5th Astyanax International Meeting 2017

March 12

6.00 – 6:15	Nicolas Rohner **Welcome remarks**
6.15 – 7.00	Katie Peichel **Genetic and molecular basis of phenotypic diversity in sticklebacks**
7.00 – 10.00	Welcome Reception Dinner

March 13

Moderator:	Nicolas Rohner
8.30 – 9.00	Sylvie Rétaux
9.00 – 9.25	James Jaggard
9.25 – 9.50	Vânia Fernandes
9.50 – 10.15	Jorge Torres-Paz
10.15 – 10.45	Coffee Break
Moderator:	Patricia Ornelas García
10.45 – 11.10	Ernesto Maldonado
11.10 – 11.35	Amanda Powers
11.35 – 12.00	Julius Tabin
12.00 – 12.25	Carole Hyacinthe
12.25 – 1.45	Lunch
Moderator:	Alex Keene
1.45 – 2.15	Masato Yoshizawa
2.15 – 2.40	Suzanne McGaugh
2.40 – 3.05	Misty Riddle
3.05 – 3.30	Ariel Aspiras
3.30 – 4.00	Coffee Break
Moderator:	Sylvie Rétaux
4.00 – 4.30	Richard Borowsky
4.30 – 4.55	Mathilda Mommersteeg
4.55 – 5.20	Robert Peuss
5.20 – 5.45	Lillian Tuttle
5.45 – 7.45	Poster
8.00 – 10.00	Dinner

March 14

Moderator:	Ernesto Maldonado
9.30 – 10.00	Patricia Ornelas García
10.00 – 10.30	Bill Jeffery
10.30 – 10.55	Aniket Gore
10.55 – 11.25	Alex Keene
11.25 – 11.55	Josh Gross
11.55 – 2.00	Lunch/PI Meeting
Moderator:	Joshua Gross
2.00 – 2.30	Luis Espinasa
2.30 – 2.55	Carly Heitz
2.55 – 3.20	Joseph Kopp
3.20 – 3.45	Jenna Robinson
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Moderator:	Daphne Soares
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4.45 – 5.15	Yahui Zhao
5.15 – 5.45	Marta Barluenga
5.45 – 6.30	Discussions/concluding remarks
7.00 – 10.00	Banquet Dinner

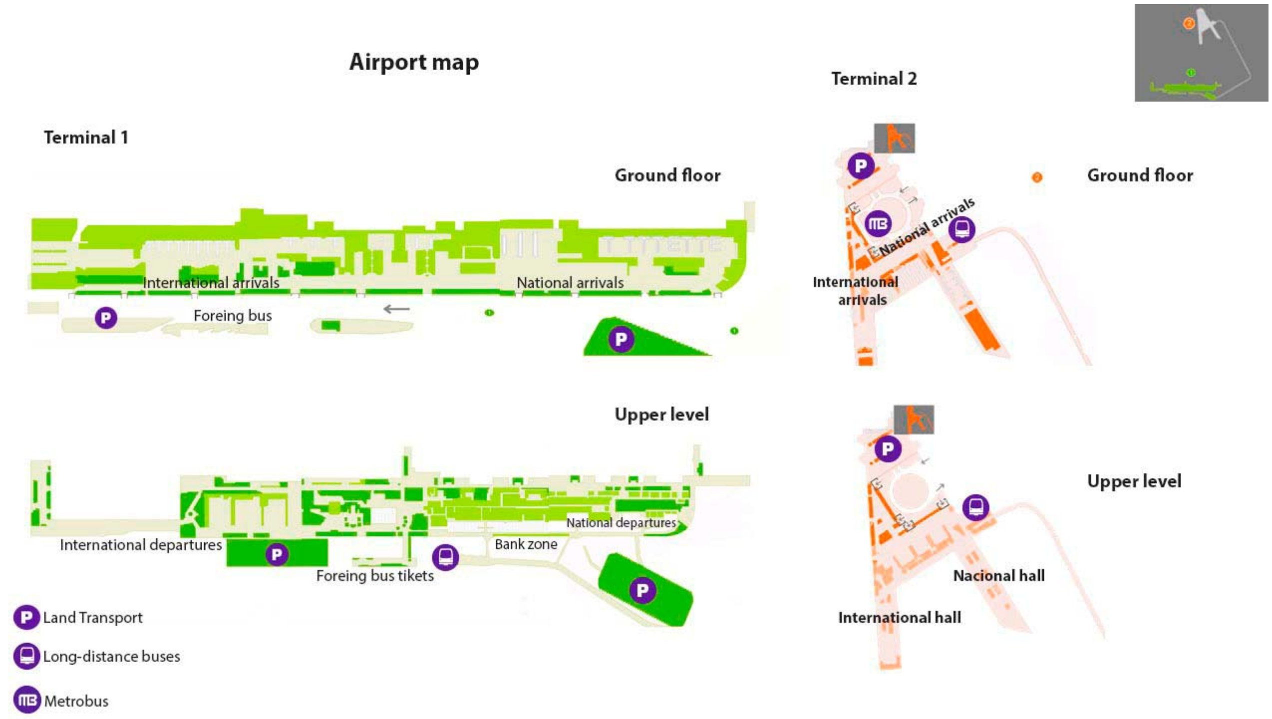
Useful Information

Passports and Visas

A valid passport is required for all foreign travelers. The visa entry requirements for Mexico vary according to your country of origin. Please contact the Embassy or consulate of Mexico in your respective country for further details

Aeropuerto Intercontinental de Querétaro

(Intercontinental Airport of Querétaro)
Address:
Carretera Estatal 200 Querétaro-Tequisquiapan, No. 22500, Colón
Querétaro, Querétaro, México. C.P. 76270
Phone: (442) 192 55 00 | www.aiq.com.mx



