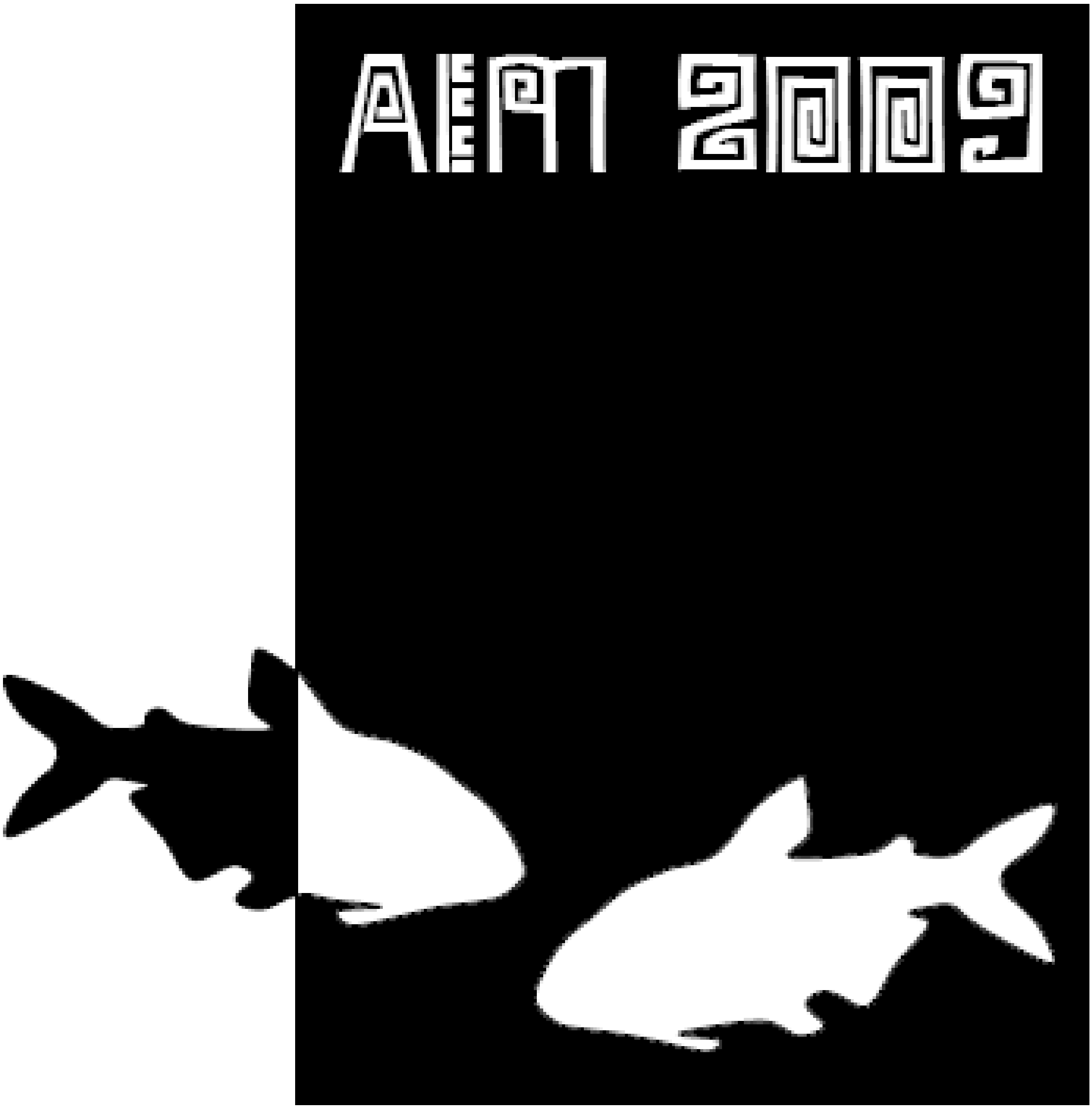


# Astyanax International Meeting

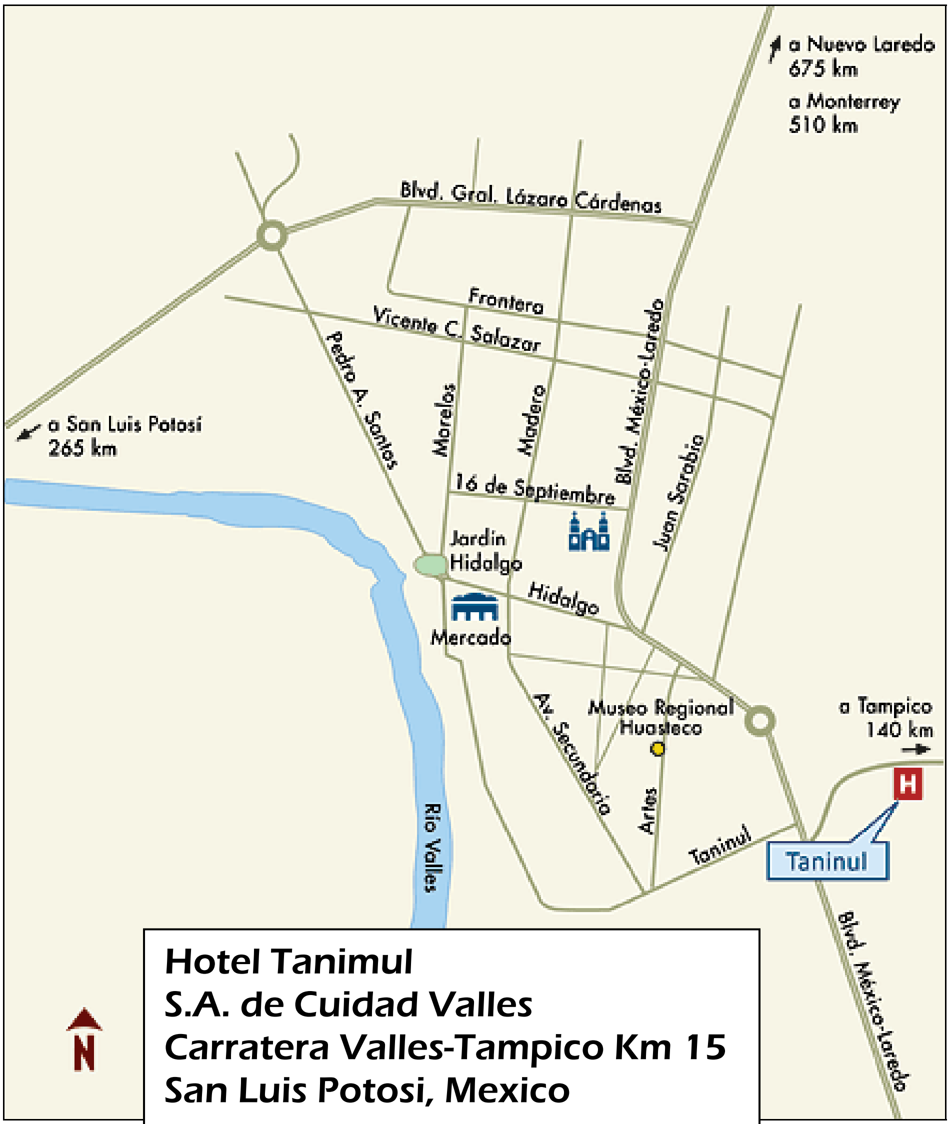
March 15-18, 2009 Ciudad Valles, Mexico

# Meeting program, March 15th-19th, 2009

## Welcome to AIM 2009 in Mexico

# Meeting venue informations

## Hotel Taninul in Ciudad Valles:



**This map is not to scale!**
**Do not try to walk from Ciudad Valles to Hotel Taninul !**
**See next map for a larger view of the area at scale.**

Hotel Tanimul
S.A. de Cuidad Valles
Carratera Valles-Tampico Km 15
San Luis Potosi, Mexico

TEL: (52)-481-381-4619
FAX: (52)-481-381-4616
Email: taninul@avantel.net or
hotel_taninul@hotmail.com


El Pachon
Tampaspaque
Las Granjas
León García
A
Ciudad Valles
Tanzacalte
Tonotilco
Río Micos
San Luis Potosí
MEX 70
MEX 70
MEX 70
El Abra
Taninul
Los Dolores
Las Palmas
Río Florido
El Caseral
Estación Tamuín
Tamuín
Tampico
Antiguo Tamuín
La Fortaleza
Río Tampaon
Ctra Cd Valles-Tampico
El Aserradero
Nueva Primavera
Santa Elena
Tampacoy
Primavera
El Desengano
5km
5mi
Saucede
Rancho Nuevo
Los Nopales
Ojo de Agua
MEX 85
Álvaro Obregón
El Detalle
El Cuiche
La Concepción
El Chuchupe
La Pila
Rancho Nvo.
La Subida
La Lima
Santa Fe

## *“Donde Nace el Agua“*: Hotel Taninul and its Local Environment

Hotel Taninul is a former hacienda with long engaging corridors and sweeping balconies set in a lush oasis at the base of the eastern slope of the Sierra de El Abra, about 15 km east of Cuidad Valles. The oasis lies within a 2.8km² reserve dotted with springs, streams, gardens, meadows, and sugar cane fields. The main feature of the oasis is its springs, the reason for the hotel slogan “donde nace el agua” (where the water is born). There are two springs, a warm sulfurous spring (actually several individual springs) used for bathing, and a fresh water limestone spring, which used to be or might still be the home of an alligator, and definitely not for bathing. The springs were discovered by Europeans in 1808 but of course had already been in use for centuries by the Native Americans of La Huasteca. Soon people traveled here from all over Mexico to bath in the warm sulfurous water, which was believed to have healing properties.

In 1945 the Taninul hacienda was converted into a hotel, which received many famous visitors. The signatures of seven Mexican presidents, a soccer team, and Hollywood stars like Burt Lancaster can be seen in the guest book of the old hotel. In this era, the hotel had a museum of regional antiquities, an amphitheater, a small zoological garden, and a disco made from the mouth of a cavern (see below). Later, the hotel and its surroundings fell into disrepair and became disused. Now the hotel has new management and is being restored, the museum is usually open, although the disco is still closed. The disco cave is now rented out only for private parties (we are trying to get it), and at all other times may be freely visited by hotel guests. The hotel has a spa, a bar (the famous round bar) a restaurant, tennis courts, a gift shop, and bicycles are available. But there are no money distributing machines (the closest are in Cuidad Valles) and internet and telephone service is very limited. The most frequent activity is relaxing, swimming, and bathing in the warm sulfurous spring, especially in the early evening hours, when it has a dreamlike appearance.

## *Amusements*

**Hotel Gardens**.

The hotel and springs are surrounded by lush gardens featuring huge banyan trees. Rest for a while in hammock (provided) under a banyan tree or even sleep there one night for the experience of a tame jungle.

**Swimming and Bathing**.

The warm sulfurous pool offers a tremendous opportunity for swimming, bathing, and general gathering site for our meeting, both during the day and in the evening (the springs are lighted). We are visiting during the dry season, so it is very unlikely to rain, and there will be pleasant bathing for certain. Soak in the slightly sulfurous waters, which are reputed to have healing properties. The sulfurous odor, which sometimes pervades the hotel, is mild and not particularly obnoxious. If you bathe in it, it will stay with you for days, even after showers, and help keep the insects away. You can take a warm water massage by sitting in a chair below the pool's overflow, something that is sure to put you to sleep after a hard day of discussion with your colleagues.

