Revista Mexicana de Biodiversidad 81: S177- S185, 2010
http://dx.doi.org/10.22201/ib.20078706e.2010.0.221

Unique 16S rRNA sequences of *Eurythenes gryllus* (Crustacea: Amphipoda: Lysianassidae) from the Gulf of Mexico abyssal plain

Secuencias únicas 16SrRNA de *Eurythenes gryllus* (Crustacea: Amphipoda: Lysianassidae) de la planicie abisal del Golfo de México

Elva Escobar-Briones[1*], Eduardo Nájera-Hillman[2] and Fernando Álvarez[3]

[1]*Universidad Nacional Autónoma de México, Unidad Académica Sistemas Ecología Marina, Instituto de Ciencias del Mar y Limnología, Unidad Académica Sistemas Ecología Marina, Universidad Nacional Autónoma de México. Apartado postal 70-305, Ciudad Universitaria; 04510 México D.F., México.*
[2]*The School for Field Studies, Center for Coastal Studies. Puerto San Carlos, México.*
[3]*Colección Nacional de Crustáceos, Instituto de Biología, Universidad Nacional Autónoma de México,. Apartado postal. 70-530, México 04510 México, D. F., México.*
**Correspondent: escobri@cmarl.unam.mx*

Abstract. Amphipods of the species *Eurythenes gryllus* were collected at 2 locations on the abyssal plain (~3 400 m) of the Gulf of Mexico in order to test whether or not these scavenger amphipods are isolated in this peripheral sea or show connectivity by their predominant swimming behavior, moving horizontally along the abyssal water masses in the region. Partial sequences of the mitochondrial 16S rRNA gene from 2 individuals of *E. gryllus* were determined and showed small differences when compared to sequences of other amphipods of the same species from the Atlantic Ocean (3.6 to 3.9%) and Pacific Ocean (4.0 to 4.1%) and increasing (4.2 to 9.5%) when compared to sequences of specimens from sites of less than 500 m. The largest differences (18%) were observed when the sequences were compared to that of *Eurythenes* from the Tongue of the Ocean in spite of its closer geographical distance in the region. Isolation in the deep Gulf of Mexico could be attributed to limited genetic exchange with the western tropical Atlantic through the Caribbean over the 2 040 m deep sill and inexistent at abyssal depth through the Florida Straits.

Key words: amphipods, deep-sea fauna, genetic diversity, scavenger assemblages, Intra-Americas Sea, Yucatán Channel.

Resumen. Se recolectaron ejemplares de los anfípodos de la especie *Eurythenes gryllus* en 2 localidades de la planicie abisal (~3 400 m) en el golfo de México con el objeto de evaluar si estos carroñeros se encuentran aislados en el mar marginal o presentan cierta conectividad por su conducta natatoria, desplazándose horizontalmente en las masas de agua que caracterizan la región. Las secuencias parciales del gen mitocondrial 16S rRNA que se obtuvieron de 2 individuos de *E. gryllus* mostraron diferencias ligeras al compararse con las secuencias de otros anfípodos de la misma especie procedentes de otras zonas del Atlántico (3.6 a 3.9%) y del Pacífico (4.0 a 4.1%), incrementándose (4.2 a 9.5%) al compararse con secuencias de organismos de aguas someras (<500 m). Las diferencias mayores (18%) se observan en la comparación de ejemplares de *Eurythenes* procedentes del canal noroeste de las Bahamas a pesar de la gran cercanía geográfica en la región. El aislamiento que pudiera existir en el mar profundo del golfo de México podría atribuirse a un intercambio genético limitado al Atlántico tropical occidental solamente por el Caribe a través del canal de Yucatán con un umbral de 2 040 m e inexistente a profundidades abisales a través del estrecho de Florida.

Palabras clave: anfípodos, fauna de mar profundo, diversidad genética, asociaciones de carroñeros, Mar Intra Americano, canal de Yucatán.

Introduction

The species *Eurythenes gryllus*, 1 of 2 scavenger species described in the genus, lives in deep-sea habitats with an extended geographic distribution and has been studied extensively world wide (Barnard and Karaman, 1991; Ingram and Hessler, 1987). Studies since the 19th century have contributed information on population structure, age, size spectrum, abundance, and the changes in space and time in the Pacific, Arctic and Atlantic Oceans (Smith and Baldwin, 1984; Charmasson and Calmet, 1987) and in the southeastern Pacific Atacama trench

Recibido: 26 abril 2010; aceptado: 15 junio 2010

(Thurston et al., 2002).

The scavenger assemblages, mainly composed of fish and lysianassid amphipods, have been recorded on the shelf in smaller numbers than those reported from traps moored at higher latitudes and greater depths (Christiansen et al., 1990; Priede et al., 1991; Biernbaum and Wenner, 1993), where amphipods vary spatially and seasonally in size and in relative abundance (Ingram and Hessler, 1987; Charmasson and Calmet 1987; Thurston et al., 2002), this is usually directly related to the available food supply on the deep-sea floor (Jan*β*en et al., 2000). The information on scavenger amphipods in the tropics is limited. Among the Mediterranean scavenger amphipods, 91% of the bathyal and abyssal species are endemic (Bellan-Santini, 1990).

Studies focused on genetic diversity have been carried out at the population level as well as at the species level in deep-sea species and are exemplified by the vestimentiferan tubeworms from the western Pacific (Kojima et al., 2002, 2003). The genetic variability within populations and species can be estimated directly from changes in the allozyme frequency and DNA differences (Mackenzie et al., 1998). The advances in molecular biology have increased the use of genetic analyses in the last 15 years and have provided molecular tools to answer ecological and evolutionary questions (France and Kocher, 1996b). The amplification by polymerase chain reaction (PCR) and sequencing of homologous genes are tools used to determine genetic divergence in closely related species and establish population differences within species (Palumbi, 1996).