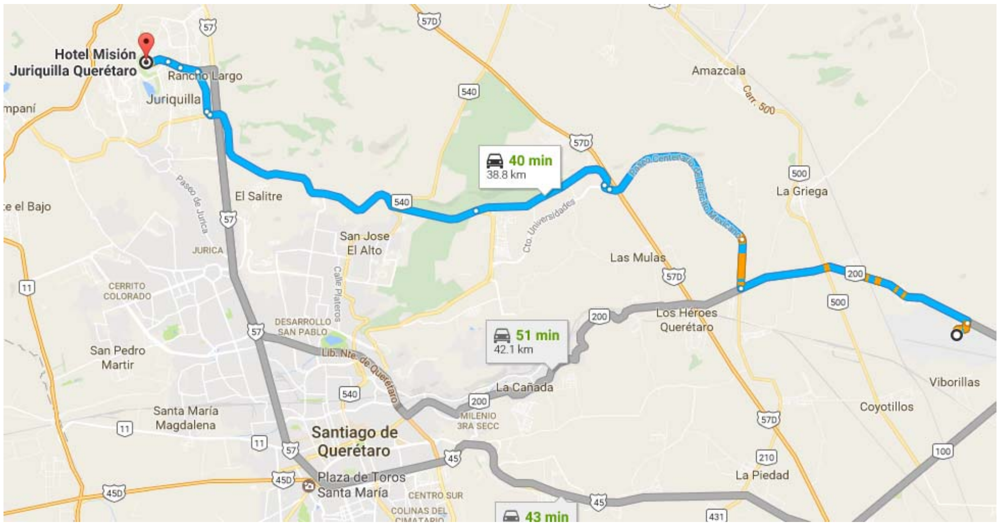
Hotel Misión Juriquilla Querétaro

Hotel Mision Juriquilla Queretaro is located within an exclusive residential zone toward the North of Queretaro City Mexico, in front of the Juriquilla Golf Club. The hotel offers first class accommodation in a renovated eighteenth century farmhouse north of the city of Queretaro, surrounded by gardens and buildings that evoke the colonial Mexico. There is a golf course, soccer fields and tennis courts, pool (be sure to bring your swimsuit,) spa, gym, playgrounds and silverwork, 10 minutes away from the University, 45 minutes away from the city and the airport and 2 hours from Mexico City. There are many taxi services in Santiago de Queretaro that will gladly drive you to the hotel.

Directions from the airport to the hotel:
Querétaro Intercontinental Airport
Qro, Estatal 200, Grande, 76270 Querétaro, Qro., Mexico

- Continue to Carr. Querétaro-Tequisquiapan
  2 min (650 m)
- Turn left onto Carr. Querétaro-Tequisquiapan
  12 min (8.5 km)
- Follow Paseo Centenario del Ejército Mexicano and Cto. Universidades to Anillo Vial Fray Junipero Serra
  11 min (14.2 km)
- Follow Anillo Vial Fray Junipero Serra to Av. de las Ciencias. Take the exit toward Juriquilla/México 57 from Anillo Vial Fray Junipero Serra
  9 min (11.9 km)
- Follow Av. de las Ciencias and Blvd. Villas del Meson to your destination in Juriquilla
  7 min (3.4 km)

Hotel Misión Juriquilla Querétaro
Boulevard Villas del Mesón No. 56, Juriquilla, 76230 Santiago de Querétaro, Qro., Mexico
Phone:(442) 234-0000
Fax:(442) 234-029



Querétaro

Queretaro is a privileged area because of its excellent location, modern infrastructure and connectivity, quality of life and diversity of tourism products. It is a fascinating state, a place that fuses centuries of history, art and culture, in addition to extraordinary natural beauty.
Tourism: www.visitmexico.com/en/queretaro
Events: www.asomarte.com/ or www.queretaro.gob.mx/carteleradeeventos.aspx

Language

Spanish is the official language in Mexico. English is spoken and understood to some degree in most tourist areas. Mexicans are very accommodating with foreigners who try to speak Spanish, even in broken sentences.

Time

Central Time prevails throughout most of Mexico, including the Yucatán, Tabasco, and Chiapas. The states of Baja California Sur, Chihuahua, Nayarit, Sinaloa, and Sonora fall in the Mountain Time Zone, while Baja California uses Pacific Time. **Please note that Mexico observes daylight saving time starting April 2, so we will be on a different time schedule.**

Water

Tap water in Mexico is generally not potable and it is safest to drink purified bottled water. Some hotels and restaurants purify water, but you should ask rather than assume this is the case. Use ice with caution as it may also come from tap water.

Tipping

Most service employees in Mexico count on tips for the majority of their income, and this is especially true for bellboys and waiters. Bellboys should receive the equivalent of 5 to 15 pesos per bag; waiters generally receive 10% to 15%, depending on the level of service. It is not customary to tip taxi drivers, unless they are hired by the hour or provide touring or other special services.

Smoking

In early 2008, the Mexican president signed into law a nationwide smoking ban in workplaces and public buildings, and on public transportation. Under this ground-breaking law, private businesses are permitted to allow public smoking only in enclosed ventilated areas.

Emergencies

In case of emergency, dial tel. 066 from any phone within Mexico. Dial tel. 065 for the Red Cross. The 24-hour Tourist Help Line in Mexico City is tel. 01-800/987-8224 in Mexico, or 55/5089-7500, or simply dial tel. 078. The operators don't always speak English, but they are always willing to help.

AIM 2017 Field Trip, Ciudad Valles, March 15-18

We will go on one day to Pachón and one day to Rio Subterraneo. The beauty of these two caves is that the first shows the most troglomorphic and classic cave population and the second shows evolution as it is taking place. Rio subterraneo drastically shows how the epigean morph is starving while the cave morph is doing well. These two are also the easiest caves to explore. Even though they have been visited in past meetings, new caves other than the visited ones would require rope techniques, which is not feasible for 30+ novice cavers. Furthermore, since it is four years since the last AIM at Taninul, it is likely that most of the young people are new and have never been to either of the caves.

If we want to create a little extra variability, what we could do is that the day of Pachón, since it is a cave that can be explored fairly fast, we could also drive to Nacimiento Rio Mante for people to snorkel with the surface fish, so be sure to bring a snorkel and swim suit.

For Pachon cave we drive about 85 km north towards Mante and then deviate to the town of "Praexedis Guerrero". It requires a short 1.5 km drive in excellent dirt road. Return trip according to mapquest is 60 miles for about 1 hr 8 min.

To go to Rio Subterraneo we drive 14 km west of Cd Valles on the main highway to San Luis Potosi until we arrive to the town of "Otates". From there we drive on a good dirt road for 3.5 km to where we start walking to the cave. From Pachon it is a further about 30 km drive north to reach "El Nacimiento" of river Mante.

If some people have enough with one cave, we recommend that they independently go to Tamul waterfall. This is Rio Gallinas, with holds the "old stock" fish from Rascon and are isolated by this 100 m waterfall from the "new stock" found in river below and all over the rest of the area. I am sure Taninul hotel can organize private trips if desired.

Field Trip Itinerary

March 15

9-10am	Load up vans and depart Hotel Mision Juriquilla
afternoon	Stop for lunch on the way
4-5pm	Arrive at Hotel Taninul in the early evening

March 16

8am	Depart Hotel Taninul for the caves
	Arrive at Pachón cave for caving/exploration
	When everyone is done, we will depart Pachón cave
	Drive to Nacimiento Rio Mante for snorkeling with surface fish
	Depart Nacimiento Rio Mante
dusk	Arrive at Hotel Taninul

Note: The excursions on March 16 and 17 will leave at about 8 AM from Hotel Taninul. There will be a Mexican police checkpoint along the way to the caves, when we cross into the state of Tamaulipas, so you are REQUIRED to bring your passport on the trip. We should be back at Hotel Taninul by dusk.

March 17

8am	Depart hotel for the caves
	Arrive at Rio Subterraneo for caving/exploration
	Depart cave when everyone is done
dusk	Arrive at Hotel Taninul

March 18

8am	Depart Hotel Taninul
afternoon	Stop for lunch on the way
4-5pm	Arrive at Querétaro International Airport for passenger drop off

We will depart Hotel Taninul on March 18 at 8am and will return to the Queretaro International Airport by about 4 or 5pm. We recommend staying the night and departing Mexico on March 19 to account for any travel delays that may be encountered in the return from the field trip.