Give yourself and a colleague a relaxing “mud” bath using the “ooze” (see conference delegate Megan Porter for scientific description) from the pool, as many people you will see there are doing (to find out how to do it see the You Tube internet site http://au.youtube.com/watch?v=SXY8XmfouLY). The “ooze” is the greenish stuff usually floating along the edges. It is the sediment from the pool's bottom 10m below, uplifted by the pressure of the springs. The “ooze” is used for beautifying facial and body masks and you will probably see other guests covering themselves with it during your visit to the pool.

If you don't choose to try out the water, then just sit at one of the tables under the thatched huts surrounding the pool, and let the attendant serve you a Mexican beer, or a “vampiro” (a special drink named after bats that are common in La Huasteca).

**Visit Museo Lariab**.

There is a lovely stone museum with a gift shop next to the hotel, which has limited hours but well presented exhibits on ancient and contemporary Huastecan culture. Photography is allowed.

**Go Birding**.

The grounds of the hotel are excellent for birding and wildlife observations. Snuggled up against the base of a mountain ridge that is part of the first range of foothills to the rugged Sierra Madre Oriental, Hotel Taninul offers a good introduction to the tropical birds of La Huasteca. Some of the birding highlights are nesting elegant trogons, which sit for hours in the lush vegetation near the thermal springs, tawny-collared nightjars coursing through the skies at dusk, rufous-capped brush-finches in the thickets on the hillsides, ferruginous pygmy owls, and the plaintive serenade of thicket tinamous in the cooler hours of the day. And of course, parakeets and parrots will be screeching overhead through the palms.

**Follow the Stream**.

The sulfur spring at Hotel Taninul is very active upwelling a large volume of warm water per hour. It exists into a sulfur stream, which is eventually joined by a tributary emanating from the cold fresh water limestone spring. After the confluence, the stream moves rapidly through the jungle into the meadows, becoming cooler and cooler, and eventually harboring some nice aquatic animals, including large turtles. Pathways border both sides of the stream. This is a nice little hike through the jungle.

**Take a Little Cave Trip**.

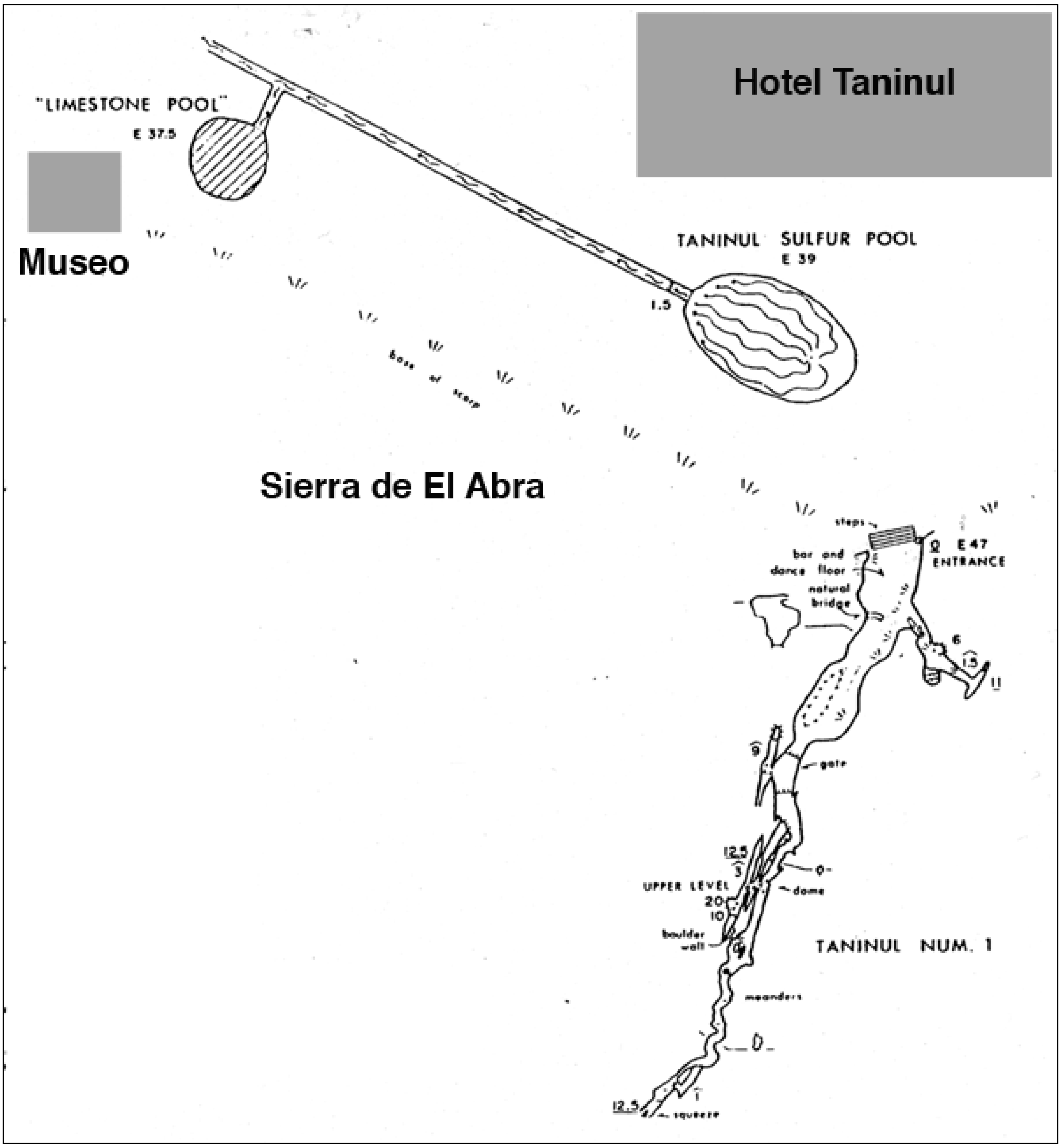
Bring a light and follow the stone staircase at the back of the pool up to the Disco Cave (see above), officially known as La Cueva de las Quilas (The Cave of the Parrots) or (to local cavers) La Cueva de Taninul Numero 1. Here you will find a cavern with a natural bridge, a converted dance floor, and a built-in stone bar. There is a wild cave past the barred gate in the back but please talk to some of the experienced cavers at the meeting before you try it. If you are thinking that since there is a Numero 1 there must be a Numero 2, you are correct. La Cueva de Taninul Numero 2 is a larger cave than Numero 1 that requires a scramble up the steep rocky side of the sierra to get to its main entrance above the sulfur spring. Please let us know if you want to go there and take one of our experienced cavers with you, or just settle for the guided cave tour on the March 19 field trip.

## Other Activities.

There are numerous sights located farther away from Hotel Taninul and requiring a vehicle (and sometimes a hike) to explore. Nacimiento del Río Choy, a large and beautiful resurgence of water collected from the many blind cavefish caves located to the west has a large population of *Astyanax* surface fish. Please speak to one of the meeting organizers for instructions and directions to this location. The ruins of the ancient city of Tamtoc with sculptures, reliefs and an observatory are located south of the village of Tamuín, about 15 km east of Hotel Taminul. The waterfalls of the Río Tampaón and the Río Naranjo are located to the west of Cuidad Valles. Descriptions of the latter places can be found in brochures available in the hotel gift shop.

Several tour guide companies offer day tours to these and other locations, and the hotel is a perfect base for climbers, kayakers, cavers, and regular tourists.

## Hotel and cave map for exploration at Taninul:



# Program at a glance

## Sunday 15th March

**Arrival and evening welcome reception, Diner at 19h.**

**Welcome address by Bill Jeffery**

## Monday 16th March

**Introduction to the first International *Astyanax* meeting by Bill Jeffery**

**Morning session: population biology and ecology**

**Afternoon session: genetics and evolution**

## Tuesday 17th March

**Morning session: development and evolution**

**Free afternoon: resting, natural spring bathing, informal discussions by the pools and the garden.**

**Evening plenary lecture by David Culver at 20h30.**

## Wednesday 18th March

**Morning session: behavior**

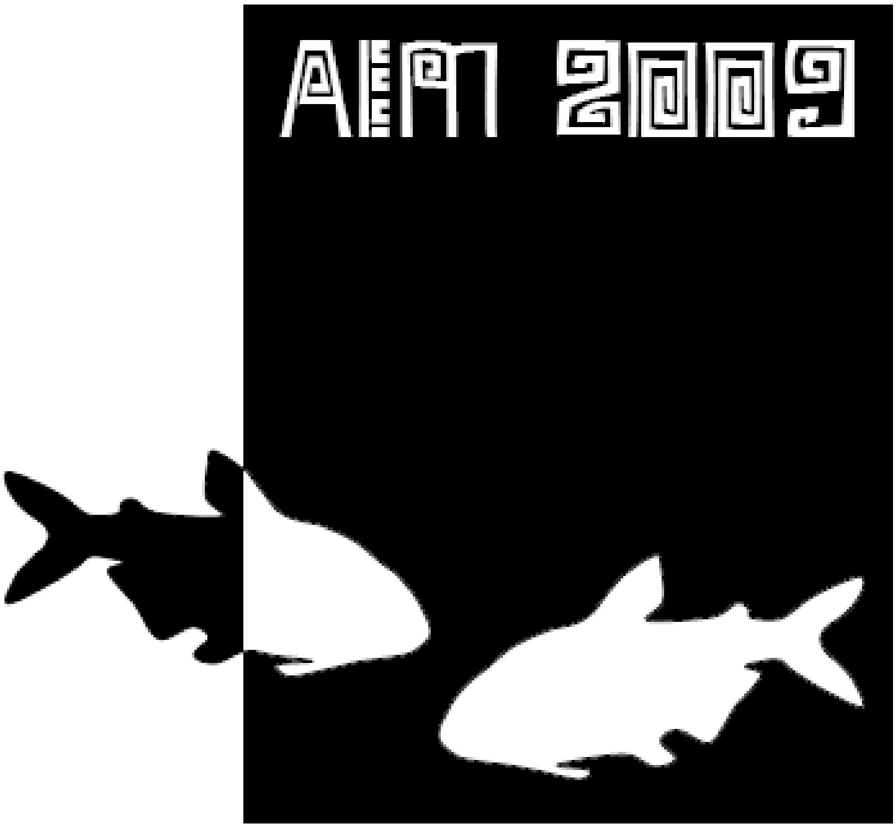
**Afternoon session: behavior continued,**
**genomics and perspectives/projects**

**Closing banquet at 20h**

## Thursday 19th March

**Departure, or Field trip to visit cavefish at the La Cueva de El Pachón.**

# Scientific conference program



## Monday 16th March: population biology, ecology, genetics, evolution.

### Introduction to the meeting

**9h30-10h: William Jeffery (Maryland) "AIM 2009: The First International *Astyanax* Meeting"**

### Morning session: population biology and ecology (Chair: Bill Jeffery)

**10h05-10h35: Victor Hugo Reynoso (Mexico City) "Conservation issues in the blind cave fishes of the genus *Astyanax* in northeastern México"**

**10h40-11h10: Claudia Patricia Ornelas-García (Madrid) "Phylogeny and biogeography of the genus *Astyanax* in Mesoamerica"**

**11h15-11h40: break and photograph**

**11h40-12h: Daniel Sepulveda (Mexico City) "Morphologic variation of *Astyanax mexicanus* in the Atlantic slope of Mexico"**

**12h05-12h25: Sarai Esquivel (La Paz) "Microsatellite polymorphisms on *Astyanax mexicanus* at Mexican Atlantic slope"**

### Lunch at 12h30

### Afternoon session: other cavefish, evolution, genetics (Chair: Yoshiyuki Yamamoto)

**14h-14h30: Didier Casane (Gif) "Molecular phylogeny and phylogeography of Cuban cave fishes, genus *Lucifuga*"**

**14h35-15h05: Megan Porter (Maryland) "Estimating divergence times in *Astyanax mexicanus*"**

**15h10-15h40: break**

**15h40-16h10: Masato Yoshizawa (Maryland) "Evolution of adaptive behaviors in the cavefish *Astyanax"***

**16h15-16h45: Johanna Kowalko (Boston) "Genetic approaches to studying morphological and behavioral traits in *Astyanax mexicanus*"**

### Diner at 19h

## Tuesday 17th March: development and evolution

**Morning session: development and evolution** (Chair: David Stock)

9h30-10h: Megan Dufton and Tamara Franz-Odendaal (Halifax) "Sensory structures and developmental modules within Astyanax"

10h05-10h20: Karen Pottin (Gif) "Interactions between signalling centres for anterior neural plate patterning in *Astyanax*"

10h25-10h55: David Stock (Boulder) "*Astyanax mexicanus* and the developmental genetic mechanisms of dental evolution"

11h-11h20: break

11h20-11h50: Yoshiyuki Yamamoto (London) "How cavefish lost their eyes. Midline signaling and eye development. "

11h55-12h25: Sylvie Rétaux (Gif) "Why do cavefish first develop eyes? A forebrain development hypothesis"

Lunch at 12h30

Free afternoon: resting, natural spring bathing, informal discussions by the pools and the garden.