Genetic differences between specimens of *E. gryllus* with different geographic and depth distribution patterns have been analyzed by comparing mitochondrial 16S rRNA gene sequences (France and Kocher, 1996a). The identification of barriers for genetic flow that can lead to the reproductive isolation of a species is a challenge in the study of deep–sea populations (France and Kocher, 1996a). Alloenzymatic frequencies of specimens collected in the Pacific abyssal plain were compared to frequencies of specimens from the seamount slope, showing a limited vertical genetic exchange (Bucklin et al., 1987). The variability in allozyme frequencies has been commonly used to determine genetic differences between deep-sea amphipod populations, i.e. *E. gryllus* (Bucklin et al., 1987) and Ventiella sulfuris (France et al., 1992). The mitochondrial gene sequences most often used to study genetic differences in deep-sea faunal components have been cytocrome oxidase I (COI), in endemic gastropods of hydrothermal vents, i.e. Ifremeria nautilei (Kojima et al., 2000), and 16S rRNA gene in *E. gryllus* (France and Kocher, 1996a). Speciation of *E. gryllus* in isolated basins has been suggested in the Atacama trench based solely on slight morphological differences among the specimens collected (Thurston et al., 2002). A study from the Mediterranean Sea recorded high endemicity in deep-sea amphipod species (Bellan-Santini, 1990).

This study contributes with sequences of the mitochondrial 16S rRNA gene of 2 specimens of *E. gryllus* collected at abyssal locations in the Gulf of Mexico and compares them to published *E. gryllus* sequences from other deep sea regions with the hypothesis that the deep Gulf of Mexico, considered a peripheral sea, is topographically isolated from the western Atlantic, a condition that would favor a separate phylogenetic history of mtDNA of *E. gryllus* from the other deep-sea locations. Previous studies have shown a homogeneous distribution of mitochondrial DNA gene variants throughout world oceans, suggesting substantial gene flow of the species, with local heterogeneity occurring in specific depth zones.

Materials and methods

Area of investigation. The Gulf of Mexico is a peripheral sea with an area of 1.5 x 10^6 km^2, an average depth of 2 500 m, and a large abyssal plain that reaches 3 800 m which encompasses an area of 103 600 km^2. The basin is located in the western tropical Atlantic (18° N - 30° N, 80° W - 98° W). It is connected to the Atlantic through the Yucatán Channel and the Caribbean Sea in the southeast, and with the northwestern Atlantic through the shallow Florida Straits (Wiseman and Sturges, 1999). Based on its isolation from the Atlantic and its characteristic hydrographic regime, Sherman (1994) suggested that the Gulf of Mexico should be included among the large marine ecosystems.

Oligotrophic conditions of the euphotic layer of the Gulf of Mexico from May to July (0.06mg m^{-3}; Müller-Karger et al., 1991) and offshore fishery have suggested limited biogenic carbon (C_{BIO}) export to the deep sea floor. It has been proposed that primary production on the surface is seasonal with the largest production over deep water occurring from December to February (>0.18 mg m^{-3}; Müller-Karger et al., 1991). This limited C_{BIO} export explains the low abundance and biomass values recorded in the abyssal infaunal benthos (Escobar et al., 1997, 1999) and may influence the scavenger abyssal populations in the Gulf of Mexico.

All of the deeper layer exchanges take place within the Caribbean over the 2 400 m deep sill and play an important role in renewing the deep water in the Gulf of Mexico with colder, saltier, denser, and oxygen-richer water (Sheinbaum et al., 2002). The topographic seafloor

structures, such as the sills, act as barriers in the peripheral seas of the western Atlantic and may provide adequate conditions for faunal isolation that may lead to endemism and speciation resulting from limited genetic exchange with other populations and local adaptation.

Field work. Specimens of the amphipod *E. gryllus* were collected at 5 locations, 2 at which the sequences were obtained in the central Gulf of Mexico using scavenger traps that were baited with fish during expeditions in June and August of 2002 (Table 1). Abyssal crustacea have small amounts of muscle tissue, the volume is mostly provided by water and therefore after inspection of organisms from all stations the specimens from localities 2 and 4 were the only ones used for genetic studies (Fig. 1). The trap array included a flotation sphere of 43.18 cm diameter with a buoyancy of 25.4 kg and an acoustic release model 865-A adjusted to release a disposable weight after twelve hours, 2 crab traps of 0.5 x 0.3 x 0.6 m, and 1 smaller trap 0.4 x 0.2 m, all with 2.54 cm wire mesh and frozen tuna as bait. The trap array was recovered at night, after 9 to 12-hour deployment time, using a radio tracking system and a light strobe to find the array on the water surface.

A total of 94 specimens were captured at all 5 locations. Two to 5 specimens of different sizes from locations 2 and 4 were selected for genetic studies based on their integrity and healthy aspect. These specimens were fixed in cold absolute ethanol and kept at 4 °C onboard. Other specimens were used for taxonomic, systematic, SEM photography and trophic studies and will not be cited herein.

Species identification. All specimens were identified based on the morphological characters for *E. gryllus* as described in Barnard and Karaman (1991) and compared to specimens from the northern Atlantic on loan from the Natural History Museum, in London, UK, and the National Oceanography Centre, Southampton, UK, and a few specimens from the Tongue of the Ocean, Bahamas from Dr. Scott France, University of Louisiana. The collected specimens were deposited in the National Crustacean Collection at the Institute of Biology in UNAM (CNCR), Mexico, and were cataloged with the numbers CNCR 20582 to 20590 and CNCR 20678 to 20681.