What to bring

- Your respirator mask (you will be issued one)
- Helmet
- Flashlight with extra batteries
- A SMALL backpack, to carry extra batteries, camera, water, etc.
- Pair of cotton/leather gardening gloves to protect your hands while hiking
- Hiking shoes or boots with corrugated soles and cotton socks
- Change of clothes if you don’t feel like returning in the same (possibly) muddy trousers

What to expect

The hike to La Cueva de El Pachón from the village is short and over level ground, except for the last section (about 15 min), which is up the western slope of Sierra de El Abra along a fairly steep rocky trail to the cave entrance. The upward slope is slightly strenuous, although brief, and should be manageable for anyone in reasonable health. There will be a hands and knees crawl (not particularly tight) downward into the entrance of the cave. Once inside, it will immediately open to walking passage with plenty of headspace until the end, where the cavefish are located. The passage may be muddy and slippery in spots, particularly at the end near the cavefish lake. We will rest at the underground lake for a while, view the cavefish, and then return to the surface in the opposite direction. The temperature in the cave will be warm (the mean annual temperature of the region, about 80ºF or 27ºC), a little cooler than outside but not uncomfortable, although the humidity will be high (your glasses might fog). Please note, there is absolutely no collecting of any kind allowed in the cave, although photography is permitted.

Notice

In Mexican caves, especially those harboring bats, there is risk of infection with histoplasmosis. La Cueva de El Pachón is not a particularly risky cave in this regard, as it does not contain a large bat population and our visit will be relatively short. However, to be cautious, each participant will be issued a 3M 8000 Particle Respirator N95 approved mask at the meeting and asked to wear it on their face throughout their visit to the cave. The N95 air-purifying respirator offers 95% protection from solid particles larger than 3 microns, including fungal spores.

Hotel Taninul and its Local Environment

Hotel Taninul is 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 is within 2.8km² reserve including springs, streams, gardens, meadows, and sugar cane fields. The main feature of the oasis is the 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 were already 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 had many famous visitors. The signatures of seven Mexican presidents, a soccer team, and Hollywood stars like Burt Lancaster can be seen in 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 and at all other times may be freely visited by hotel guests. The hotel has a spa, a bar (the famous “ronda 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 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 order, which sometimes pervades as much as half 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 caving with your colleagues.

Give yourself and a colleague a relaxing "mud" bath using the "ooze" from the pool, as many people you will see there are doing. The "ooze" is the greenish stuff usually floating along the edges. It is sediment from the pool's bottom 10 m 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).

Go Birding. The grounds of the hotel are an excellent spot 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 unwilling lots of warm water per hour. It exist over a tough into a sulfur stream, which is eventually joined by a tributary emanating from a cold fresh water spring, the limestone pool. After the confluence, the stream moves rapidly through the jungle into the meadows, becoming coller and coler, and eventually harboring some nice aquatic animals, including large turtles. Pathways border both side of the stream. This is a nice little hike through the jungle.

Others. There are numerous sights located more distantly from Hotel Taninul and requiring a vehicle (and sometimes a hike) to explore. Nacimiento del Rio Choy, the large and beautiful resurgence of water from the many blind cavefish caves located across the sierra spine in Valle de Antiguo Morellos 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 the village of Tamuin, about

15 km east of Hotel Taminul. The waterfalls of the Rio Tampaon and the Rio 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 home base for climbers, kayakers, cavers, and regular tourists.

La Cueva de El Pachón

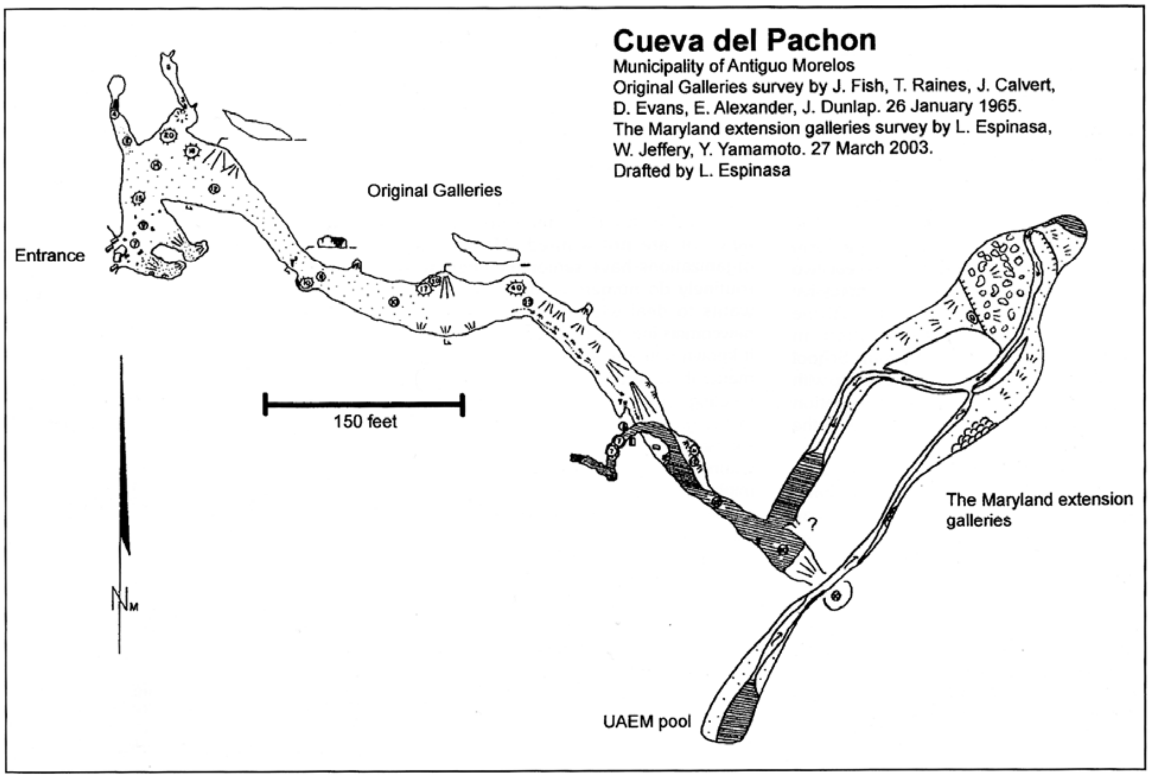
La Cueva de El Pachón, along with La Cueva Chica and La Cueva de los Sabinos, are probably the three best-known *Astyanax* cavefish caves. Pachón Cave was named after the nearby village of El Pachón, now called Praxedis Guerrero, Tamaulipas. The cave was discovered by a Mexican expedition led by J. Alvárez 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*.

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 2.5 m high and 6 m wide. Inside the entrance the cave quickly enlarges into a passage of about 5 m by 10 m, 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 200 m 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.