Diner at 19h

Evening plenary lecture

20h30-21h30: David Culver (Washington DC) "Caves as Evolutionary Laboratories and *Astyanax mexicanus* as a Model of Evolution in Caves"

## Wednesday 18th March: behavior and perspectives

**Morning session: behavior (1)** (Chair: Sylvie Rétaux)

**9h30-10h: Daphne Soares (Maryland) "Changes in sensory strategies during development of cavefish"**

**10h05-10h35: Christophe Guibal (London) "Biological clocks in *Astyanax mexicanus*"**

**10h40-11h10: break**

**11h10-11h40: David Whitmore (London) "Circadian clocks and light sensitivity in *Astyanax* cell lines"**

**11h35-12h05: Matthew Mc Henry (Irvine) "Do blind *Astyanax* have highly sensitive lateral line receptors? "**

**Lunch at 12h30**

**Afternoon session 1: behavior continued (2)** (Chair: Yoshiyuki Yamamoto)

**13h30-14h: Shane Windsor (Oxford) "Hydrodynamic imaging in blind Mexican cave fish"**

**14h05-14h35: Robert Holbrook (Oxford) "Spatial orientation of two morphs of *Astyanax fasciatus*"**

**14h40-15h10: Paul Patton (Bowling Green) "Active wall following in the Mexican blind cavefish (*Astyanax sp.*)**

**15h15-16h45: Sheryl Coombs (Bowling Green) "Short-range, sensorimotor adaptations for acquiring spatial knowledge".**

**16h50-17h20: break**

**Afternoon session 2: genomics and perspectives/projects**

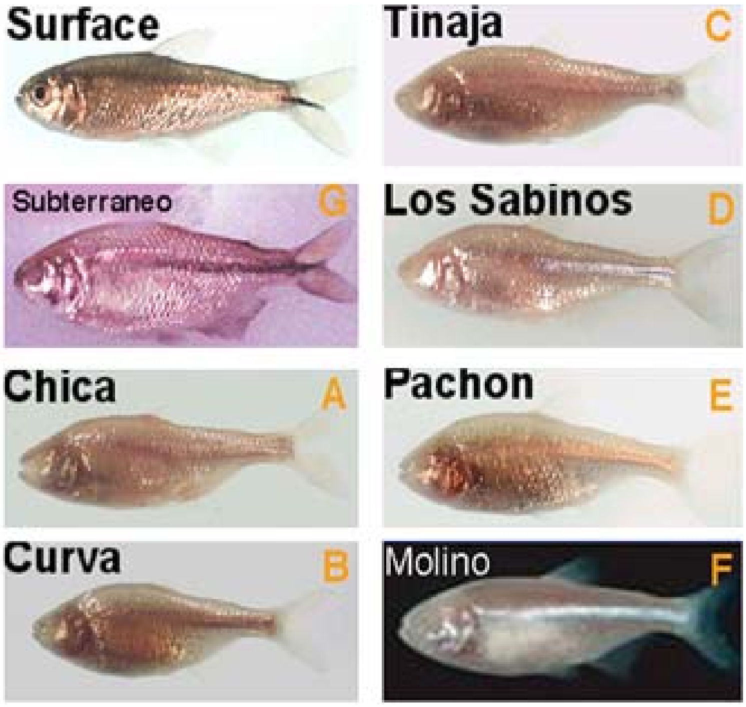
**17h20-17h40: Richard Borowsky (New York) "An *Astyanax* Community Website"**
**17h45-19h30: Round Table**

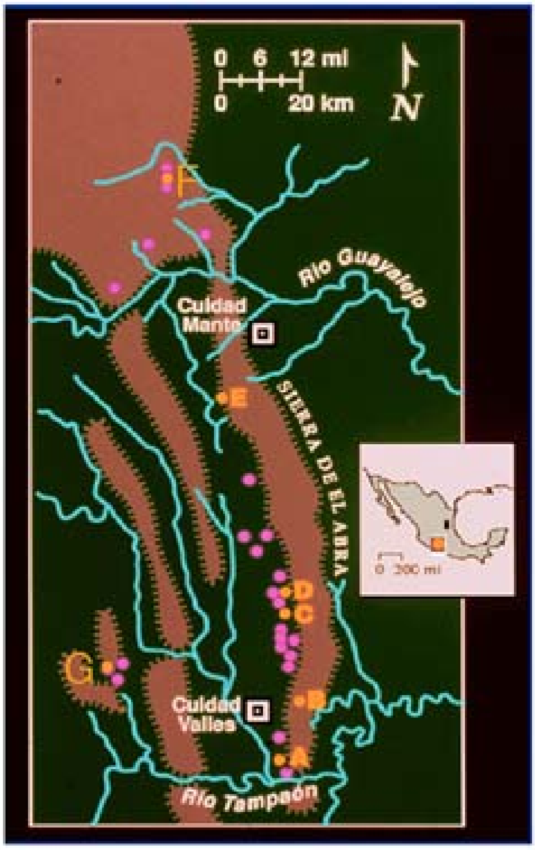
**Closing banquet at 20h**

## Thursday 19th March: trip to Pachón

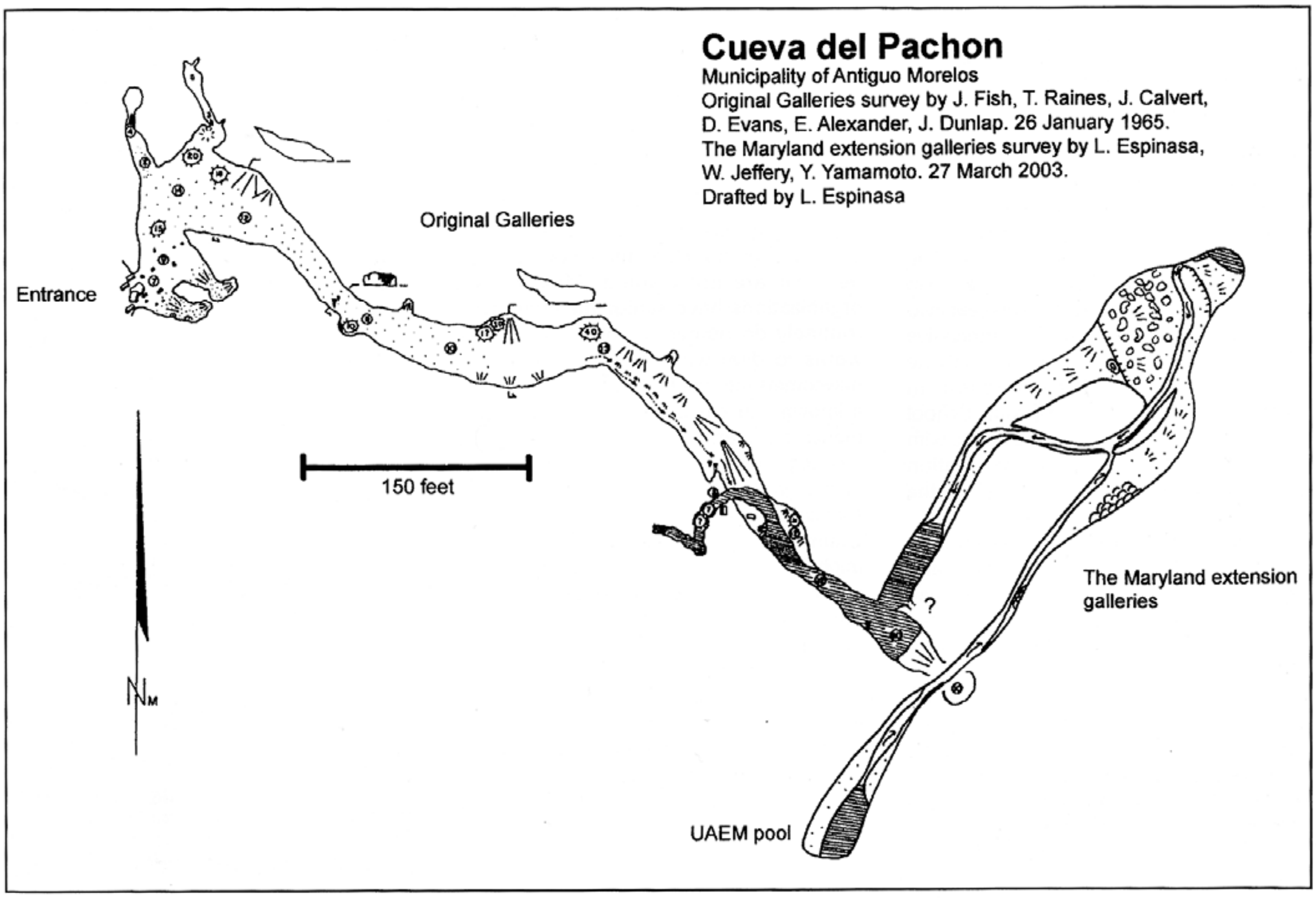
### La Cueva de El Pachón

La Cueva de El Pachón, along with La Cueva Chica and La Cueva de los Sabinos, are probably the three best-known *Astyanax* cavefish caves. Pachón Cave was named after the nearby village of El Pachón, now called Praxedis Guerrero, Tamaulipas. The cave was discovered by a Mexican expedition led by J. Alvárez in 1946, and the population of eyeless fish it contained was originally named *Anoptichthys antrobius* (Alvárez, 1946). After it was realized that this cavefish is the same species as other *Astyanax* cavefish and nearby surface dwelling *Astyanax*, this name has fallen out of use. Now we call it the Pachón cavefish population of *Astyanax mexicanus* (= *fasciatus*).





The entrance to Pachón Cave is located a short distance from the village on the western slope of the Sierra de El Abra, about 70 km north of Cuidad Valles, San Luis Potosí (Russell and Raines, 1967). To reach the cave, one walks from the north side of the village through fields and then climbs a steep rocky arroyo (seasonal stream) leading from the base of the sierra to the entrance. The entrance is a wet-weather resurgence (the exit of a seasonal cave stream) about 2.5m high and 6m wide. Inside the entrance the cave quickly enlarges into a passage of about 5m by 10m, which takes sharp bends to the left and right before heading straight into the hill. The short entrance passage is simple in structure and floored by black soil. About 200m into the cave one reaches the shore of a wall-to-wall pool. The pool serves as a water source for the village (note the pipeline) and fluctuates in size and depth according to seasonal and yearly average precipitation. Based on mark-recapture studies, about 10,000 eyeless fish, one of the largest populations in the region, inhabit this pool (Mitchell et al., 1977). Pachón cavefish show vibration attraction behavior. Dropping a small clay ball into the water and watching them swim toward the disturbance to investigate is a nice way to observe this behavior (Parzefall, 1983). Only large mature fish are seen in this pool.