Genetic analyses. A small portion of abdomen and antennal tissue was excised and placed in small centrifuge tubes, minced, excess liquid was blotted in a sterile disposable towel, and then the tissue was digested in 500 µL of STE homogenization buffer, 25 µL of proteinase K (20 mg mL^{-1}; Sigma) and 75 µL of 10% SDS solution were added to produce tissue homogenates. Each sample was well mixed and incubated for 24 h at 57°C. The DNA extraction was made following a modified version for crustaceans of the basic phenol-chloroform technique described in Hillis et al. (1996). In an extraction hood, 600 µL of phenol were added to each sample, mixed gently, incubated at room temperature for 5 min, and centrifuged (2 800 rpm) for 5 min in an Eppendorf 5415C centrifuge. The supernatant liquid was retrieved using a micropipette and deposited in a clean 1.5 mL Eppendorf microtube; 600 µL of phenol were added and the incubation and centrifugation steps were repeated, as above. The supernatant was transferred to a clean 1.5 mL microtube and 600 µL of chloroform were added. The mixture was incubated at room temperature for 2 min and centrifuged at high speed for 3 min; the supernatant was transferred to a clean 1.5 mL microtube. DNA precipitation was accomplished by adding 450µL of sample, 50 µL of 2M NaCl and 1 000 µL of 96% cold ethanol, keeping the sample at –20°C for 24 h. The sample was spun for 10 min at 2800 rpm and the DNA pellet was washed with 70% ethanol and dried at room temperature. The DNA was resuspended in 100 µL double distilled water (ddH_2O) and stored at –20°C (Palumbi, 1996).

The samples were run in a TBE, 1% agarose gel, using a DNA standard marker, in a Thermo AC Minicell Primo AC320 electrophoresis gel system for 20 min at 100V to visualize the extracted DNA in a transilluminator. High molecular weight DNA was obtained as the band in the sample co-migrated with the largest band of the marker.

Table 1. Deep Gulf of Mexico (DGoMB) cruise data. Sampling date (day.mo.yr), position, and depth of the locations. Size range (in cm) of specimens of *Eurythenes gryllus* collected and number of individuals in parenthesis (n). nd=no data available

Sampling date	*Location number*	*Latitude N*	*Longitude W*	*Depth (m)*	*Females (cm)*	*Males (cm)*	*Juveniles (cm)*
03.08.02	1	25°00’	92°00’	3 530	nd	5.03 - 5.74 (n=5)	2.33 (n=1)
06.06.02	2	23°30’	91°59’	3 732	3.43 - 6.42 (n=9)	2.81 - 5.50 (n=12)	2.03 - 3.05 (n=20)
06.08.02	3	24°45’	90°45’	3 678	5.50 - 5.81 (n=2)	4.25 - 5.72 (n=5)	2.1 - 3.46 (n=4)
10.08.02	4	24°15’	85°30’	3 410	4.40 - 5.75 (n=6)	2.90 - 6.16 (n=9)	2.16 - 3.20 (n=11)
12.06.02	5	25°29’	88°15’	3 308	4.25 - 5.10 (n=3)	4.30 - 5.83 (n=7)	nd

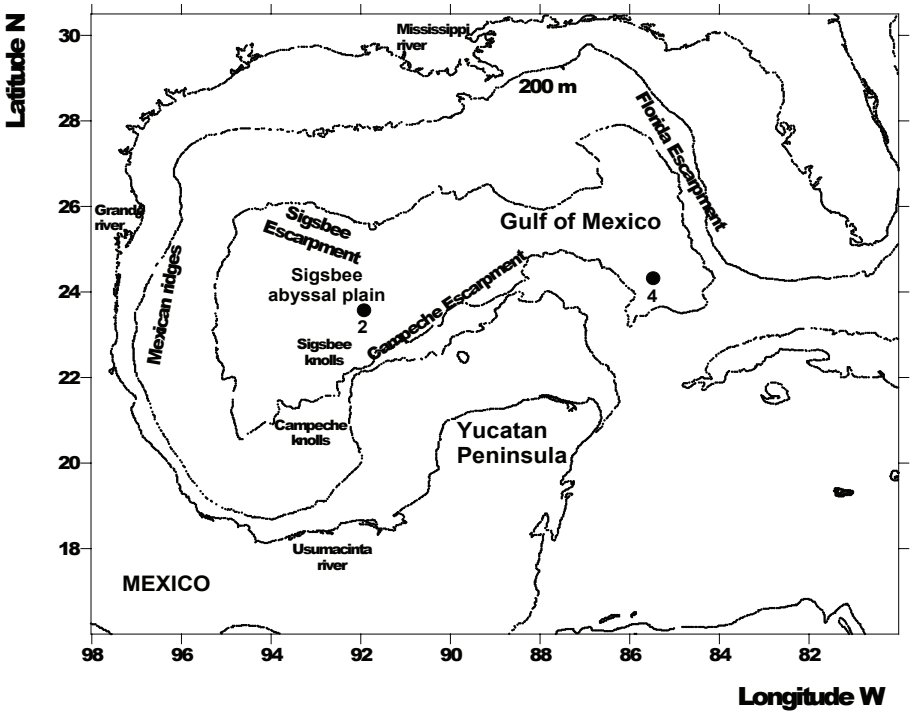


Figure 1. Area of study, major topographic features of the basin. Markers depict sampling locations; black solid markers depict samples with specimens of *Eurythenes gryllus*.

Amplification protocol. A portion of the 16S rRNA gene was amplified by means of PCR in an Eppendorf Mastercycler Gradient thermal cycler using the universal primers 16Sar and 16Sbr (Palumbi et al., 1991). These primers had previously been used for the same species (France and Kocher, 1996a; Kojima et al., 2000).

The thermal cycler protocol was modified from that described in France and Kocher (1996a) based on 3 gradient PCRs that were completed to determine the optimum annealing temperature. The final protocol included an initial 2 min denaturation step at 94°C, followed by 32 cycles with denaturing periods of 1 min 45 s at 94°C, annealing for 1 min at 49°C, extension of 1 min 15 s at 72°C, and a final extension period of 7 min at 72°C. PCR products were analyzed by electrophoresis and the molecular weights of the bands were determined and compared to the molecular weight of the desired gene product. The second electrophoresis of the 500 bp section of the 16s rRNA region followed the same protocol described above to obtain products marked with fluorescent dideoxynucleotides. The bands were excised and transferred to 2 mL microtubes for sequencing. Double-stranded PCR products obtained and amplified were purified using a QIAGEN QIAquick PCR Purification Kit, with final elution of 50 µl per individual.