KEYNOTE TALK: GENETIC AND MOLECULAR BASIS OF PHENOTYPIC DIVERSITY IN STICKLEBACKS

Katie Peichel
Institute of Ecology and Evolution, University of Bern, Switzerland

What are the genetic and genomic changes that underlie phenotypic evolution? How do these changes lead to adaptation to new environments and the formation of new species? Although these questions are of longstanding interest to evolutionary biologists, until recently they have been intractable, particularly in vertebrate species. In order to address these questions, we are using the threespine stickleback fish (*Gasterosteus aculeatus*) as a model system. In particular, we have investigated the genetic mechanisms that underlie morphological, behavioral, and physiological traits that differ between stickleback populations that have adapted to divergent habitats. I will discuss how these studies in my lab and others have provided insight into long-standing questions about the genetic and molecular architecture that underlies rapid and repeated adaptation to divergent environments.

My laboratory has been particularly interested in the genetic basis of behavioral evolution, particularly in social grouping behavior. The formation of social groups is prevalent across the animal kingdom, but can vary dramatically within and among species. One dramatic example of social grouping behavior is found in shoals and schools of fish. We have developed novel assays and analytical methods to study the genetic basis of shoaling and schooling. Using our “model school assay”, we have demonstrated that sticklebacks adapted to different habitats have heritable and genetically unlinked differences in the two key components of schooling behavior: the tendency to school and coordination of body position when schooling. Using transgenic methods, we have demonstrated that variation in the *Eda* gene contributes to variation in both the lateral line neurosensory system and the ability of sticklebacks to school. Together, these experiments have provided mechanistic insight into how and why fish form schools and identified one of the first genes known to shape behavior in an evolutionary context.

Abstracts for Oral Presentation

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MECHANISMS UNDERLYING THE EVOLUTION OF OLFACTORY CAPACITIES IN BLIND CAVEFISH.

Sylvie Rétaux, Yoni Bibliowicz, Maryline Blin, Yannick Elipot,
Luis Espinasa[1]*, Hélène Hinaux, Eugène Tine.*

Institut de Neuroscience Paris-Saclay, Gif sur Yvette, France.
[2]Marist College, New York, USA

We use *A. mexicanus* cavefish to understand developmental, genetic and cellular mechanisms involved in brain and behavioral evolution. We have particularly focused on the evolution of the olfactory sensory system, by a comparative approach at several levels between cavefish and their surface-dwelling conspecifics.

First, we have assessed the olfactory capabilities of larvae and adults of the two *Astyanax* morphs. Experiments performed in the lab in controlled conditions and using amino-acids as olfactory cues show that Pachón cavefish have a much better olfactory discrimination threshold than surface fish. Experiments performed inside the Subterráneo cave (Mexico) show that cave morphs strongly respond to food-related olfactory cues and also to low concentrations of amino-acids. This suggests that increased olfactory capacities have evolved independently in two caves.

In search for the origins of the outstanding olfactory capacities of cavefish, we have then compared the development and the neuroanatomy of the olfactory epithelium/organ in Pachón embryos and juveniles, we have manipulated its development, and we have tested the impact of the lack of vision on olfactory capabilities. The results suggest that the size and the neuronal composition of the olfactory epithelium, as well as some degree of developmental plasticity of the olfactory system are responsible for the excellent cavefish olfaction.

Such a change in the cavefish olfactory sensory system can probably be considered as adaptive in the extreme dark environment where cavefish live.

THE LATERAL LINE CONFERS EVOLUTIONARILY DERIVED SLEEP LOSS IN THE MEXICAN CAVEFISH

James Jaggard[1]*, Beatriz G. Robinson*[1]*, Bethany A. Stahl*[1]*, Ian Oh*[2]*, Pavel Masek*[3]*, Masato Yoshizawa*[4]*, and Alex C. Keene*[1*]

1. Department of Biological Sciences, Florida Atlantic University, Jupiter, FL 33458, USA 2. Davidson Academy of Nevada, Reno, NV, 89557, USA 3. Department of Biology, SUNY Binghamton, Binghamton, NY, USA 4. Department of Biology, University of Hawaii, Manoa, Honolulu, HI 96822, USA

Sleep is an essential behavior exhibited by nearly all animals, and disruption of this process is associated with an array of physiological and behavioral deficits. While sleep is defined by changes in sensory gating, where sensory input to the brain is reduced during sleep little is known about the neural basis for interactions between sleep and sensory processing. The blind Mexican cavefish is comprised of an extant surface dwelling form and 29 cave morphs that have independently evolved increased numbers of mechanoreceptive lateral line neuromasts and the convergent evolution of sleep loss. Ablation of the lateral line enhanced sleep in the Pachón cavefish population suggesting that heightened sensory input underlies evolutionarily derived sleep loss. Targeted lateral line ablations and behavioral analysis localized the wake-promoting neuromasts in Pachón cavefish to superficial neuromasts of the trunk and cranial regions. Strikingly, lateral line ablation did not affect sleep in four other cavefish populations, suggesting distinct neural mechanisms regulate the evolution of sleep loss in independently derived cavefish populations. Cavefish are subject to seasonal changes in food availability, raising the possibility that sensory modulation of sleep is influenced by metabolic state. We found that starvation promotes sleep in Pachón cavefish, and this is not enhanced by lateral line ablation, suggesting functional interactions between sensory and metabolic regulation of sleep. The wake-promoting neuropeptide, hypocretin/orexin (HCRT) is unregulated in Pachón cavefish, and pharmacological inhibition of HCRT signaling restores surface-like sleep in Pachón. Both starvation and ablation of the lateral line curtailed HCRT expression in Pachón cavefish, suggesting that enhanced sensory signaling promotes HCRT. Taken together, these findings support a model where sensory processing contributes to evolutionarily derived changes in sleep that are modulated in accordance with food availability.

HOW ENHANCEMENT OF SENSORY SYSTEM IS INTEGRATED TO GENERATE ADAPTIVE BEHAVIOR

Vânia Fernandes, Christian Macaspac, Masato Yoshizawa

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Evolving animals shift their sensory systems to acquire environmental information and express adaptive behaviors. However, the evolutionary mechanism of its integration into the brain is largely unknown. *Astyanax mexicanus* includes eyed surface populations (surface fish) and isolated populations living in caves (cavefish). Cavefish evolved from surface fish ancestors millions years ago and quickly adapted to their new environment by acquiring specific traits such as eye loss and albinism. A significantly enhanced trait, Vibration Attraction Behavior (VAB) – ability to swim towards prey in darkness – is found in cavefish making this Mexican tetra a unique model for sensory evolution. The superficial neuromasts (SN) present at the eye orbit are the mechanosensory units responsible for VAB. Since SN, covered by mucus cupula, are scattered on entire skin surface, neuromasts can have distinct sensing properties based on their position. Here, we present the millisecond resolution recording of the cupula movements using fluorescent microspheres. The phase-locked movement of cupula we observed suggest that these could stimulate SN neurons in applied frequency. We are then testing whether this mechanical property of cupula differs depending on the cranial areas in different cave populations. We are also testing how stable the neuromast development is in surface fish and cavefish and the difference in the neural wirings. The pharmacological inhibition of Endothelin signaling induced more neuromasts in the cranial region of cavefish than in surface fish, suggesting that cavefish lost 'safety-lock' stabilizing the number of SN. In addition, neuromast enhanced-cavefish did not increase VAB, suggesting an additional factor to establish high-level VAB. We are also generating a brain atlas for both morphs and mapping neural wirings of the cranial lateral line system. Above all, we are underway to reveal the evolutionary mechanism of the specialized eye orbit-SN and its impact on VAB.