The pool is usually as far into the cave as one can easily penetrate under normal circumstances. In 2003, a very dry year, a virgin passage leading from the lower left of the pool was discovered, which leads to several hundred meters of additional large passages, dubbed the Maryland extension galleries (Espinasa and Jeffery, 2003). The new passage is more complex than the entrance passage, well decorated with flowstone, stalactites and stalagmites (unlike the entrance passage), and has several small pools containing juvenile cavefish and various cave-adapted invertebrates. An episode of introgression with surface fish in about 1985 was reported in Pachón Cave (Langecker et al, 1991). It is difficult to believe that the surface fish could have entered the cave from the dry arroyo leading up the steep escarpment. Thus, they could have come in either through the Maryland extension from a source of surface fish higher in Sierra de El Abra or from a (pre-adapted hybrid) source far below near the water table. This highlights the complicated evolutionary history of the Pachón (and probably other) cavefish populations.

When exiting the cave, there is a good view of the Valle de Antiguo Morelos from the side of the Sierra de El Abra with the nearest cavefish caves located either 30 km to the north (Bee Cave, El Sótano de Vásquez, and El Sótano de Caballo Moro) or the south (El Sótano de Venadito). Here one can reflect on the extent of isolation of Pachón cavefish with respect to the other 28 known cavefish populations in La Huasteca.

Alvarez, J. (1946). Revision del generero Anoptichthys con description de una especies nueva (Pisc. Characidae).An. Esc. Nac. Cien. Biol. Mexico 4: 263-282.

Espinasa, L., Jeffery, W. R. (2003). Scientists restore eyes in blind cave fish and find virgin passage. Nat. Spel. Soc. News 61: 254-255.

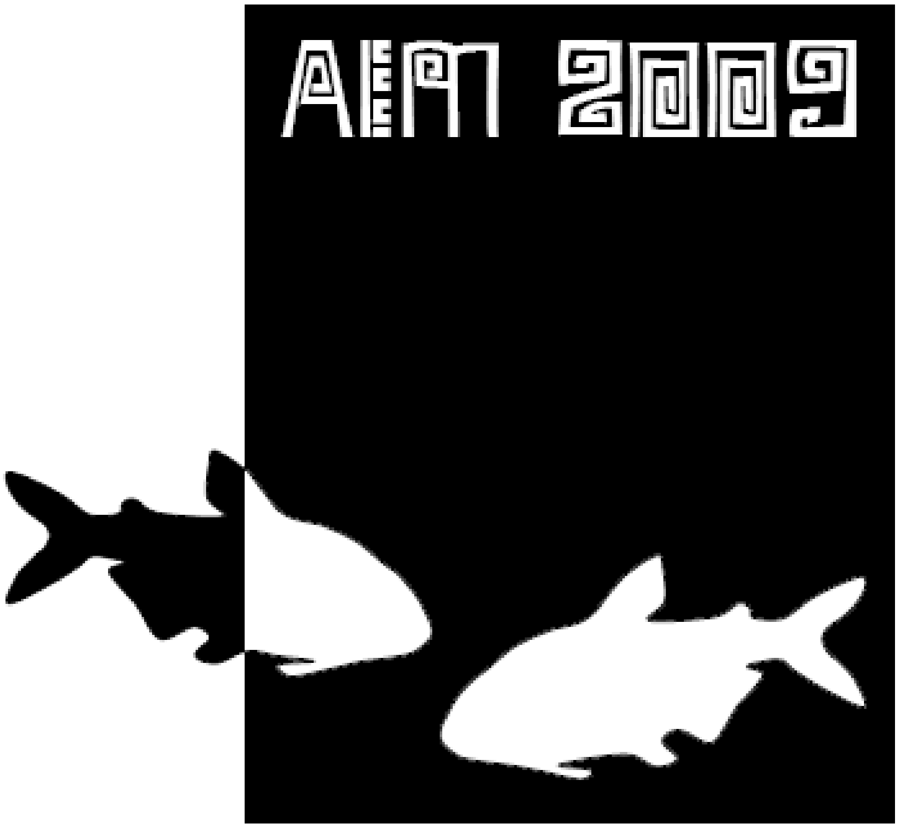
Langecker, T., Wilkens, H., Junge, P. (1991). Introgressive hybridization in the Pachon cave population of *Astyanax fasciatus* (Teleostei, Characida). Ichthy. Explor. Freshwaters 2: 342-344.

Mitchell, R. W., Russell, W. H., Elliot, W. R. (1977). Mexican eyeless characin fishes, henus Astyanax: Environment, distribution, and evolution. Spec. Pub. Mus. Texas Tech Univ, 12: 1-89.

Parzefall, J. (1983). Field observation in epigean and cave populations of Mexican characid *Astyanax mexicanus* (Pisces, Characidae). Mém. Biospéléol. 10:171-176.

Russell, W. H. Raines, T. W. (1967). Caves of the Inter-American Highway. Assoc. Mex. Cave Studies, Austin, TX.

# Abstracts



# Introduction

## AIM 2009: The First International *Astyanax* Meeting

William Jeffery

Laboratory of Evolutionary Developmental Biology, Department of Biology, University of Maryland, College Park, MD 20742 USA. Jeffery@umd.edu

*Astyanax mexicanus* (=*fasciatus*) has emerged as a model species for several different types of biological studies. As the first speaker at AIM 2009, the *Astyanax* International Meeting, I will address four questions: (1) Where are we? (2) Where are the cavefish? (3) How did cavefish become a popular research animal? (4) What will we do here? The brief answers are as follows. We are at the base of the eastern slope of Sierra de El Abra, a cavernous limestone escarpment running through the states of San Luis Potosí and Tamaulipas, México. Sierra de El Abra extends in an unbroken line for about 100 km to the north and 15 km to the south of Hotel Taninul, dividing the coastal plain from the foothills and high valleys of Sierra Madre Oriental and defining a cultural region known as La Huasteca. Caves with blind cavefish populations are found in Sierra de El Abra, Valle de Antiguo Morelos and Sierra de Colmena to the west, and in Sierra de Guatemala and Sierra de Nicolás Pérez to the north and northwest, respectively. A total of 29 caves harboring different *Astyanax* cavefish populations have been described here. The most famous are La Cueva Chica, 15 km to the south, La Cueva de los Sabinos, 20 km to the north, La Cueva de El Pachón – our March 19 field trip destination – 75 km to the north, and El Sótano de Molino, 130 km to the north. *Astyanax* surface fish are also abundant, inhabiting the rivers, streams, lakes, and springs (nacimientos) of La Huasteca. The timeline of *Astyanax* cavefish in research begins in 1936, when Salvador Coronado discovered them in La Cueva Chica. Through subsequent discoveries of additional populations and their popularization for scientific studies, first by Carl Breder and then by Horst Wilkens and Jakob Parzefall, the overview brings us to the present meeting. AIM 2009 was conceived as an opportunity for researchers of *Astyanax* to meet each other – some for the first time, to present our research, to discuss our ideas and plans, to foster collaborations, and to socialize in the lush tropical setting that is the home of our favorite research animal.

## Population biology and ecology

# Conservation issues in the blind cave fishes of the genus *Astyanax* in northeastern México

Víctor Hugo Reynoso, Ricardo Paredes-León y Denisse Arroyo Lambaer.

Instituto de Biología, Universidad Nacional Autónoma de México. Ciudad Universitaria, México, D.F. C.P. 04510. vreynoso@ibiologia.unam.mx.

*Astyanax fasciatus (mexicanus* group*)* is a clade of broad distribution in northern México. Blind populations in caves in northeastern México, are referred to this species. Since surface populations are quite large, the nominal species *A. fasciatus* is not included in protection and trade lists, such as the IUCN, the Mexican NOM-059-ECOL-2008 or CITES. The peculiar habitat condition and independent lineage evolution of blind fishes deserve particular attention and isolated cave populations should be considered with special conservation concern, even though their taxonomic name is the same as that of the abundant surface fish. We performed capture and recapture studies in most popular blind cave fishes population at Pachón and Chica caves. Data were compared to surface fish population at Micos River. Captures were carried out exhaustively by two people during two consecutive hours using specially built fishing nets. Captures ($n_1$) were marked by fin clipping the lower lobule of the caudal fin and immediately released in the ponds where they were captured. Recaptures were done two days later with the same effort counting new captures ($n_2$) and fin clipped recaptures ($m_2$). Population size (P) was estimated using the Peterson modified method for closed populations. Results are as follow: Chica ($n_1$=36, $n_2$=14, $m_2$=0) P=540 + 368.89; Pachón ($n_1$=50, $n_2$=54, $m_2$=0) P=2750 + 1926.8; Micos ($n_1$=333, $n_2$=432, $m_2$=3) P=36047.25 + 16046.18. These indicate that population sizes estimated for both caves are very low. Although it seems not to be any particular threat for these populations in their sites, their small populations situated in restricted confined places, make them vulnerable to human or stochastic environmental impact. We conclude that, the scientific community should be concerned about the vulnerability of blind cave fish populations, since fishes are constantly extracted or populations are manipulated for scientific purposes. Since Mexican Government usually grants permits based on NOM-059 names listed at the species level, there is no true protection for cave populations. IUCN considers blind Chica´s *A. fasciatus* as *A. mexicanus jourdani* as Vulnerable (A1ac+2c, B1+2c, D2), and NOM-059 as *A. jourdani* as threatened. A severe decline of Chica and Pachón cave populations can be concluded from available data.

## Population biology and ecology

# Phylogeny and biogeography of the genus *Astyanax* in Mesoamerica

Ornelas-García Claudia Patricia[1], Domínguez-Domínguez Omar[2] and Ignacio Doadrio[1]

[1.] Museo Nacional de Ciencias Naturales, Madrid Spain,[2.] Universidad Michoacana de San Nicolás de Hidalgo

*Astyanax* is between the most widely distributed genera of freshwater fish fauna in the American continent. Due to its distribution *Astyanax* represents a good model in phylogeographic analyses. Moreover, its interesting morphological plasticity has made of *Astyanax* one of the most important groups in the study of the evolution of phenotypic traits. In order to access the evolutionary history of the genus in the Mesoamerican Region we used mitochondrial and nuclear DNA data (3.8 kpb), comprising the entire mitochondrial cytochrome *b* (*Cytb*) gene for 210 individuals from 147 localities and three mitochondrial genes (*Cytb*, 16 S, and *COI*) and a single nuclear gene (*RAG1*) for a subset of individuals. Phylogenetic analyses were conducted with Maximum Parsimony and Bayesian Inference. The topologies obtained were concordant with the geographic distribution, divided in three major groups: North and Upper Central America, Middle Central America and Lower Central America. All *Bramocharax* samples grouped with their sympatric *Astyanax* lineages (in some cases even with allopatric *Astyanax* populations), with less than 1% divergence between them. This evidence supports "*Bramocharax*" as a morphotype included in the genetic variation of *Astyanax*, and should represent another example of the morphological homoplasies of the genus, probably as a result of ecological adaptation related to trophic specializations. The first invasion of the genus from South America to Mesoamerica was dated around 8 Mya, in accordance to other primary freshwater fish. This dating contrasts with previously proposed dates for the expansion of the genus in Mesoamerica.