Sequencing strategy. Sequencing reactions followed the protocol of Perkin Elmer, using an ABI BigDye Terminator Mix, reaction with custom primers (Applied Biosystems). The product was sequenced in both directions to resolve ambiguities. The number of thermal cycles was 25 with cycles of denaturing at 96°C for 10 s, annealing at 50°C for 5 s and extension at 60°C for 4 min all in a Gene Amplifier PCR System 9700. The products obtained were stored at 4°C. Clean PCR products were run on an ABI Prism 310 automated sequencer. Each sample was resuspended in dd water. The sample was denatured at 95°C for 3 min and placed in ice for 10 min to stop the reaction.

Sequence analysis. The resulting sequences were visualized and both directions assembled using Sequencher™ version 3.1 (Gene Codes Corporation). The consensus sequences of the Gulf of Mexico amphipods were compared to sequences in the GenBank database (NCBI). In order to confirm their identity as *E. gryllus,* these sequences were compared to those of *E. gryllus* from other locations. In addition, GenBank sequences of another species of the same genus and those of the abyssal amphipod of the genus *Abyssorchomene* (the outgroup) were used for comparisons. A multiple alignment and paired distances were determined using corrected distances with ClustalX 1.8. A neighbor-joining (NJ) analysis was carried out using PAUP v.4.0b10 for Macintosh (Swofford, 2002). Maximum parsimony (MP) analysis was performed using a heuristic search, based on branch swapping with tree-bisection-reconnection. Insertion and deletion gaps were treated as missing data in all the tree reconstructions with PAUP v.4 Ob10. Confidence in the nodes of MP and NJ trees were assessed using the bootstrap procedure with 100 replications, showing values equal or larger than 50%. As the outgroup we selected the genus *Abyssorchomene*, in order to emphasize the genetic variation within the genus *Eurythenes*. This outgroup was also chosen to make the phylogenetic interpretation consistent with previous reports.

Results and discussion

The amplification reactions that produced partial sequences of the 16S rRNA gene of 2 specimens of *E. gryllus* from locations 2 and 4 showed unique, clean bands, with products of 418 and 464 base pairs (bp), respectively, with no differences between the homologous fragments. These amphipod sequences have the GenBank accession numbers AY943568 and AY943569, respectively.

The distance matrix showed that the 16S rRNA sequences of *E. gryllus* from 2 distant locations in the Gulf of Mexico were identical, and they were very similar to published sequences of amphipods of the same genus from the Atlantic Ocean (3.6 to 3.9%) and from the Pacific Ocean (3.9 to 4.1% and up to 9.5% in sites with less than 500 m depth). The largest differences (18%) were observed when our sequences were compared to *Eurythenes sp.* from the Tongue of the Ocean. The differences with respect to *Abyssorchomene spp.,* the outgroup taxa, were 25%. Comparisons among the abyssal amphipod species from other locations using paired corrected distances are shown on Table 2. The geographic position and depth records of *E. gryllus, Eurythenes sp.* and *Abyssorchomene spp*., from which sequences were used in this study were obtained from France and Kocher (1996a). Additional sequences have been cited by France and Kocher (1996b) and Blankenship and Yayanos (2005).

The existence of communication between the Protoatlantic and the Pacific Oceans from the Triassic through the Cretaceous period may have allowed a common population of *E. gryllus*, with free genetic flow that has been maintained between the Gulf of Mexico and the Western Tropical Atlantic through the Yucatán Channel and the Caribbean Sea. Similarity between the sequences from the North Atlantic and the Gulf of Mexico suggests that these populations were in contact in a recent past. The genetic similarity with populations of *E. gryllus* from the North Atlantic can be related to the geographical and hydrographical connectivity between the deep-water masses in the Northern Atlantic and the Sigsbee abyssal plain in the central Gulf of Mexico. A large amount of the north Atlantic Deep Water mass enters the Venezuelan and Colombian Basins from the Atlantic through the Anegada-Jungfern Passage at 1900 m (Morrison and Nowlin, 1982) and the Windward Passage (Sturges, 1965) and slides down the Yucatán Channel slope into the deep Gulf of Mexico (Sheinbaum et al., 2002). The residence time for the deep waters of the Golf is about 250 years (rivas et al., 2005).

In contrast, the lower genetic similarity between specimens of *E. gryllus* from the Gulf of Mexico and from the Tongue of the Ocean can be explained by the fact that the sill at the Florida Straits which connects the eastern Gulf of Mexico with the western Atlantic lies at a depth of only 740 m. These 2 areas are isolated, with no exchange, nor connectivity between the deep-water masses below the Antarctic Intermediate Water. Exchange