DEVELOPMENTAL EVOLUTION OF THE PROSENCEPHALON IN BLIND CAVEFISH: ORIGINS OF NATURAL VARIATIONS IN NEUROPEPTIDERGIC PATTERNING AND THEIR BEHAVIORAL CONSEQUENCES

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Drastic changes in life style and habitat through vertebrates' evolution have brought along functional and morphological brain modifications. During colonization of caves, *Astyanax* cavefish have evolved behavioral adaptations in order to ensure their survival in such extreme conditions. Most of these behaviors, important for maintaining body homeostasis, are controlled by complex circuits within the hypothalamus.

Here we performed a comparative study on the ontogeny and organization of peptidergic neurons in the hypothalamus and neurosecretory preoptic area (NPO) of *Astyanax* surface and cave morphotypes.

Six out of the nine neuropeptides analyzed by *in situ* hybridization showed quantitative differences in terms of cell numbers in specific neuronal clusters. We found that variations where mainly restricted to clusters in the basal hypothalamus, while cell numbers in the NPO where similar in both morphs. Our interpretation of the gene expression data in the context of the prosomeric model highlights great conservation in terms of alar-basal forebrain organization from fish to mammals. Using knock-down experiments, we then showed that the increase in Hypocretin and NPY neurons in cavefish results from expanded expression domains of the transcription factors Lhx9 and Lhx7, respectively. Additionally, using pharmacological experiments, we found that early SHH and FGF signaling impact the fate specification and/or the differentiation of specific NPY and Hypocretin clusters.

Finally, we found that manipulating *Lhx9* expression has long lasting effect on Hypocretin neuron numbers in the hypothalamus, and increases sleep scores in cavefish. Thus, we propose a link between early developmental changes in brain patterning and the evolution of behavioral adaptations.

EVODEVO AND ADAPTATIONS TO PERPETUALLY DARK ENVIRONMENTS: THE LEFT-RIGHT SWIMMING PREFERENCE IN CAVE AND SURFACE MORPHS FROM *ASTYANAX MEXICANUS*

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Bilaterians organisms are, in fact, not completely bilateral. There are morphological asymmetries in many animals, with some notorious examples in fishes; like *Perissodus microlepis* with scale-eating morphs with a jaw bended to the left (or to the right) that eats scales of other fishes' right flank (or left flank) or like flounder fishes with both eyes located at the same side of the head. It has been described that *Astyanax mexicanus* cavefishes possess different craniofacial arrangements at the right side of the head, such as extensive fragmentation or fusion in suborbital bones, compared with the left side. Asymmetry of behavior or preference for the left or right side, reflects partitioning of information processing in either hemisphere of the brain and is a common feature in land and aquatic animals including fishes. Visual stimuli and response play an important role in "Handedness", therefore it will be interesting to explore if lateral dominance (either right or left) exist in blind Cavefish *Astyanax* morphs adapted to perpetual darkness. In behavioral experiments, we explored the lateral preference of *Astyanax mexicanus* cavefishes (CF) or surface fishes (SF) to navigate a maze in search for food. In experiments, carried out in complete darkness (recorded with infrared cameras) using wild caught fish, we found that SF prefer to solve the maze using the right side, while CF do not seem to show a clear preference for either side. SF trained to use the right door keep using the right door when presented with both choices, however when SF trained to use the left door were presented with both options, the majority will switch to use the right door. Further studies will be performed to understand the environmental influence on lateral preference and the importance of the visual function for handedness.

THE EVOLUTION OF CRANIAL ASYMMETRY ACROSS THE LIFE HISTORY OF THE BLIND MEXICAN CAVEFISH

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Craniofacial alterations in cavefish were among the first morphological traits to be characterized during their discovery in the 1930's. Alvarez (1947) described "fragmentations" and "fusions" that occur in the circumorbital bones surrounding the collapsed eye orbit in multiple cavefish populations. These features are not present in extant surface-dwelling forms of the same species, *Astyanax mexicanus,* providing a comparative paradigm for morphological traits. In addition to bone fragmentation and fusion, other cranial alterations impact the size, shape and positioning of cranial bones, and include bilateral asymmetries. The developmental mechanisms contributing to cranial asymmetries in cavefish remain unknown. We sought to understand when, during ontogeny, cranial asymmetries arise in cavefish. Toward that end, we implemented a geometric morphometric approach to evaluate shape variation of the chondrocranium and osteocranium across life history in surface fish and two distinct cavefish populations. Interestingly, measures of cartilaginous skull in juveniles were bilaterally symmetric and shape was conserved across all three populations. In contrast, osteocranial shape segregated into significantly distinct groups in adults. Cavefish demonstrated significant asymmetry for bones surrounding the collapsed eye orbit, and the opercle bone posterior to the orbit. Interestingly, we discovered that cavefish exhibit directional asymmetry in the form of a dorsal-cranial bend, which was biased to the left. This observation may relate to observed asymmetries with the lateral line sensory system and putative 'handedness' associated with swimming behavior. Further experimentation will reveal the extent to which sensory system expansion correlates to cranial alterations in cavefish. In sum, this work reveals dynamic changes to the craniofacial complex over the course of cavefish life history, which has evolved in response to extreme environmental pressures of the cave.

THE EVOLUTION OF TEMPERATURE PREFERENCE IN THE MEXICAN CAVE FISH *ASTYANAX MEXICANUS*

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Even though a human can survive equally well in both fifty degree weather and seventy degree weather, why is it that most would prefer to be in the latter? Everybody has preferences, but not much is known about the genetics of preferences or how they evolve. We have been studying the temperature preference of *Astyanax mexicanus* populations from different caves and of the surface population living in adjacent rivers. In principle, the temperature in the caves differs from, and is more stable than, the temperature in rivers, which could lead to selection for different preferences. We are in the process of measuring the actual temperatures throughout the year at a series of caves and the adjacent rivers in Mexico. It would make sense that the caves have different temperatures, as we have indeed found that the cave populations have different temperature preferences. For example, the Pachon fish distinctively like cooler temperatures than the surface fish. Genetic analysis indicates that these are inherited traits. Analysis of F2s suggest that the trait is not Mendelian but rather is more complex. We inadvertently discovered a second type of temperature preference in these populations when our colony was transiently afflicted with parasites (gill flukes). The parasites had the effect of making the fish prefer warmer temperatures (akin to a fever response in humans). However, this effect was found to be significantly less pronounced in the cave fish than the surface fish. We are now attempting to map the genes underlying temperature preference in Astyanax by QTL analysis, the results of which will be discussed.