**Population biology and ecology**

# Morphologic variation of *Astyanax mexicanus* in the Atlantic slope of Mexico.

Sepulveda, Daniel[1] and Espinosa, Hector[1]

[1]Instituto de Biologia, UNAM
dasj@ciencias.unam.mx

The population from *Astyanax mexicanus* that is located in Cuatro Cienegas, has an evolutionary process different from the rest of the population from the north of México. The physical barriers and the change in the environment, from river stream to cienega, had produce changes in the morphometry of this population. In this study we use morphometry to establish where those changes are produced. 16 landmarks where selected using a standard method for characids, the results are changes in the body depth, and significant changes in the head form. Both set of changes make the Cuatro Cienegas population a unique morph. Also this method is compared with traditional morphometry used to describe new species in the rest of America.

# Microsatellite polymorphims on *Astyanax mexicanus* at Mexican Atlantic slope

[1]S. Esquivel Bobadilla, [2]R. Borowsky, H. [3]Espinosa Pérez and [1]F.J. García de León.

[1]Laboratorio de Genética de la Conservación, Programa de Planeación Ambiental y Conservación, CIBNOR, La Paz, Baja California Sur (México).
[2]Department of Biology, New York University, Washington Square, NY 10003.
[3]Facultad de Ciencias. UNAM (México). sesquivel@cibnor.mx

The Mexican tetra, *Astyanax mexicanus* is successful species with high capacity to dispersion and adaptation to different habitats, including aquifers. Along its range this species have two morph-types; a normal epigean and other ones hipogean blind and without pigmentation habiting aquifers in Northeastern of Mexico. The morphological monotony a long its range had raised confusion on specie's taxonomic status; however it is possible there is cryptic species. We are interested to know the genetic structure along the most part of its range along Mexican Atlantic slope. Here we are present the polymorphism of two microsatellite loci (Ast-10 y Ats-09) on seven epigean populations of *Astyanax mexicanus* form Coahuila (COA), Tamaulipas (TAM), San Luis Potosí (SLP) and Veracruz (VER). We studied a total of 203 individuals. The polymorphism observed was moderated; nine alleles were detected on Ast-10 and eight in Ast-09; average observed heterocigosity was of 0.79. We found a genetic differentiation (Fst = 0.18, P = 0.001) and an isolation by distance structure. We found three different groupings; Northern ones and very different (Cuatrocienegas, COA); other intermediate ones (San Fernando y Soto la Marina, TAM) and finally a southern ones (Pánuco, SLP and Tuxpan, VER).

Key word: *Astyanax mexicanus*, microsatellites, population genetic.

# Molecular phylogeny and phylogeography of Cuban cave fishes (genus *Lucifuga*)

Didier Casane[1], Damir Hernández[2], Alfredo García[3], Pedro Chevalier-Monteagudo[4], Louis Bernathez[5] and Erik García-Machado[2]

[1]Laboratoire Evolution, Génomes et Spéciation, CNRS, 91198 Gif-sur-Yvette
[2]Centro de Investigaciones Marinas, Universidad de La Havana, [3]Sociedad Cubana de Zoología [4]Acuario Nacional de Cuba, Ciudad de La Havana, CP 11300, Cuba
[5]Département de biologie, Université de Laval, Quebec QC, G1K 7P4, Canada.
Didier.casane@legs.cnrs-gif.fr

Morphological characters, that is, the number of caudal fin rays, the number of vertebrae, the size of the eyes, the presence of palatine dentition, the connection of dorsal/anal/caudal fins and the shape of the copulatory organ, were used to identify seven species in the genus *Lucifuga*: four from Cuba, two from the Bahamas and one from Galapagos. The latter, *L. inopinata*, was assigned to the genus with some uncertainty. Our analysis of a large number of widely distributed samples of the four Cuban species (*L. dentata, L. subterranea, L. simile* and *L. teresinarum*) showed that the morphological characters are highly variable within species. In addition, the highly fragmented distribution of the populations in regions where the possibility for migration is low or very low suggests that there may also be cryptic species, difficult to distinguish morphologically. For these reasons, we undertook a molecular analysis of the phylogeny of the Cuban species using mitochondrial and nuclear markers. We identified three lineages, putatively new species, and found a taxonomical synonymy (*L. subterranean* and *L. teresinarum*). Phylogeographic analyses of mitochondrial DNA, using a large fragment of cytb gene, were performed for two species: *L. dentata*, widely distributed, and *L. subterranea/L. teresinarum*, with a narrower distribution. We found strong geographical organization at different geographic scales that can be explained by periods of population expansion followed by population fragmentation and restricted gene flow. At a larger temporal scale, these processes could also explain the distribution of the different species identified. Finally, we correlated the distribution of the different species and the distribution of the polymorphism within species with the paleogeographic history of the Caribbean basin, particularly the timing of cave development in karts regions and sea level fluctuations.

# Estimating divergence times in *Astyanax mexicanus*

Megan L. Porter[1], William R. Jeffery[2], & Katharina Dittmar[3]

1 – Dept. of Biological Sciences, University of Maryland Baltimore County, Baltimore MD 21250,
2 - Dept. of Biology, University of Maryland, College Park MD 20742,
3 – Dept. of Biological Sciences, SUNY Buffalo, Buffalo NY 14260,
porter@umbc.edu and kd52@buffalo.edu

In evolutionary studies of cave adaptation, *Astyanax mexicanus* has become a model system. The presence both surface and cave-adapted populations makes *A. mexicanus* useful for investigating the timing of acquiring the troglomorphic form. In a previous study we used published mitochondrial and nuclear sequence data to estimate the divergence times of the cave populations of *Astyanax mexicanus*. A combination of population-level analyses using molecular clock methods and broad phylogenetic analyses using fossil calibrations indicated that acquisition of the troglomorphic form in the cave populations was younger than 2.2-5.2 Ma. However, these analyses were hindered by the number of cave populations previously sampled (10 of 30) and the number of sequences available for each gene from each population. Furthermore, analyses were conducted on three different sets of sequence data, with no way to combine the information. Here we extend the previous study of *A. mexicanus* divergence time estimates. Individuals from three additional, previously unsampled, cave populations have been sequenced and new individuals from previously studied populations have been included. Additionally, the same set of sequences has been obtained from all populations, including mitochondrial and nuclear genes. Using these new datasets, divergence times will be recalculated to provide better estimates of the time necessary to obtain the troglomorphic form.

# Evolution of Adaptive Behaviors in the Cavefish *Astyanax*

Masato Yoshizawa, Daphne Soares, Špela Gorički, Go Ashida, and William R. Jeffery
Department of Biology, University of Maryland, 1210 Biology-Psychology Building, College Park, MD 20742, USA. yossy@umd.edu

Cavefish are faced with the challenge of finding food and mates in a completely dark environment. They are also devoid of natural predators, which may have relaxed constraints that normally prevent the evolution of behaviors that are detrimental in lighted environments. Here we describe studies designed to reveal the physiological and genetic bases of two behaviors in *Astyanax mexicanus*, vibration attraction behavior and feeding posture behavior. We define vibration attraction behavior as the tendency of fish to be attracted to a vibrating probe. It is measured by (1) the time it takes a fish to initially enter a 2 cm circle around the probe, and (2) the number of times it enters the circle within a 3 minute interval. Cavefish showed significantly augmented vibration attraction behavior relative to surface fish, which is presumably adaptive for detecting water vibrations that may be a source of food and/or mates. Frequency analysis ranging from 2Hz to 500Hz and pharmacological analysis using lateral line inhibitors suggested that the lateral line system of neuromasts, which is enhanced in cavefish, is critical for vibration attraction behavior. Mating experiments between surface fish and cavefish suggested that vibration attraction behavior is inherited paternally as a polygenic trait. This paternal inheritance can explain differential survival and mitochondrial DNA replacement in the Pachón cavefish population, which has experienced natural introgression with surface fish (Strecker et al., 2003). QTL mapping using 125 F2 offspring showed a unique genetic basis for vibration attraction behavior. There was a major epistatic interaction between QTLs on LG1 and LG5, which explained 29% of the phenotypic variance in vibration attraction behavior. To our knowledge, this is the first example of a behavioral trait controlled by an epistatic interaction. We will also briefly present the results of QTL mapping of feeding posture behavior, which may be controlled by 1-3 genes (Schemmel, 1980). In darkness cavefish pick up food while swimming forward along the substrate at a 55˚ angle, whereas surface fish feed at an 80˚ angle while spinning along their body axis, and thus frequently fail to pick up food. We propose that the evolution of vibration attraction and feeding posture behaviors has been instrumental in adapting cavefish to life in perpetual darkness.

Schemmel, C. (1980). Studies on the genetics of feeding behaviour in the cave fish *Astyanax mexicanus* f. *Anoptichthys*. An example of apparent monofactorial inheritance by polygenes. *Z. Teirpsychol*. 53: 9-22.

Strecker, U., Bernatchez, L., and Wilkens, H. (2003). Genetic divergence between cave and surface populations of *Astyanax* in Mexico (Characidae, Teleostei). *Mol. Ecol*. 12: 699-710.

**Other cavefish, evolution, genetics**

## Genetic approaches to studying morphological and behavioral traits in *Astyanax mexicanus*

Johanna Kowalko, Josh Gross, Meredith Protas, Richard Borowsky and Cliff Tabin

Department of Genetics, Harvard Medical School, Boston MA

The genetic basis of vertebrate morphological evolution has traditionally been very difficult to examine in naturally occurring populations. However the fact that the Mexican cave tetra can interbreed with its conspecific river-dwelling relatives opens the door to a classical genetic approach to this problem. Several years ago, we constructed a genome-wide linkage map using microsatellite markers to allow quantitative trait analysis of evolutionarily-derived morphologies. We initially focused on regressive traits. In studies now published, we identified instances of convergent evolution in the loss of pigmentation in cave populations through coding and non-coding mutations in the *Oca2* gene (causing an albinism phenotype) and in the *Mc1r* gene (causing a decrease in melanin production per cell, the "Brown" phenotype). We also examined the genetic architecture of several polygenic regressive traits and found evidence of selection in the loss of eyes, but not for the observed decrease in melanophore number in the cave environment. Current work in the lab has turned towards understanding the nature of the genetic variation responsible for constructive traits, including increases in number of taste buds and neuromasts, and to mapping several behavioral traits such as differences in schooling and feeding behaviors seen in the cave populations.

## Sensory structures and developmental modules within *Astyanax*

Megan Dufton[1] and Tamara Franz-Odendaal[2]

[1]Department of Biology, Life Science Centre, Dalhousie University, 1355 Oxford Street, Halifax, Nova Scotia Canada B3H 4J1 [2]Mount Saint Vincent University Bedford Highway Halifax, Nova Scotia Canada B3M 2J6 Tamara. mcoxhead@dal.ca and Franz-Odendaal@msvu.ca

We believe that understanding the interactions between developmental units is critical to understanding phenotypic variation. We have argued that the sensory structures can be considered developmental modules that are interacting. Our research focus is to further explore this aspect of *Asytanax* development by focusing on two interactions.

The first is between the developing eye (soft tissue) and the ocular skeleton. We are conducting lens ablations in sighted tetras in order to determine what the developmental window is during which the lens can affect the ossification within the eye. The second interaction is between the developing eye and other sensory structures (e.g., taste buds, neuromasts, and olfactory bulbs). We have optimized the immunohistochemistry protocols for these structures and are investigating the interaction between the structures required for vision, taste and smell. We hope to use this methodology to determine whether developmental modules are interacting and to determine whether there is a trade-off between these structures during development.

These studies will lay the foundation for future studies investigating the genes/gene networks underlying development and interactions between these developmental modules.

# Interactions between signalling centres for anterior neural plate patterning in *Astyanax*.

Karen Pottin and Sylvie Rétaux.