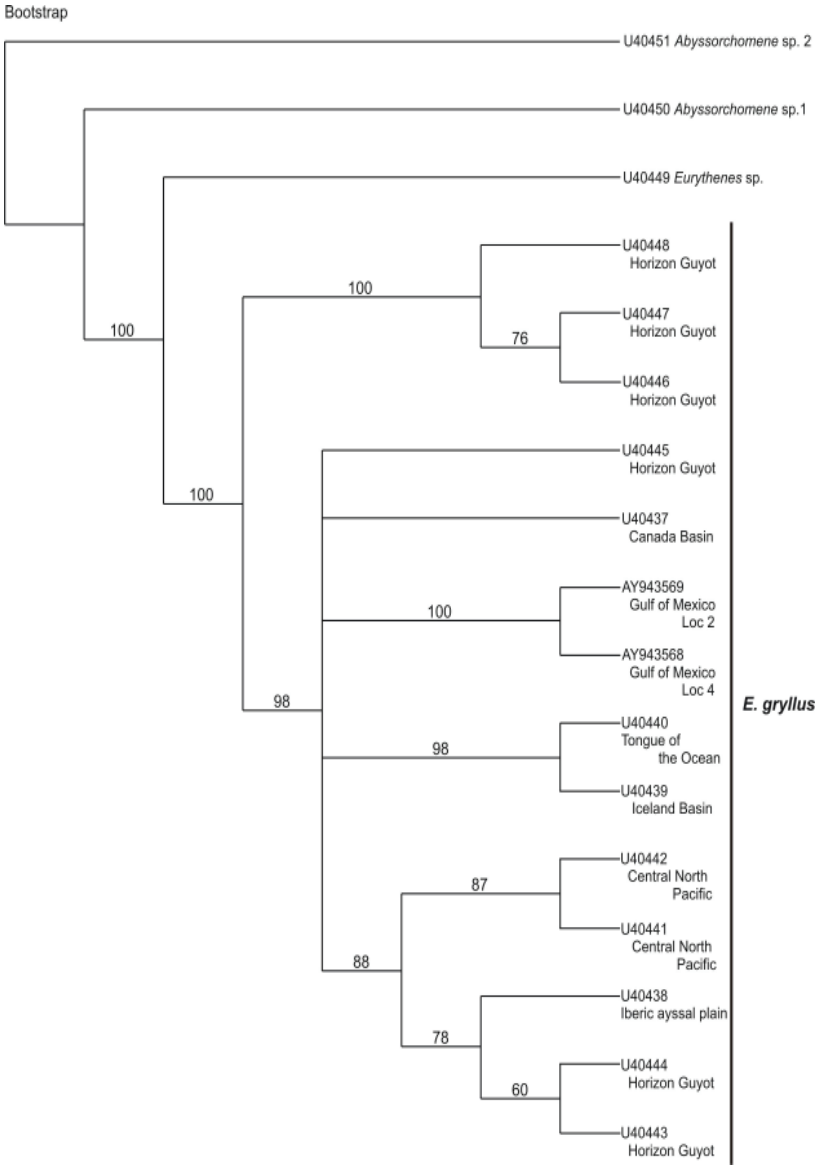
Table 2. Matrix of genetic distances. Above the diagonal line, absolute paired distances; below the diagonal line, total number of different base pairs. ID numbers correspond to those shown in Table 3

ID	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*
1	-	0	0.19	0.25	0.25	0.09	0.09	0.10	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
2	0	-	0.19	0.25	0.25	0.09	0.10	0.10	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
3	76	76	-	0.28	0.26	0.19	0.19	0.19	0.19	0.18	0.18	0.19	0.19	0.19	0.19	0.18	0.19
4	101	101	113	-	0.07	0.03	0.25	0.25	0.24	0.25	0.25	0.25	0.25	0.24	0.24	0.25	0.25
5	99	99	105	30	-	0.24	0.25	0.25	0.24	0.25	0.25	0.25	0.25	0.24	0.24	0.25	0.25
6	37	38	78	101	98	-	0.00	0.01	0.09	0.08	0.08	0.09	0.08	0.08	0.08	0.09	0.08
7	38	38	79	102	99	1	-	0.00	0.09	0.08	0.08	0.09	0.09	0.08	0.08	0.09	0.09
8	39	40	79	102	99	2	1	-	0.09	0.09	0.08	0.09	0.09	0.08	0.08	0.09	0.09
9	17	17	78	98	97	36	37	38	-	0.04	0.04	0.04	0.04	0.04	0.05	0.04	0.04
10	17	17	75	100	100	33	34	35	18	-	0.00	0.01	0.01	0.03	0.03	0.01	0.03
11	16	16	75	100	100	32	33	34	17	1	-	0.01	0.01	0.03	0.03	0.01	0.03
12	17	17	78	102	102	35	36	37	18	6	5	-	0.00	0.04	0.04	0.01	0.03
13	16	16	77	101	101	34	35	36	17	5	4	1	-	0.03	0.04	0.01	0.03
14	15	15	77	98	98	31	32	33	18	13	12	15	14	-	0.00	0.04	0.03
15	16	16	76	98	97	32	33	34	19	14	13	16	15	1	-	0.04	0.03
16	15	15	75	100	100	35	36	37	16	4	3	6	5	15	16	-	0.03
17	16	16	77	100	100	34	35	36	17	14	13	14	13	13	14	12	-

of this deepwater mass with the upper layers in the Gulf of Mexico occurs only through diffusion and vertical mixing; therefore, the deep sea fauna of the Gulf of Mexico could have remained isolated for long periods of time from the nearby Western Tropical Atlantic, unless having a wide vertical mobility, which would allow the genetic exchange among populations.

The processes by which the deep-water transport occurs within the Gulf of Mexico include the coupling with the Loop Current variability as described by Bunge et al. (2002), topographic Rossby waves (Oey and Lee, 2002) and eddy shedding (Ezer et al., 2003). The transport and renewal of the deep water mass in the Gulf seems to be a continuous process occurring in the order of weeks, months, up to a few hundred years (Rivas et al., 2005), implying that deep sea fauna such as *E. gryllus* could be genetically renewed throughout the Intra Americas Sea (IAS) region and the deep western tropical Atlantic.

Regarding the phylogenetic relationships among taxa, 1 common clade appeared in both trees, it was supported by high bootstrap values, and it was sorted out from the undescribed species of *Eurythenes,* collected on the slope of the Bahamas Tongue of the Ocean, and the outgoup, *Abyssorchomene spp.,* from 2 different sites in the Pacific Ocean. The 2 Gulf of Mexico specimens were grouped together in a cluster from abyssal sites. The specimens from shallower locations were aggregated into 2 separate subgroups, Horizon Guyot in the Pacific (U40446 to 48) and Tongue of the Ocean and Iceland Basin (U40439 and 40) (Fig. 2). DNA sequences from the mitochondrial large-subunit ribosomal RNA gene (16S rRNA) of 2 individuals in the Gulf of Mexico were obtained and compared with existing sequences that used the same subunit ribosomal RNA gene. The physical uniformity of the deep sea was used as the hypothesis of genetic homogeneity tested in this study for this eurygraphic cold-water stenotherm species in which we suggest a lack of environmental barriers to faunal dispersal between an open ocean (western Atlantic) and a marginal sea (Gulf of Mexico).



