Castilho, A. L., Bertini, G., & Fransozo, A. (2014). Vertical distribution by demographic groups of ghost crab *Ocypode quadrata* (Crustacea: Brachyura). *Biologia*, *69*, 905–915.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, *9*, 772.

Diaz, H., & Costlow, J. D. (1972). Larval development of *Ocypode quadrata* (Brachyura: Crustacea) under laboratory conditions. *Marine Biology*, *15*, 120–131.

Excoffier, L., Laval, G., & Schneider, J. A. (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, *1*, 47–50.

Fisher, J. B., & Tevesz, M. J. S. (1979). Within-habitat spatial patterns of *Ocypode quadrata* (Fabricius) (Decapoda, Brachyura). *Crustaceana*, *5*(Supplement): 31–36.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Biology and Biotechnology*, *3*, 294–299.

Freeland, J. R. (2005). *Molecular ecology*. West Sussex, England: John Wiley and Sons.

Gomes, V. V., Neves, G., & Almeida, C. L. (2011). Sensitivity of a cirolanid isopod to human pressure. *Ecological Indicators*, *11*, 782–788.

Gouy, M., Guindon, S., & Gascuel, O. (2010). SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution*, *27*, 221–224.

Guindon, S., & Gascuel, O. (2003). Simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, *52*, 696–704.

Hobbs, C. H. III, Landry, C. B., & Perry, J. E. (2008). Assessing anthropogenic and natural impacts on ghost crabs (*Ocypode quadrata*) at Cape Hatteras National Seashore, North Carolina. *Journal of Coastal Research*, *24*, 1450–1458.

Liu, Y., Liu, R., Ye, L., Liang, J., Xuan, F., & Xu, Q. (2009). Genetic differentiation between populations of swimming crab *Portunus trituberculatus* along the coastal waters of the East China Sea. *Hydrobiologia*, *618*, 125–137.

Lucrezi, S., Schlacher, T. A., & Robinson, W. (2009a). Human disturbance as a cause of bias in ecological indicators for sandy beaches: Experimental evidence for the effects of human trampling on ghost crabs (*Ocypode spp*.). *Ecological Indicators*, *9*, 913–921.

Lucrezi, S., Schlacher, T. A., & Walker, S. (2009b). Monitoring human impacts on sandy shore ecosystems: A test of ghost crabs (*Ocypode spp*.) as biological indicators on an urban beach. *Environmental Monitoring and Assessment*, *152*, 413–424.

Monteiro, N. F., & Bemvenuti, C. E. (2006). The ghost crab *Ocypode quadrata* (Fabricius, 1787) as a potential indicator of anthropic impact along the Rio Grande do Sul coast, Brazil. *Biological Conservation*, *1*, 431–435.

Moss, D., & McPhee, D. P. (2006). The impacts of recreational four-wheel driving on the abundance of the ghost crab (*Ocypode cordimanus*) on a subtropical sandy beach in SE Queensland. *Coastal Management*, *34*, 133–140.

Nei, M. (1987). *Molecular evolutionary genetics*. Nueva York, NY: Columbia University Press.

Noriega, R., Schlacher, T. A., & Smeuninx, B. (2012). Reductions in ghost crab populations reflect urbanization of beaches and dunes. *Journal of Coastal Research*, *28*, 123–131.

Ocaña, F. A., Vega, A., & Córdova, E. A. (2012). Distribución espacial de *Ocypode quadrata* (Decapoda: Ocypodidae) en ocho playas de la zona norte oriental de Cuba (Spatial distribution of Ocypode quadrata (Decapoda: Ocypodidae) on eight beaches in the north eastern part of Cuba). *Revista de Biología Tropical*, *60*, 1177–1186.

Petit, R. J., Mousadik, A. E., & Pons, O. (1998). Identifying populations for conservation on the basis of genetic markers. *Conservation Biology*, *12*, 844–855.

Ragionieri, L., & Schubart, C. D. (2013). Population genetics, gene flow, and biogeographical boundaries of *Carcinus aestuarii* (Crustacea: Brachyura: Carcinidae) along the European Mediterranean coast. *Biological Journal of the Linnean Society*, *109*, 771–790.

Schlacher, T. A., & Thompson, L. (2008). Physical impacts caused by off-road vehicles to sandy beaches: Spatial quantification of car tracks on an Australian Barrier Island. *Journal of Coastal Research*, *14*, 234–242.

Schlacher, T. A., & Lucrezi, S. (2010). Compression of home ranges in ghost crabs on sandy beaches impacted by vehicle traffic. *Marine Biology*, *157*, 2467–2474.

Schlacher, T. A., Lucrezi, S., Connolly, R. M., Peterson, C. H., Gilby, B. L., Maslo, B., . . . Schoeman, D. A. (2016). Human threats to sandy beaches: A meta-analysis of ghost crabs illustrates global anthropogenic impacts. *Estuarine, Coastal and Shelf Science*, *169*, 56–73.

Silva, W. T. A., & Calado, T. C. S. (2013). Number of ghost crab burrows does not correspond to population size. *Central European Journal of Biology*, *8*, 843–847.

Steiner, A. J., & Leatherman, S. P. (1981). Recreational impacts on the distribution of ghost crabs *Ocypode quadrata* Fab. *Biological Conservation*, *20*, 111–122.

Tajima, F. (1983). Evolutionary relationship of DNA sequences in finite populations. *Genetics*, *105*, 437–460.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*, 2725–2729.

Templeton, A. R., Crandall, K. A., & Sing, C. F. (1992). A cladistics analysis of phenotypic associations with haplotypes inferred

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from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, *132*, 619–633.

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*, 4673–4680.

Ugolini, A., Ungherese, G., Somigli, S., Galanti, G., Baroni, D., Borghini, F., . . . Focardi, S. (2008). The amphipod *Talitrus saltator* as a bioindicator of human trampling on sandy beaches. *Marine Environmental Research*, *65*, 349–357.

Wieman, A. C., Berendzen, P. B., Hampton, K. R., Jang, J., Hopkins, M. J., Jurgenson, J., . . . Thurman, C. L. (2013). A panmictic fiddler crab from the coast of Brazil? Impact of divergent ocean currents and larval dispersal potential on genetic and morphological variation in *Uca maracoani*. *Marine Biology*, *161*, 173–185.

Yepes, P. V. (1999). Las playas en la gestión sostenible del litoral (The beaches in the sustainable management of the coast). *Cuadernos de Turismo Universidad de Murcia*, *4*, 89–110.

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