EVOLUTION OF ACOUSTIC COMMUNICATION IN THE BLIND CAVEFISH *ASTYANAX MEXICANUS*.

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Acoustic communication is an essential feature to exchange information related to social cohesion and coordination (i.e.: alarm calls, mother-youth interaction, sexual selection) and thus, survival, in many vertebrates. In fish, behaviours like territory defence, reproduction and speciation processes involve acoustic functions. However, little is known on how acoustic communication evolved. The physiological, morphological and behavioural adaptations reported in blind cavefish (CF) compared to its river-dwelling conspecific surface fish (SF), makes *Astyanax mexicanus* a good model to investigate the evolution of acoustic communication. We hypothesised that acoustic communication has evolved in CF, to compensate for the lack of visual modality in the dark.

We first recorded and analysed SF and Pachón CF sounds in laboratory controlled conditions. We identified a panel or repertoire of 6 specific simple and complex sounds. Qualitative analyses of acoustic parameters showed that SF and CF share a common acoustic repertoire but, quantitative measures revealed a differential use of this repertoire in the two morphotypes, which also depends on the context. We are now investigating the "meaning" of these sounds in terms of communication in various contexts (i.e.: solo, duo or groups discovering new environments, after habituation period and with *Astyanax*-playback stimulations). Moreover, long acoustic and video recordings performed in Pachón, Subterráneo and Tinaja caves during field expeditions revealed similar sounds produced by CF in their natural environment. Altogether, the results of this work suggest that acoustic communication could play an important role in CF adaptation to survive after drastic environmental changes.

RAPID EVOLUTION OF THE GUT SYMBIONTS AND ASD-LIKE BEHAVIOR IN THE EXTREME CAVE ENVIRONMENT

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The recent human studies indicate an association between autistic symptom and gut microbiome. In general, gut biota is regulated by gut morphology, physiology and ingesting diet. We therefore started investigating the relationship between gut biota, gut morphology and autism spectrum disease (ASD)-like behaviors by using Pachón and Molino cave fish, surface fish and their F1 hybrids. From the one-month-old juvenile stage, the five morphs were fed three diets: nutrient-poor spirulina algae, nutrient-rich brine shrimp and bat guano. Cavefish gut morphology showed significantly less pyloric caeca, longer microvilli, and a wider gut diameter but showed the same gut length comparing with surface fish in the same diet groups. The gut diameters of two cave morphs were widen with the brine shrimp and bat-guano diets comparing with spirulina, whereas surface fish gut morphology stayed as the same, suggesting that the gut plasticity may be evolutionarily acquired in cavefish lineage. Analysis by 16S ribosomal DNA sequencing of the gut biota revealed surface fish and cavefish have significantly different gut biota and F1 hybrids are at intermediate between them. Surprisingly, gut biota is stable in each group regardless of diet, suggesting a major involvement of host genetics. We further looked at short- and medium-chain fatty acids in the intestinal tissue, which are critical nutrients for multiple tissues including nervous system that microbiota supply and the host cannot synthesize by itself. Surface fish gut contains 15.3 times more fatty acids versus cavefish on the same diet, suggesting cavefish may not absorb fatty acid which in turn may lead ASD-like symptom. We are currently further investigating the relationship among the gut plasticity, gut biota, fatty-acid and ASD-like symptom.

POPULATION GENOMICS IN ASTYANAX

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The genetic underpinnings of many cave-associated traits are different among cave populations even within the new and old lineages, and we are examining divergent outlier loci between cave and surface fish within caves among these lineages to infer commonalities about the evolutionary process across multiple origins of the cave phenotype. To address these goals, we generated whole genome resequencing data from three cave populations and two surface populations, including surface and cavefish populations from both the new and old lineages. We also sequenced a closely related congener A. aeneus to serve as outgroups for polarizing changes in the A. mexicanus cavefish. In total, we have 45 resequenced genomes (7.3x-19x per individual aligned to the reference genome), and have thoroughly explored the relationships between populations, revealing extensive gene flow between new and old cavefish lineages, while the surface fish lineages remain distinct. This gene flow shapes how we interpret adaptation to the cave environment, and the interpretation of outlier loci, and will provide fruitful avenues for future research into gene-flow mediated adaptation.

IT TAKES GUTS TO LIVE IN A CAVE: INVESTIGATING THE GENETIC BASIS OF CAVE-SPECIFIC GASTROINTESTINAL (GI) AND METABOLIC ADAPTATIONS

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Animals that thrive in low-nutrient environments evolved to efficiently consume and utilize energy in ways that are not fully understood. Cave forms of *Astyanax mexicanus* survive long periods of starvation, weigh more than surface counterparts on the same diet, and have a higher fat content. We aim to identify quantitative trait loci (QTL) for these characteristics by genotyping and phenotyping 225 individually-housed F2 Surface/Tinaja hybrids fed precisely the same diet. Surface and Tinaja cavefish have quantitative differences in the GI tract; Tinaja cavefish have a significantly longer GI tract and fewer, but larger, digestive appendages called pyloric caeca. These features likely alter metabolism by increasing digestive surface area and thus capacity for nutrient absorption. Interestingly, intestine length in the F2 population correlates with fat content. Also in line with evidence for greater nutrient absorption, Tinaja appear to accumulate food-derived carotenoids in their visceral fat. Carotenoids are yellow vitamin A precursors important for vision and pigmentation. Yellow fat is however independent of eyes or melanin production; Molino cavefish and some eyeless F2 Surface/Tinaja hybrids have white fat, and vice versa some F2 hybrids with eyes have yellow fat, leading us to hypothesize that yellow fat is due to increased capacity for carotenoid absorption. In addition to GI morphology, the relative proportion of intestinal cell types and their proliferation dictates the function and energetic cost of the GI tract. We are using a combination of histology and flow cytometry to quantify cellular-level differences in the GI tract of our F2 population with the goal of uncovering the genetic control of intestinal cell-type maintenance and its role in metabolism. Taken together our results will lead to a better understanding of physiological adaptation in a low nutrient environment.

INSULIN RESISTANCE IN CAVEFISH AS AN ADAPTATION TO A NUTRIENT-POOR ENVIRONMENT

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We see tremendous variation in metabolism among different populations; however, how these traits evolved remain poorly understood. Mutations in glucose regulation can lead to severe pathologies, yet we still see variation in glucose regulation in nature. The cavefish *Astyanax mexicanus* provides us a unique opportunity to investigate how metabolic traits like glucose regulation can evolve among different populations. Here we show that cave-adapted populations of the *A. mexicanus* have insulin resistance in the skeletal muscles. As a consequence, the cavefish exhibit drastically altered blood glucose profiles which coincidentally lead to increased body weight. We identified a coding mutation in the insulin receptor of the Tinaja and Pachon cave populations. The mutation is linked to increased body weight in surface/Tinaja F2 hybrids carrying the mutation. Humans carrying the same mutations have Rabson-Mendenhall syndrome (a severe form of insulin resistance). We point out that we were unable to detect negative health effects in the cavefish commonly associated with diabetes. Not only do the cavefish tolerate dramatic acute changes in blood glucose levels better than the surface fish, but also do not accumulate advanced glycated end-products (AGE)'s associated with chronic hyperglycemia and the progression of diabetes-associated pathologies. We hypothesize that the cavefish have a acquired compensatory mechanisms that allow them to avoid the pathologies with aberrant glucose regulation. This study provides novel insight to the larger question of metabolic evolution and may lead to understanding relevant to human metabolic disease.