CNRS-UPR2197, Institut de Neurobiologie Alfred Fessard,
DECA group Avenue de la terrasse, 91198 Gif-sur-Yvette, France.
pottin@inaf.cnrs-gif.fr

Eye regression in cavefish is a consequence of increased hedgehog signalling (including Shh) at the ventral midline of early embryos (Yamamoto et al, 2004). In fact, Shh expression domain is expanded throughout development, although global forebrain patterning is not affected at later stages.

We have investigated whether other forebrain signalling centres may compensate for ventral Shh expansion in cavefish. We isolated *Astyanax Fgf8*, *Bmp4* and *Wnt1*, three major factors secreted by the anterior and dorsal signalling centres, respectively, and compared their expression time course in cavefish and surface fish embryos. We focused on the heterochrony of Fgf8 expression between the two populations that is observable at the anterior telencephalic midline, and we asked whether interactions between the Shh and Fgf8 signalling centres could explain this heterochrony. We incubated cavefish embryos in SU5402 (an inhibitor of Fgf signalling) at various times of development. SU-treated cavefish embryos showed a dose-dependent reduction of Shh expression, thereby mimicking the “surface-type” Shh pattern. Thus, earlier expression of Fgf8 in cavefish could maintain Shh expansion in the anterior ventral midline. Moreover, SU-treated embryos showed expanded expression of Fgf8 at the anterior neural plate. To analyse potential reciprocal interactions, we performed cyclopamine treatments and found that fgf8 was down-regulated in the dorsal forebrain of treated cavefish. These results highlight complex interactions between these two signalling centres. To investigate the impact of such modifications of midline signalling in cavefish on neural plate patterning, we used *Lhx2 and Lhx9*, two anterior neural plate markers (and crucial eye development genes). We found that Lhx2/Lhx9 expression patterns were significantly different in size and shape in cavefish versus surface fish at 10-12 hpf. Lhx2/Lhx9 are therefore used as an index of anterior neural plate patterning and morphogenesis to analyse interactions between above-cited signalisation centres.

# *Astyanax mexicanus* and the developmental genetic mechanisms of dental evolution

David W. Stock

University of Colorado; Boulder, CO 80309-0334; USA;
David.Stock@Colorado.edu

Dentition in fishes exhibits enormous diversity in shape, number, and location of individual teeth. My laboratory is investigating the developmental genetic mechanisms underlying this diversity through comparative analyses of tooth development focused on the zebrafish, *Danio rerio*. *Astyanax mexicanus* is an ideal species for comparison to this biomedical model because of its relatively close relationship and significantly different dentition. Teeth in the zebrafish are restricted to a single pair of elements of the pharyngeal skeleton, the fifth ceratobranchials, and are exclusively unicuspid. In contrast, *A. mexicanus* possesses teeth on the premaxillary, maxillary, and dentary bones of the oral jaws, and on gill rakers, upper pharyngeal tooth plates, and fifth ceratobrachials of the pharyngeal skeleton. In addition, unicuspid jaw teeth in *A. mexicanus* larvae are replaced by teeth with increasing numbers of cusps during ontogeny. The dentition of the zebrafish and other cypriniforms is thought have undergone reduction from the state present in their last common ancestor with *A. mexicanus*, while the multicuspid teeth of the latter species represent an evolutionary novelty of the order Characiformes. Comparisons of gene expression during oropharyngeal development of the zebrafish and *A. mexicanus* revealed several candidate genes for involvement in cypriniform dentition reduction. Transgenic and pharmacological manipulation of the expression of these genes in both species produced altered numbers of teeth and tooth cusps. Taken together, these results provide insight into mechanisms of dentition reduction in cypriniforms and the origins and ontogenetic distribution of multicuspid teeth in characiforms.

# How cavefish lost their eyes. Midline signaling and eye development.

Xiaoyun Ren and Yoshiyuki Yamamoto

University College London, London, UK
yoshiyuki.yamamoto@ucl.ac.uk

In the cave population, the expression of shh is expanded at the anterior midline. Furthermore, artificial elevation of shh in the surface fish leads to eye loss. However, it is not clear how expanded shh expression is achieved.
Previously, we have compared the sequence of shh enhancer element, ar-C, between the two populations and found very little difference. The ar-C element is responsible for anterior midline shh expression in Zebrafish. The cavefish ar-C is 98% identical to that of the surface fish. Another way of achieving expression differences is to change the expression of up-stream regulatory proteins. We examined the expression of goosecoid and cyclops mRNA at the tailbud stage and found that their expression is also expanded in the prechordal plate region in cave populations. Cyclops is a member of the nodal family of signaling molecules, and directly regulates shh expression in the zebrafish. To investigate whether increased nodal signaling is responsible for expanded shh expression, we treated cavefish embryos with an inhibitor of nodal signaling SB431542 (SB). We found SB treatment can decrease anterior midline shh expression at the tailbud stage, as well as significantly increase lens and eyecup size in 48hr embryos. Conversely, increasing nodal signaling in the surface fish can significantly decrease lens and eye-cup size. Interestingly, SB treatment is more efficient when used early in development. Inhibitor treatment needs to start before shield formation to be effective, and short treatment before shield formation is sufficient to increase lens and eyecup size. These results suggest that the specification and patterning of the prechordal plate is important for the anterior midline shh expression expansion in the cavefish.

## Development and evolution

# Why do cavefish first develop eyes? A forebrain development hypothesis.

Sylvie Rétaux, Alessandro Alunni, Arnaud Menuet and Karen Pottin

CNRS-UPR2197, Institut de Neurobiologie Alfred Fessard,
DECA group Avenue de la terrasse, 91198 Gif-sur-Yvette, France.
retaux@inaf.cnrs-gif.fr

Eye regression in cavefish is a consequence of increased hedgehog signalling (including Shh) at the ventral midline of early embryos (Yamamoto et al, 2004). We have further shown that Shh expression domain is expanded throughout development, although global forebrain patterning is not affected at later stages, probably due to compensation mechanisms by other signalling centres.

We have found several consequences for the long-lasting and increased Shh expression on cavefish forebrain development. First, there is an increase in the migration of Gabaergic interneurons towards the olfactory bulbs, and an increased proliferation of the olfactory bulb territory itself, suggesting an augmented olfactory component of the cavefish forebrain, visible also on the sizes of the bulbs at adult stage. Second, there is an increase in the size of the presumptive hypothalamic territory, an augmented proliferation rate in this region, and a specific increase in some neuronal populations such as serotoninergic neurons in the preoptic region. These changes appear like trade-off modifications, increasing certain sensory-motor modalities to compensate for the loss of eyes. We will discuss how we think these modifications of cavefish forebrain may be advantageous for their lives in darkness, and how we are now testing the impact of these modifications of neuronal network development on changes of behaviour in cavefish.

A question remains however: why do cavefish develop normal eyes in the first 24 hours of their development, before undergoing a relatively slow process of eye degeneration? We will discuss evidence that from a developmental and morphogenetic point of view, it is impossible to construct a well-developed, well-organised, and well-adapted forebrain without specifying an eye territory and undergoing the first steps of eye development during the first day of embryogenesis. Work supported by ANR-Neuro.

**Plenary**

# Caves as Evolutionary Laboratories and *Astyanax mexicanus* as a Model of Evolution in Caves

David C. Culver

Department of Environmental Science, American University, 4400 Massachusetts Ave. NW Washington DC 20016

The appeal of caves as model systems for the study of evolution lies in (i) their highly replicated nature, (ii) their environmental similarity and severity, (iii) the selective factors that are a consequence of these extreme environments, and (iv) the species-rich, convergent cave fauna, the result of thousands of independent invasions of the surface fauna. Superficial subterranean habitats, such gravel banks in rivers and shallow seeps in forests, harbor eyeless de-pigmented species with elaborated extra-optic sensory structures (termed troglomorphic species) and they call into question the nature of the environments in which selection can result in the evolution of troglomorphic species. The only major shared environmental feature of caves and superficial subterranean habitats is darkness. The long debate about the importance of natural selection relative genetic drift and neutral mutation in the evolution of troglomorphy needs to be framed in the context of evolution in darkness.

The potential of *Astyanax mexicanus* as a model for evolution in caves has often been challenged because it seems only to have lost features, that it is too recent a colonist to be typical, and that the caves in which it occurs are atypical because they are not energy- and nutrient-poor. Elaborated features of *A. mexicanus* are quite extensive, the age of colonization is likely millions rather than tens of thousands of years, and darkness rather than energy limitation is likely the key selective factor. *A. mexicanus* remains the only cave-limited animal that can be easily bred in the laboratory and that can be crossed with eyed relatives. Evolutionary developmental studies of *A. mexicanus* eye systems have already yielded the most convincing evidence of the role of natural selection in eye loss. *In situ* estimates of natural selection in both cave and stream populations could provide an important link in the elucidation of the role of natural selection.

**Behavior-1**

# Changes in sensory strategies during development of cavefish

Amy Streets and Daphne Soares

Biology department, University of Maryland, College Park, MD 20742 USA
daph@umd.edu

Cavefish are born with eyes and lenses. But are they functional?
Here we show that, not only *Astyanax* larva can see, but also that they use their visual system for behaviorally significant tasks. We have used electroretinograms (ERGs), transmission electron microscopy and a behavioral paradigm to examine the progressive degeneration and functionality of the retina in cavefish. Cavefish have photoreceptors in their retina at an early age, show positive ERGs for a few days after hatching and use visual information to localize and consume prey.
More interestingly as the retina degenerates fish change their sensory preferences and adjust their hunting strategies to a mechanosensory modality. This is the first time it has been shown that larval fish are able to localize small, intermittently moving prey in a low Reynolds number environment.

**Behavior-1**

# Biological clocks in *Astyanax mexicanus*

Christophe Guibal, Yoshiyuki Yamamoto, Victor Reynoso (1) and David Whitmore.

Department of Cell and Developmental Biology, University College London, Gower street, London WC1E 6BT, UK. (1) Coleccion Nacional de Anfibios y Reptiles Departamento de Zoologia Instituto de Biologia, UNAM Ciudad Universitaria Mexico D.F. 04510. c.guibal@ucl.ac.uk

Biological clocks are required to facilitate the synchronization of organisms with their environment. They are involved in timing various biological processes, such as sleep patterns, reproduction and cell cycle. Although biological clocks have been found in each of the species that have been studied so far, including plants and bacteria, there is no report of a functional clock in organisms that live in a relatively constant environment. Using cave dwelling and surface dwelling forms of *Astyanax*, it is possible to compare within the same species the effects of long-term light deprivation on biological rhythms.

Non invasive approaches were used to study the *Astyanax* clock in their natural environment, in North-Eastern Mexico. Fish were filmed continuously along the length of the day and their activity was quantified. Homologues of genes known to be involved in the clock of zebrafish were cloned and sequenced in *Astyanax*, and the regulation of these genes was quantified at different time of the day in the caudal fin by Reverse Transcriptase quantitative Polymerase Chain Reaction (RT-qPCR).

In *Astyanax* surface-dwelling fish, the expression of clock genes *period 1*, *period 2* and *cry1a* varies significantly during the day. The quantity of their transcripts appears to oscillate similarly to that previously found in zebrafish. No such evidence was found in fish from the cave, averaged data suggesting a much smaller variation of their expression. It appears therefore that the amplitude and/or the synchrony of biological rhythms is significantly smaller is cavefish than in surface-dwelling fish. Video analysis shows that cavefish are preferentially found close to the water surface during mid afternoon and few fish are spotted at the surface in the early morning. Analysis from three separated individuals reveals that each fish exhibits a change of activity during the day, but this does not appear to be synchronized. Finally investigations in the laboratory show that the clock genes oscillate in cavefish when artificially exposed to a light-dark cycle or to a tight feeding schedule. This suggests that cavefish have conserved a functional light-dependent and food-dependent clock despite their 100,000 years of evolution in absolute darkness.