As cited by France and Kocher (1996) the excessive lumping more than splitting, characterizes the current systematic situation in many deep sea groups. Morphologically similar species are often quite distinct genetically, suggesting that morphological stasis may be involved. Sympatric or parapatric taxa typically exhibit ecological differences, suggesting that species are less generalized than previously assumed. Most genetic analyses in deep sea Crustacea reveal the existence of cryptic species,

Figure 2. Molecular phylogeny of *Eurythenes gryllus* inferred by analysis of maximum parsimony (MP) and B) neighbor-joining (NJ) methods of ca. 500 bp fragments of 16S mtDNA. *Abyssorchomene* was used to root the tree. The sample designations of the haplotypes downloaded from GenBank have been included and are indicated at the tips of the trees (see Table 3).

Table 3. Geographic position and depth records of *Eurythenes gryllus, Eurythenes* spp. and *Abyssorchomene* spp., incluidng 16S rRNA gene sequences cited in France and Kocher (1996a). ID = identification number

ID	*Species*	*Geographic position*	*GENBANK accession number*	*Depth (m)*
1	*Eurythenes gryllus*	Gulf of Mexico, station 2 (23°N; 91°W)	AY943568	3732
2	*Eurythenes gryllus*	Gulf of Mexico, station 4 (24°N; 85°W)	AY943569	3410
3	*Eurythenes* sp.*[1]	Northwestern Channel, Bahamas (25°N; 78°W)	U40449	1122
4	*Abyssorchomene* sp. *1*	St. Nicholas Basin, northern Pacific (33°N; 119°W)	U40451	1176
5	*Abyssorchomene* sp. *2*	San Clemente Basin, northern Pacific (32°N; 118°W)	U40450	1930
6	*Eurythenes gryllus*	Slope of the Horizon Guyot, seamount in the northern Pacific (20°N; 169°W)	U40448	3193
7	*Eurythenes gryllus*	Slope of the Horizon Guyot, seamount in the northern Pacific (20°N; 169°W)	U40447	3193
8	*Eurythenes gryllus*	Slope of the Horizon Guyot, seamount in the northern Pacific (20°N; 169°W)	U40446	3193
9	*Eurythenes gryllus*	Slope of the Horizon Guyot, seamount in the northern Pacific (20°N; 169°W)	U40445	3982
10	*Eurythenes gryllus*	Base of the Horizon Guyot, seamount in the northern Pacific (20°N; 169°W)	U40444	4920
11	*Eurythenes gryllus*	Base of the Horizon Guyot, seamount in the northern Pacific (19°N; 168°W)	U40443	5178
12	*Eurythenes gryllus*	Central North Pacific (31°N; 159°W)	U40442	≈ 0775
13	*Eurythenes gryllus*	Central North Pacific (31°N; 159°W)	U40441	≈ 0775
14	*Eurythenes gryllus*	Tongue of the Ocean (25°N; 78°W)	U40440	1309
15	*Eurythenes gryllus*	Iceland Basin (59°N; 21°W)	U40439	≈ 0092
16	*Eurythenes gryllus*	Iberian Abyssal Plain (46°N; 17°W)	U40438	4695
17	*Eurythenes gryllus*	Alfa Escarpment, Canada Basin (86°N; 111°W)	U40437	2076

*[1]Suggested as a different species (France and Kocher, 1996a).

some of which are distinguished by surprisingly large genetic differences given their morphological similarity (e.g., Bucklin et al., 1995; Knowlton and Weigt, 1998; Sarver et al., 1992). Some reproductively isolated taxa with abbreviated development exhibit only small levels of genetic differentiation. Recent genetic analyses in amphipod crustaceans include *Themisto* (Schneppenheim and Weigmann- Haass, 1986), *Abyssorchomene* (France, 1994) and *Eurythenes* (France and Kocher, 1996).

Our observations suggest genetic homogeneity between the specimens of the 2 sites in the deep Gulf of Mexico and differences within the samples from the Atlantic bathyal zone. This could be the result of different ecological conditions or hydrographic isolating mechanisms between the Gulf of Mexico and other western Atlantic locations.

E. gryllus has been considered an eurygraphic species, with a widespread distribution (Menzies et al., 1973). Populations of *E. gryllus* in the Gulf of Mexico are restricted to the Sigsbee abyssal plain, which is relatively isolated. Morphological differences between North Pacific individuals of *E. gryllus* and Atlantic specimens of the species have been described by Ingram and Hessler (1983), who found that females from the Pacific Ocean had 5 pairs of oosteguites, whereas females from the Atlantic had 4. The total length ranged from 1.7 to 13.9 cm (mean 12.7 cm) for the North Pacific specimens (Ingram and Hessler, 1987) and from 1.4 to 12.1 cm (mean 12.1 cm) for individuals from the Northern Atlantic (Charmasson and Calmet, 1987). In contrast, the total length of specimens from the Southeastern Pacific ranged from 2.4 to 7.6 cm (Thurston et al., 2002). The Gulf of Mexico specimens could be considered an isolated population.

The topographic complexity of the abyssal seafloor, exemplified by mini-basins, seamounts, and minimum oxygen zones, represents a barrier for genetic flow among benthic species with limited dispersion, such as the amphipods, which can lead to divergence among isolated populations (France, 1994). Amphipods lack pelagic larval stages, limiting their dispersal capabilities,

hindering genetic flow, and promoting isolation and speciation of the populations. Most adults of scavenger species have strong swimming abilities, giving them widespread distribution patterns. However, they may display morphological variability on a geographical scale, i.e. sea cucumbers, tanaids (Larsen, 2001, 2003), gastropods, and isopods (Wilson and Hessler, 1987). A few of the widespread deep-sea species have been split into species and subspecies that have limited distributions in basins. Examples of these patterns have been recorded for the isopod *Eurycope complanata*, which has been described as a complex of 12 species (Wilson, 1983), and the deep-sea fish *Coryphaenoides armatus*, initially considered an eurytopic species and currently recorded as a set of subspecies with limited geographical distribution (Wilson and Waples, 1984). In the Gulf of Mexico abyssal plain, a speciose tanaid assemblage is represented by endemic species restricted geographically to mini-basin distribution (Larsen, 2003). Allozyme (Bucklin et al., 1987) and 16S rRNA sequence (France and Kocher, 1996a) analyses have suggested that populations of *E. gryllus* from seamounts and continental slopes, where oxygen, food supply and other factors vary significantly, can represent different species. We therefore conclude that the *E. gryllus* complex should be reevaluated and local processes reconsidered, especially where larger phylogenetic differences have been recognized.