QTL ANALYSIS OF SLEEP IN *ASTYANAX* CAVEFISH

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Meaningful QTL analysis of behavioral traits is often difficult because behavior is so sensitive to environmental influence. Thus, the genetic inputs into behavioral phenotypes are typically obscured by considerable noise. One approach to solving this problem is to start with a set of weak QTLs containing many false positives and to use multiple criteria to screen them, with improvement of the quality of the set with each subsequent screen. This approach was tested with a mapping panel of Pachón/Surface BC hybrids which had been phenotyped for sleep as larvae. Putative QTL were screened by (1) polarity, (2) significant SNP population data, and (3) genetic content of the QTL. This approach yielded seven plausible QTL containing three genes involved with β-adrenergic signaling, known from previous work on this system to be a crucial component of the regulation of sleep. Sequencing of these candidates revealed differences between the cave and surface alleles which may affect functionality.

HEART REGENERATION IN ASTYANAX MEXICANUS

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Whereas the human heart cannot regenerate cardiac muscle after myocardial infarction, zebrafish efficiently repair their hearts. Identification of the fundamental mechanisms driving natural heart regeneration in fish could lead to the development of strategies to heal the human heart after injury. However, inter-species differences have confounded direct translation of advances in our understanding of fish heart regeneration into designing therapies for human heart repair. Here, we present a fish model, Astyanax mexicanus, that not only uniquely allows comparison of an adult regenerative and scarring response within one species, but also provides the opportunity to link this response directly to the genome using forward genetic screening. While Astyanax mexicanus surface fish regenerate their heart after injury, their Pachón cave-dwelling counterparts cannot and form a permanent fibrotic scar, similar to the human heart. Myocardial proliferation peaks at similar levels in both surface fish and Pachón one week after injury. However, in Pachón this peak coincides with a strong scarring and immune response and, ultimately, cavefish cardiomyocytes fail to replace the scar. Furthermore, using Quantitative Trait Locus (QTL) analysis, we have linked the degree of heart regeneration to 3 loci in the genome, identifying novel candidate genes fundamental to the difference between scarring and regeneration. Our study provides evidence that, unlike findings in zebrafish, myocardial turnover is not the sole driver of heart regeneration and that successful heart regeneration entails a delicate interplay between myocardial proliferation and scarring.

APPROACHING “OLD FRIENDS” WITH A NEW MODEL: HOW PATHOGEN DIVERSITY SHAPED THE ADAPTATION OF *ASTYANAX MEXICANUS* TO THE CAVE

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Host-parasite interactions are one of the major driving forces in evolution. The loss of parasite diversity in modern societies in the last 200 years, however, strongly correlates with an increase of autoimmune diseases caused by immune-regulatory defects in humans. This indicates, that these interactions between host and parasite not only created a co-evolutionary dynamic, but also represent a necessity for a vertebrate host to develop a functional immune phenotype. So far, this “Old Friends” hypothesis is largely based on descriptive data from humans since there are no suitable model organisms available that adapted to an environment with low parasite diversity. Here, we introduce *Astyanax mexicanus* as a new model organism for host-parasite interactions to specifically shed light on the question of how the loss of parasite diversity influences the evolution of the vertebrate immune system. We found that not only key innate immunological functions like phagocytosis are strongly decreased in cave populations of *A. mexicanus*, but inflammatory responses towards bacterial antigens are highly increased. Furthermore, we found striking differences in the organization of immune cell populations between cave dwelling and surface *A. mexicanus*. Future investigation in this direction will not only provide important insights into the immune system of the emerging model organism *A. mexicanus* in general, but will also help to elucidate the evolutionary trajectory of vertebrate hosts in an environment with low parasite diversity.

BLIND CAVEFISH SHOW SIMILARITY WITH HUMAN PSYCHIATRIC DISEASE IN GENE ARCHITECTURE, BEHAVIORAL PHENOTYPES, AND DRUG RESPONSES

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Psychiatric diseases such as autism spectrum disease (ASD) and schizophrenia (SCZ) are common but difficult to treat due to their multigenic nature. The presence of several psychiatric disease-like behaviors in Mexican cavefish (*Astyanax mexicanus*), many of which are also multigenic, led us to test the potential for cavefish as a new model for psychiatric disease. Of the 55 human genes strongly associated with ASD, our genome survey revealed 51 (92.7%) homologs in cavefish descended from the Pachón cave population. Forty-one of these homologs of ASD-risk genes (80.4%) were significantly up- or down-regulated at 72 hours post-fertilization in cavefish, relative to their surface-dwelling conspecifics. Genetic divergences of ASD-risk gene homologs indicated that many of these genes are under selection, and that most of those under selection have multiple down-stream targets, especially at the intervals of known quantitative trait loci (QTL). This suggests that ASD-risk genes at QTL intervals may act as “master genes” that regulate other ASD-risk genes. In addition to genetic similarities with ASD, treatment of cavefish with antipsychotics mitigated their disease-like behaviors, including sleeplessness, hyperactivity (swimming distance), and adhesion to a stimulus (vibration). Both aripiprazole and clozapine increased sleep duration, decreased swimming distance, and decreased adhesion to a vibration stimulus. Another drug, fluoxetine, increased sleep duration and decreased swimming distance, but did not affect adhesion to vibration. Lastly, risperidone decreased adhesion to vibration, but did not alter other behaviors. In summary, due to their overlap with humans in gene architecture, behavior, and responsiveness to psychiatric drugs, Mexican cavefish may serve as a new multigenic model for ASD. To better understand the commonalities between humans and cavefish, future studies should investigate the relative development and physiology of their nervous systems.