**Behavior-1**

# Circadian clocks and light sensitivity in *Astyanax* cell lines.

David Whitmore, Christophe Guibal, Kathy Tamai and Yoshiyuki Yamamoto.

University College London, Dept. of Cell and Developmental Biology, Gower Street, London, WC1E 6BT, UK.
d.whitmore@ucl.ac.uk

One of the more unusual aspects of zebrafish circadian clock organization is that the majority of cells and tissues not only contain a circadian clock, but are themselves directly light responsive. This fact is true also for early stage embryos, prior to major differentiation of classical light detecting structures, and for cell lines generated from zebrafish embryos. The whole circadian system appears to be highly decentralized in these teleosts.

A major question, following on from our whole animal studies in *Astyanax*, is whether this phenomenon is also true for cell lines generated from a variety of cavefish embryos. Do *Astyanax* cell lines also contain functional circadian pacemakers that are directly light responsive? The short answer to this question is yes. However, the amplitude and robustness of rhythms measured in cells from cave strains, i.e. Pachon and Steinhardt, show significantly lower amplitude oscillations than cells from surface strains. We have also examined the light responsiveness of these cells not only in terms of the acute regulation of circadian clock genes, but also the activation of DNA repair processes. All of these cells retain the direct light responsiveness seen in zebrafish cells, but with particular differences in specific gene activation. This issue will be discussed further, as well as the potential utility of these cell lines for broader scientific analysis, including their possible utility for genome sequencing efforts.

**Behavior-1**

## Do blind *Astyanax* have highly sensitive lateral line receptors?

Matt McHenry, Karla Feitl, Gilberto Cardenas

Department of Ecology & Evolutionary Biology
University of California Irvine, CA
mmchenry@uci.edu

Blind *Astyanax mexicanus* have large lateral line receptors that are thought to be highly sensitive. This idea was tested by mathematically modeling the mechanics of these receptors, known as superficial neuromasts. Using classical beam theory and fluid dynamics, we considered the sensitivity of superficial neuromast in an oscillatory pressure field over a range of frequencies. We also considered how sensitivity varies at high stimulus intensities using a computational finite-element model. The predictions of these models suggest that the inertial hydrodynamics of the superficial neuromasts help to offset the saturation the sensory hair cells within the neuromast at high stimulus intensities. Furthermore, the large diameter and great material resistance of the neuromasts makes them less sensitive than the smaller neuromasts of other fishes. However, the neuromasts of *A. mexicanus* filter signals in a similar manner along the length of the body. This property may aid the ability of the central nervous system to accurately interpret the spatial cues detected by numerous neuromasts across the lateral line system.

**Behavior-2**

# Hydrodynamic imaging in blind Mexican cave fish

Shane Windsor

Department of Zoology, University of Oxford, Oxford OX1 3PS
shane.windsor@zoo.ox.ac.uk

The hypogean form of *Astyanax fasciatus*, commonly known as blind Mexican cave fish, lack a functioning visual system but are capable of moving through complex environments without colliding with obstacles. They do this by using their mechanosensory lateral line system to sense how the flow field that they create while swimming is altered by the presence of nearby objects; an ability termed hydrodynamic imaging. Little is known about the fluid mechanics involved with this ability. Automated digital video analysis was used to measure the swimming kinematics of the fish as they explored novel environments. Particle image velocimetry (PIV) was then used to measure the flow fields around the fish in similar situations. A series of computational fluid dynamic (CFD) models were created in order to estimate the stimulus to the lateral line. The fish reacted to avoid head-on collisions with a wall at a remarkably short mean distance of 0.09±0.01 body lengths (BL). This agreed with the PIV and CFD results, where the stimulus to the lateral line was estimated to be sufficient for the fish to be able to detect the wall at 0.10 BL, but decreased rapidly at increasing distances. The ability of the fish to react was dependent on whether they were beating their tail as they approached the wall. When following surfaces, such as a wall, the fish changed their swimming kinematics significantly and used both tactile and hydrodynamic information. Interestingly, the swimming velocity of the fish was not correlated with the distance at which they reacted to walls. This was supported by the CFD models, which indicated that the relative change in the stimulus to the lateral line was nearly independent of the velocity of the fish. The combined results of these three methods showed that hydrodynamic imaging is a short range sensory ability and that blind cave fish require their sensitive lateral line and fast reactions in order to be able to use it to sense the world around them and avoid collisions.

**Behavior-2**

# Spatial orientation of two morphs of *Astyanax fasciatus*

Robert Holbrook and Theresa Burt de Perera

Department of Zoology, South Parks Road, Oxford, OX1 3PS

The ability to orient through familiar environments is key to the success of many animal groups, underpinning their survival and success by aiding foraging, reproduction and avoiding predation. The ultimate aim of our research is to unravel the principles of aquatic orientation by discovering how fish acquire, learn and memorise their complex surroundings and how they act on this spatial information to enable efficient orientation. In two broad, but overlapping streams of research we study the way that fish encode space and how the sensory systems of fish constrain the mechanisms of orientation that they use.

To do this, we rely heavily on *Astyanax fasciatus* as a model system. Using the eyed and the blind morph of *A. fasciatus*, we have begun to investigate how their different sensory systems shape the orientational and social aggregation strategies they use. The aim of this stream of research is to understand the evolution of these mechanisms, to investigate whether they are constrained by the sensory cues available and hence the type of information that animals are able to glean from their surroundings.

Again, using both morphs of *A. fasciatus*, our cognitive work focuses on identifying which pieces of information are learnt and remembered by the fish, and how these are fitted together to enable these fish to successfully navigate. The blind morph can orient efficiently without visual cues, using only its lateral line organ (LLO), but in order to do this, the fish needs to swim almost constantly. We have exploited this, and the cavefish’s propensity to swim more rapidly in novel environments to show that fish are capable of encoding the size and shape of landmark arrays, and the order in which they are encountered. These experiments have considered horizontal movements, but fish are not restricted to movement over surfaces like many terrestrial animals, they can move throughout their volume of water with three-degrees of freedom. This means that there is potentially much more information available for the fish to use, which in turn could lead to a processing problem. Using the eyed morph of *A. fasciatus* and novel paradigms based on rotating Y-mazes, we explore how fish encode information from three-dimensional space – a task that is difficult for humans and AI systems.

**Behavior-2**

# Active wall following in the Mexican blind cavefish (*Astyanax sp.*)

Paul Patton[1], Sheryl Coombs[1], and Shane Windsor[2]

Bowling Green State University, Bowling Green, Ohio USA[1] School of Biological Sciences, University of Auckland, New Zealand[2]
ppatton@bgnet.bgsu.edu

Spatial exploration is an important and little studied aspect of animal behavior that is likely to pose special challenges for an animal such as the Mexican blind cavefish which lacks any spatially acute long-range sense. When placed in a novel concave enclosure, the Mexican blind cavefish (Astyanax sp.) exhibits a novelty response consisting of increased swimming speed and a tendency to swim along the walls of its enclosure. It has been hypothesized that this is an exploratory behavior, allowing the animal to sequentially bring its lateral line and other short range senses within range of all portions of the limits of its environment. On the other hand, the behavior might be nothing more than an artifactual consequence of a simple obstacle avoidance strategy. If the fish swims straight whenever its path is unimpeded and then turns just enough to avoid any obstacle it confronts, wall-following behavior will arise as a consequence whenever the fish is placed in a closed concave environment. Under this reactive wall-following hypothesis, blind cavefish should not follow a section of convex wall. Eight normal Mexican blind cavefish and eight cavefish whose lateral line sense was blocked with cobalt chloride ($CoCl_2$) were tested in a goggle shaped testing arena containing two 21.2 cm radius quarter circle concave curves, and a quarter circle convex curve of the same radius. Whenever normal fish followed the concave walls, they did so through at least half of their extent in about 80% of all instances and through their full extent in 40% of all instances. The comparable numbers for lateral line blocked fishes were 60% and 18% respectively. Normal blind cavefish followed the convex wall through at least half its length in 73% of all cases and to completion in 25%. For lateral line impaired fish, these figures were reduced to 59.1% and 8% respectively. We conclude that wall-following cannot be accounted for by the reactive barrier avoidance strategy and that blind cavefish must possess an ability to actively regulate their distance from a convex wall. Wall-following impairment following $Co\ Cl_2$ treatment implies that the lateral line sense normally plays an important role in this ability. Residual wall-following during lateral line blockade must be mediated by some other sense, touch with the pectoral fin and snout being the most likely possibility. These findings are consistent with the possibility that wall-following in blind cavefish is an exploratory behavior.

**Behavior-2**

## Short-range, sensorimotor adaptations for acquiring spatial knowledge.

Sheryl Coombs and Paul Patton

Department of Biological Sciences and JP Scott Center for Neuroscience, Mind and
Behavior, Bowling Green State University, Bowling Green, Ohio USA 43402
scoombs@bgsu.edu

Mexican blind cavefish (*Astyanax sp.*) exhibit an unconditioned wall-following behavior in response to novel environments. Wall-following behaviors like this are not unique to blind cavefish and have been observed in a wide variety of vertebrate and invertebrate animals, typically those living under visually restricted or deprived conditions. Although several different biologically-significant functions, as well as artifactual explanations, have been proposed for this wide-spread behavior, its ‘true’ function remains untested for any species.

In this presentation, we compare the behavioral responses of Mexican blind cavefish and sighted Mexican tetra (*Astyanax mexicanus*) to a novel environment under dark (infrared) and well-lit conditions in order to examine the hypothesis that wall-following behavior is a sensorimotor adaptation for acquiring spatial knowledge when the use of long-range visual sensing is prevented. Consistent with this hypothesis, we show that sighted morphs exhibit dramatically different behaviors in the light and dark. Whereas they remain either motionless near the wall for long periods of time or move slowly around the center of the tank without entraining to the walls in the light, they switch to a wall-following behavior in the dark. Although both blind and sighted morphs follow walls in the dark, they exhibit subtle, but significant differences in their wall-following behaviors. Blind cavefish swim more nearly parallel to the wall, exhibit greater wall-following continuity and reach higher levels of sustained swimming speeds more quickly than sighted morphs. Because blind cavefish, unlike sighted morphs, rely entirely on non-visual, short-range senses like touch and the lateral line, they appear to have honed their wall-following/exploratory strategies over an evolutionary time scale. The enhanced wall-following abilities of blind cavefish will be discussed in terms of the active flow-sensing abilities of the lateral line and the sensorimotor strategies that would, in theory, enable these fish to learn spatial relationships among different landmark features when they cannot sense them from a single vantage point at a distance.