Acknowledgements

Specimens were collected with support of the "Deep Gulf of Mexico Benthos Study (DGoMBS) - Joint US/Mexico Studies of the Sigsbee Deep (JSSD)" MMS, USGS Contract 1435-01-99-CT-30991 to TAMU. Additional support was provided by projects CONACyT 40158 F; and DGAPA UNAM IN224503 and IN207410. The species identification was validated by Dr. J. Lowry, Natural History Museum of Australia. Technical support was provided by Ms. P. Acuña and S. Gaytán (graduate program in Marine Sciences and Limnology, UNAM) and L. Márquez (Molecular Biology Laboratory, Institute of Biology, UNAM). Advice, exchange of amplification and sequencing protocols and specimens from the Tongue of the Ocean were provided by S. France (ULL). Thanks to Drs. D. Pless (C. Neurosciences, UNAM) for editorial work, G. Pérez Ponce de León (Institute of Biology, UNAM) and L. Falcón (Institute of Ecology, UNAM) for support with the use of Clustal X, PAUP V.40B10 and Sequencher™ softwares.

Literature cited

Barnard, J. L. and G. S. Karaman. 1991. Records of the Australian Museum: the families and genera of marine gammaridean Amphipoda (except marine gammaroids). Parts I and II., Suppl. 13. The Australian Museum, Sidney. 825 p.

Bellan-Santini, D. 1990. Mediterranean deep-sea amphipods: composition, structure, and affinities of the fauna. Progress in Oceanography 24:275-287.

Biernbaum, C. K. and E. L. Wenner. 1993. Trapping of necrophagous crustaceans on the upper continental slope off South Carolina, U.S.A. Journal of Crustacean Biology 13:601-608.

Blankenship, L. E. and A. A. Yayanos. 2005 .Universal primers and PCR of gut contents to study marine invertebrate diets. Molecular Ecology 14:891-899.

Bucklin, A., R. R. Wilson and K. L. Smith. 1987. Genetic differentation of seamount and basin populations of the deep-sea amphipod *Eurythenes gryllus*. Deep-Sea Research I 34:1795-1810.

Bucklin, A., B. W. Frost and T. D. Kocher. 1995. Molecular systematics of six *Calanus* and three *Metridia* species (Calanoida: Copepoda). Marine Biology 121:655-664.

Bunge, L., J. Ochoa, A. Badan, J. Candela and J. Sheinbaum. 2002. Deep flows in the Yucatán Channel and their relation to changes in the Loop Current extension. Journal of Geophysical Research 107:3233.

Charmasson, S. S. and D. P. Calmet. 1987. Distribution of scavenging Lysianassidae amphipods *Eurythenes gryllus* in the northeast Atlantic: comparison with studies held in the Pacific. Deep-Sea Research I 34:1509-1523.

Christiansen, B., O. Pfannkuche and H. Thiel. 1990. Vertical distribution and population structure of the necrophagous amphipod *Eurythenes gryllus* in the West European Basin. Marine Ecology Progress Series 66:35-45.

Escobar, E., M. López, L. A. Soto and M. Signoret. 1997. Density and biomass of the meiofauna of the upper continental slope in two regions of the Gulf of Mexico. Ciencias Marinas 23:463-487.

Escobar, E., M. Signoret and D. Hernández. 1999. Variation of the macrobenthic infaunal density in a bathymetric gradient: Western Gulf of Mexico. Ciencias Marinas 25:193-212.

Ezer, T., L. Y. Oey, H. C. Lee and W. Sturges. 2003. The variability of currents in the Yucatán Channel: Analysis of results from a numerical ocean model. Journal of Geophysical Research 108:3012.

France, S. C. 1994. Genetic population structure and gene flow among deep-sea amphipods, *Abyssorchomene* spp., from six California continental borderland basins. Marine Biology 118:67-77.

France, S. C. and T. D. Kocher. 1996a. Geographic and bathymetric patterns of mitochondrial 16S rRNA sequence divergence among deep-sea amphipods, *Eurythenes gryllus*. Marine Biology 126:633-43.

France, S. C. and T. D. Kocher. 1996b. DNA sequencing of formalin-fixed crustaceans from archival research collections. Molecular Marine Biology and Biotechnology 5:304-313.

France, S. C., R. R. Hessler and R. C. Vrijenhoek. 1992. Genetic

differentiation between spatially-disjunct populations of the deep-sea, hydrothermal vent-endemic amphipod *Ventiella sulfuris*. Marine Biology 114:551-559.

Hillis, D. M., C. Moritz and B. K. Mable. 1996. Molecular Systematics. Sinauer, Sunderland, Massachusetts. p.563 p.

Ingram, C. L. and R. R. Hessler. 1983. Distribution and behavior of scavenging amphipods from the central North Pacific. Deep-Sea Research I 30:683-706.

Ingram, C. L. and R. R. Hessler. 1987. Population biology of the deep-sea amphipod *Eurythenes gryllus*: inferences from instar analyses. Deep-Sea Research I 34:1889-1910.

Janβen, F., T. Treude and U. Witte. 2000. Scavenger assemblages under differing trophic conditions: a case study in the deep Arabian Sea. Deep-Sea Research II 47:2999-3026.