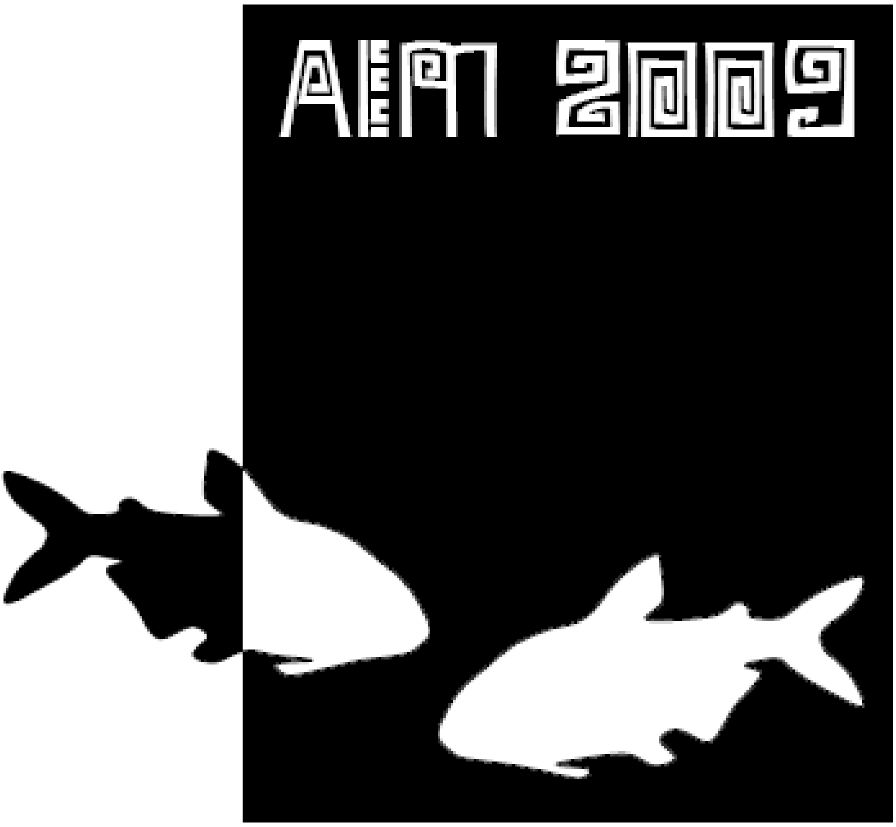
# An *Astyanax* Community Website

Richard Borowsky

Department of Biology, New York University, NY, NY 10003, USA.
rborowsky@nyu.edu

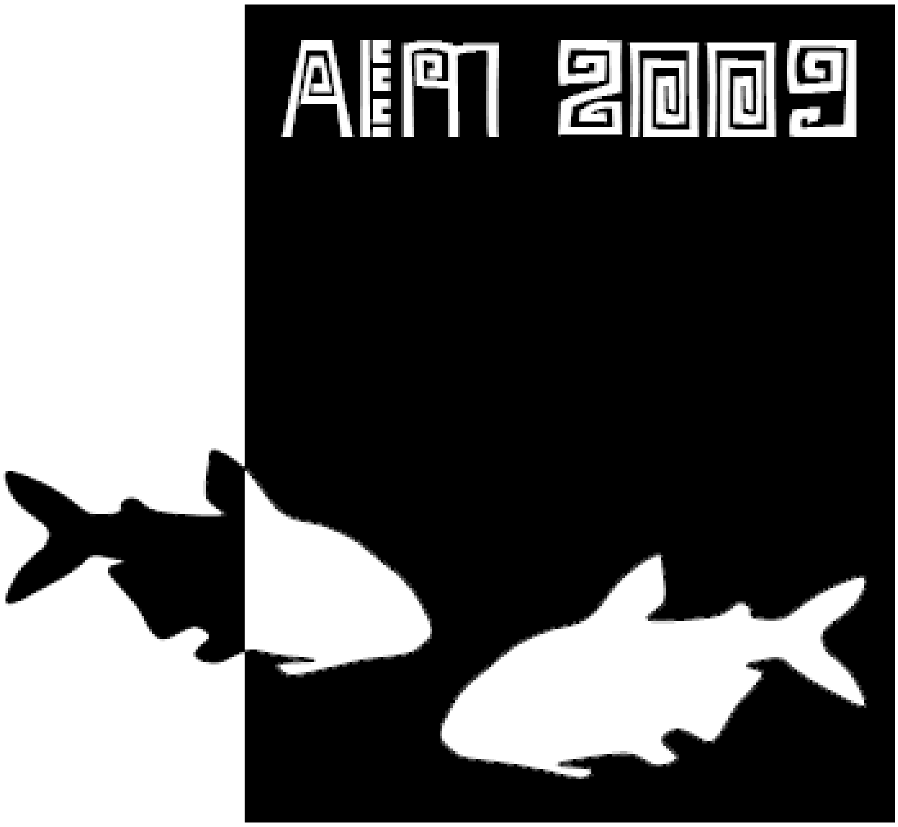
Effective communication of the aims and achievements of the *Astyanax* research community is critical for progress of this emerging model system. Good communication among researchers will facilitate collaboration and external funding; effective outreach to the broader community will foster public awareness of the general importance of biological research and the ways in which our work contributes to the understanding of evolution, development and genetics. NSF is funding the creation of a web site for the whole *Astyanax* community in order to accomplish these goals. We plan for the web site (hosted by NYU) to provide information on, among other things, literature, stock, and reagent resources for researchers, programs for potential students, and information, graphics and videos for the general public and press. The site is still under construction, so your ideas are welcome. The talk is designed to preview the site and elicit suggestions for additions and improvements. Material to be incorporated prior to the talk, including laboratory page links, photographs, pdf files of publications, etc. can be sent to the author at troglomorphic@gmail.com.

# List of attendees

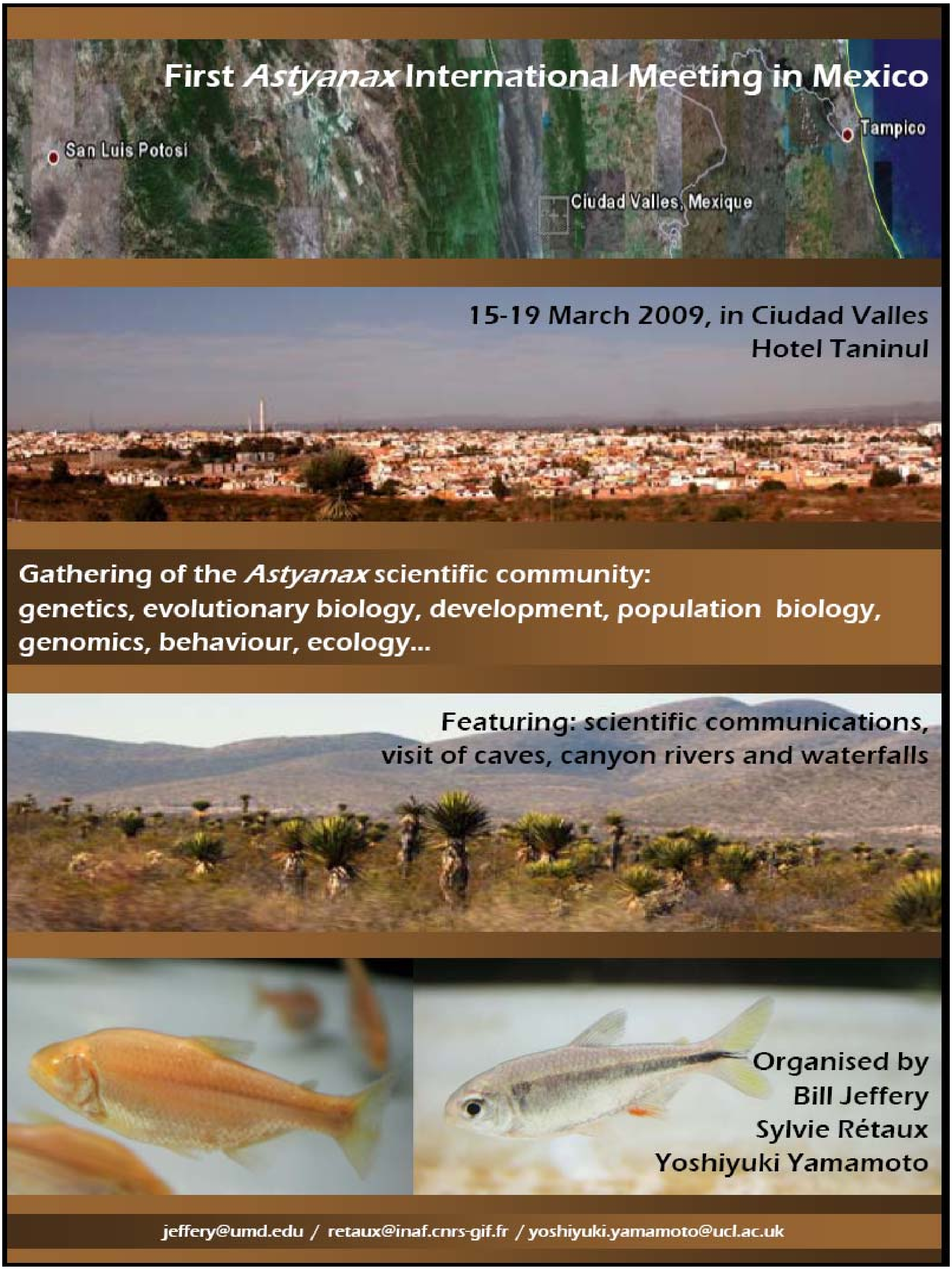


| **Name** | **email** |
|---|---|
| Borowsky, Richard | rborowsky@nyu.edu |
| Bradic, Martina | mb3188@nyu.edu |
| Cardenas, Gilberto | cardenag@uci.edu |
| Casane, Didier | Didier.Casane@pge.cnrs-gif.fr |
| Coombs, Sheryl | scoombs@bgsu.edu |
| Culver, David C. | dculver@american.edu |
| Dittmar, Katharina | katharinad@gmail.com |
| Dufton, Carl | 059324d@acadiau.ca |
| Dufton, Megan | mcoxhead@dal.ca |
| Espinosa, Hector | hector@ibiologia.unam.mx |
| Esquivel, Sarai | sesquivel@cibnor.mx |
| Evans, Benjamin | bevans12@umd.edu |
| Feitl, Karla | kfeitl@uci.edu |
| Fong, Daniel | dfong@american.edu |
| Franz-Odendaal, Tamara | Tamara.Franz-Odendaal@msvu.ca |
| Goricki, Spela | sgoricki@umd.edu |
| Guibal, Christophe | ucgacgu@ucl.ac.uk |
| Holbrook, Robert | robert.holbrook@zoo.ox.ac.uk |
| Jeffery, William | jeffery@umd.edu |
| Jones, Gerald | scoombs@bgsu.edu |
| Kowalko, Johanna | jkowalko@gmail.com |
| Legendre, Laurent | legendre@inaf.cnrs-gif.fr |
| Martasian, David | dmartasi@water.ca.gov |
| McHenry, Matthew | mmchenry@uci.edu |
| Ornelas García, Claudia Patricia | patriciaornelasg@gmail.com |
| Parkhurst, Amy | aparkhur@umd.edu |
| Patton, Paul | ppatton@bgsu.edu |
| Porter, Megan | porter@umbc.edu |
| Pottin, karen | pottin@inaf.cnrs-gif.fr |
| Rétaux, Sylvie | retaux@inaf.cnrs-gif.fr |
| Reynoso, Victor Hugo | vreynoso@ibunam.ibiologia.unam.mx |
| Sepulveda, Daniel | dasj@ciencias.unam.mx |
| Soares, Daphne | daphne.soares@gmail.com |
| Stock, David | David.Stock@Colorado.EDU |
| Whitmore, David | d.whitmore@ucl.ac.uk |
| Windsor, Shane | shane.windsor@zoo.ox.ac.uk |
| Yamamoto, Yoshiyuki | yoshiyuki.yamamoto@ucl.ac.uk |
| Yoshizawa, Masato | yossy@umd.edu |

# Meeting notes



# March 15th-19th, 2009



## First *Astyanax* International Meeting in Mexico

15-19 March 2009, in Ciudad Valles
Hotel Taninul

Gathering of the *Astyanax* scientific community:
genetics, evolutionary biology, development, population biology, genomics, behaviour, ecology...

Featuring: scientific communications,
visit of caves, canyon rivers and waterfalls

Organised by
Bill Jeffery
Sylvie Rétaux
Yoshiyuki Yamamoto

jeffery@umd.edu / retaux@inaf.cnrs-gif.fr / yoshiyuki.yamamoto@ucl.ac.uk