Kojima, S., R. Segawa, Y. Fujiwara, J. Hahimoto and S. Ohta. 2000. Genetic differentiation of populations of a hydrothermal vent-endemic gastropod, Ifremeria nautilei, between the North Fiji Basin and the Manus Basin revealed by nucleotide sequences of mitochondrial DNA. Zoological Science 17:1167-1174.

Kojima, S., S. Ohta, T. Miura, Y. Fujiwara, K. Fujikura and J. Hashimoto. 2002. Molecular taxonomy of vestimentiferans of the western Pacific and their phylogenetic relationship to species of the eastern Pacific II. Family Escarpiidae and Arcovestiidae. Marine Biology 141:57-64.

Kojima, S., S. Ohta, T. Yamamoto, T. Miura, Y. Fujiwara, K. Fujikura, J. Hashimoto and T. Yamaguechi. 2003. Molecular taxonomy of vestimentiferans of the western Pacific, and their phylogenetic relationship to species of the eastern Pacific III. Alaysia-like vestimentiferans and relationships among families. Marine Biology 142:625-635.

Knowlton, N. and L. A. Weigt. 1998. New dates and new rates for divergence across the Isthmus of Panama. Proceedings of the Royal Society of London B 265: 2257–2263.

Larsen, K. 2001. Morphological and molecular investigation of polymorphism and cryptic species in tanaid crustaceans: implications for tanaid systematics and biodiversity estimates. Zoological Journal of the Linnean Society 131:353-379.

Larsen, K. 2003. The tanaidacean fauna (Peracarida) from a deep-sea cold-seep in the Gulf of Mexico. Journal of Crustacean Biology 23:777-794.

Mackenzie, A., A. S. Ball and S. R. Virdee. 1998. Instant notes in ecology. Bios Scientific Publishers, Colchester. 321 p.

Menzies, R. J., R. Y. George and G. T. Rowe. 1973. Abyssal enviroment and ecology of the world oceans. Wiley Interscience, New York 488 p.

Morrison, J. M. and W. D. Nowlin. 1982. General distribution of water masses within the Eastern Caribbean Sea during the winter of 1972 and fall of 1973. Journal of Geophysical Research 87:4207-4229.

Müller-Karger, F. E., J. J. Walsh, R. H. Evans and M. B. Meyers. 1991. On the seasonal phytoplankton concentration and sea surface temperature cycles of the Gulf of Mexico as determined by satellites. Journal of Geophysical Research 96:12645-12665.

Oey, L. Y. and H. C. Lee. 2002. Deep eddy energy and topographic Rossby waves in the Gulf of Mexico. Journal of Physical Oceanography 32:3499-3527.

Palumbi, S. R. 1996. Nucleic acids II: the polymerase chain reaction. In Molecular Systematics, D. M. Hillis, C. Moritz, and B. K. Mable (eds.). Sinauer, Sunderland, Massachusetts. p. 205-247.

Palumbi, S., A. Martin, S. Romano, E. O. McMillan, L. Stice and G. Grabowski. 1991. The simple fool's guide to PCR. Ver. 2.0 University of Hawaii, Honolulu.

Priede, I. G., P. M. Bagley, J. D. Armstrong, K. L. Smith Jr. and N. R. Merrett. 1991. Direct measurements of active dispersal of food-falls by deep-sea demersal fishes. Nature 351:647-649.

Rivas, D., A. Badan and J. Ochoa. 2005. The Ventilation of the Deep Gulf of Mexico. Journal of Physical Oceanography 35:1763-1781.

Sarver, S. K., M. C. Landrum and D. M. Foltz. 1992. Genetics and taxonomy of ribbed mussels (*Geukensia* spp.). Marine Biology 113:385-390.

Schneppenheim, R. and R. Weigmann-Haass. 1986. Morphological and electrophoretic studies of the genus *Themisto* (Amphipoda: Hyperiidea) from the South and North Atlantic. Polar Biology 6:215-226.

Sherman, K. 1994. Sustainability, biomass yields, and health of coastal ecosystems: An ecological perspective. Marine Ecology Progress Series 112:277-301.

Sheinbaum, J., J. Candela, A. Badan and J. Ochoa. 2002. Flow structure and transport in the Yucatán Channel. Geophysical Research Letters 29, doi:10.1029/2001GL013990.

Smith, K. L. and R. J. Baldwin. 1984. Vertical distribution of the necrophagous amphipod, *Eurythenes gryllus*, in the North Pacific: spatial and temporal variation. Deep-Sea Research I 30:1179-1196.

Sturges, W. 1965. Water characteristics of the Caribbean Sea. Journal of Marine Research 23:147-161.

Swofford, D. L. 2002. PAUP. Phylogenetic analysis using parsimony (and other methods). Version 4 B10. Sinauer, Sunderland, Massachusetts.

Thurston, M. H., M. Petrillo and N. Della Croce. 2002. Population structure of the necrophagous amphipod *Eurythenes gryllus* (Amphipoda: Gammaridea) from the Atacama Trench (south-east Pacific Ocean). Journal of the Marine Biology Association of the United Kingdom 82:205-211.

Wiseman,W. J. and W. Sturges. 1999. Physical oceanography of the Gulf of Mexico: Processes that regulate its biology. *In* The Gulf of Mexico Large Marine Ecosystem: assessment, sustainability and management, H. Kumpf, K. Steidinger and K. Sherman (eds.). Blackwell Science, New York. p. 77-92.

Wilson, G. D. F. 1983. Dispersal and speciation in the deep-sea janiroidean isopods (Asellota, Crustacea). American Zoologist 23:921.

Wilson, G. D. and R. R. Hessler. 1987. Speciation in the deep sea. Annual Review of Ecology and Systematics 18:185-207.

Wilson, R. R. and R. S. Waples. 1984. Electrophoretic and biometric variability in the abyssal grenadier *Coryphaenoides armatus* of the western north Atlantic, eastern south Pacific and eastern north Pacific oceans. Marine Biology 80:227-237.