TRANSCRIPTOME ANALYSES AND 3D GEOMETRIC MORPHOMETRICS CHARACTERIZATION OF LACUSTRINE SPECIES PAIRS IN *ASTYANAX* GENUS

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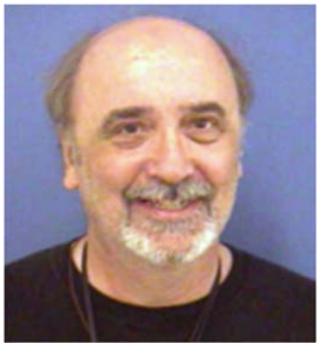
Independent lineages of lacustrine *Astyanax* species have shown convergent morphologies mainly associated with trophic habits differences. Those differences between lacustrine species corresponded to both body and trophic related traits. Despites the morphological differences, which have made taxonomist to identified the different lacustrine forms as different genera (e.g. *Astyanax* and *Bramocharax*), there is not a clear correspondence between genetic structure and morphological divergence. Partial gene flow analyses, have shown a lack of prezygotic barriers among divergent morphotypes. Based on the previously exposed problematic, the main goal of the present stifle was to characterized the morphological variation among lacustrine morphs, using Micro CT- Scans, in order to characterize the major osteological differences among lacustrine forms and evaluate at this scale convergent patterns of morphological evolution, the previous analyses were based on Geometric Morphometrics data of four lineages of Astyanax (Catemaco Lake, Metzabook, Managua Lake and Lake Nicaragua). We also carried out a RNA-seq analysis, in order to identify differences in expression levels of alternative phenotypes in the four lacustrine systems within the *Astyanax* genus, in the Illumina platform. Based on the MANOVA carried out with the geometric morphometric data, we found significant effect of both species and lakes ($P<0.05$). For the RNA-Seq analyses, we identify a correlation index between divergent lacustrine morphs of 0.83%. Among the most differentiated genes, we found 47 related to metabolic aspects. The genes that presented the highest differences in the expression levels were: Craniofacial protein 1, *CD74*, preproinsuline, galactose – 3 – O – Sulfotransferase 1b and acetyl-CoA carboxilase.

ANALYSIS OF THE EYE QTL GENE *CBSA* REVEALS THE ROLE OF CRANIAL VASCULATURE IN REGRESSION OF THE CAVEFISH VISUAL SYSTEM



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Visual system regression in *Astyanax mexicanus* cavefish is controlled by multiple genetic factors and eye QTL. The eye QTL on Pachon cavefish chromosome 21 contains the *cbsa* gene harboring a cis-acting mutation. *cbsa* encodes cystathionine ß synthase (CBS), the limiting enzyme of the transsulfuration pathway, which catalyzes the conversion of homocysteine to cystathionine, a precursor of cysteine and glutathione. Downregulation of *cbsa* during the critical period of eye degeneration significantly increases homocysteine concentration and activates expression of the stress related homocysteine-responsive transcription factor ATF3 in cavefish. CBS is also one of the major enzymes responsible for production of H_2S, a gaseous signaling factor that functions in vascular system development, and H_2S is also decreased in the cavefish embryonic eye. The known role of homocysteine in causing human cardiovascular disease prompted an investigation of the effects of *cbsa* on development and function of the cavefish vascular system. The *vegfaa* gene, encoding a VEGF ligand, the *flt1* gene, encoding a VEGF receptor, and the *Vcam1* gene, encoding a vascular cell adhesion molecule, are coordinately downregulated in the cranial region during cavefish optic degeneration. Angiography using Qdot fluorescent particles showed major leakages in the cavefish eye and brain vasculature frequently leading to hemorrhages. Cavefish hemorrhages are usually reversed within a few days, leaked blood cells are removed, and the afflicted larvae develop into normal adults. Knockdown of the *cbsa* gene in surface fish induced cranial vascular leakage and hemorrhages resembling those of cavefish. The results suggest that downregulation of *cbsa* arrests visual system development in cavefish by inducing defects in the cranial vasculature, possibly imposing anoxia and trophic deprivation on the developing eye and optic tectum.

ROLE OF DNA METHYLATION IN CAVEFISH EYE SPECIFIC GENE REPRESSION

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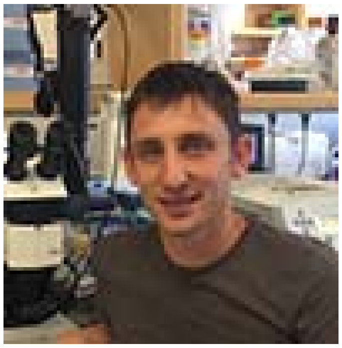
The cave morph of *Astyanax mexicanus* has a number of anatomical and physiological differences from the surface morph including increased number of facial hair cells and lack of eyes and pigmentation. Several critical eye development genes are down-regulated in the cave compared to the surface morph, however, the mechanism down-regulating these genes is largely unknown. Previous studies on eye specification genes did not identify any inactivating mutations in the coding regions. This observation suggested that other mechanisms including epigenetic and cis-regulatory changes may down-regulate gene expression in cavefish eyes. Here, we report a role for DNA methylation in cavefish eye gene repression. We performed RNA sequencing and whole genome bisulfite sequencing (WGBS) from isolated surface and cave morph embryonic eyes. Combined analyses of the RNA and WGBS revealed that more than three hundred genes show both down-regulation and promoter hypermethylation. Further analysis revealed that out of the top thirty-five down-regulated and hypermethylated genes, twenty-two genes are associated with human eye disorders. We also found that QTLs responsible for the retinal thickness in cavefish eyes contain genes from this category. These observations suggest DNA methylation based gene repression may be an important mechanism responsible for down-regulation of multiple eye-specific gene expression, and contribute to the reduced eye phenotype. We also found upregulation of Dnmt3bb.1 transcripts in isolated eyes of both the Pachon and Sabinos cave morphs, suggesting changes in DNA methylation may be a common mechanism for modulating eye-specific gene expression in cave-adapted *Astyanax*.



THE EVOLUTION OF NEURAL CIRCUITRY REGULATING SLEEP AND AROUSAL IN THE BLIND MEXICAN CAVEFISH

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Sleep is an essential behavior exhibited by nearly all animals, and disruption of this process is associated with an array of physiological and behavioral deficits. Independent populations of the blind Mexican cavefish *Astyanax mexicanus* have converged on sleep loss as a consequence of life in the subterranean environment, providing a system to examine the effect of evolutionary history and environmental perturbation on sleep-related processes. Our findings suggest the wake-promoting neuropeptide Hypocretin (Hcrt)/Orexin underlies sleep loss in cavefish. Expression of *hcrt* is increased in cavefish compared to surface fish and pharmacological inhibition of HCRT signaling restores sleep duration in cavefish to levels equivalent to the ancestral surface fish. We are currently investigating the evolutionarily derived changes in HCRT circuitry between cave and surface-dwelling fish. To genetically interrogate the neural basis for sleep loss in cavefish, we have employed tol2 transgenesis to generate *A. mexicanus* expressing the calcium indicator GCaMP6s in peptidergic neurons for subsequent comparisons of calcium imaging during sleep and wake states in whole brain or HCRT neurons. Moreover, we are working to generate a brain atlas to serve as a reference for comparison and understanding the neuroanatomy of the cavefish brain. Together, these resources will facilitate investigation of the processes governing sleep loss, and more broadly, in identifying the neural circuits that underlie the evolution of diverse traits in *Astyanax* cavefish.

EVOLUTION AND DEVELOPMENT OF CRANIAL ASYMMETRY IN *ASTYANAX* CAVEFISH

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Cave-dwelling organisms frequently evolve asymmetric features, including differences in eye size across the left-right axis. In *Astyanax* cavefish, the circumorbital bones demonstrate ossification abnormalities such as 'fragmentations' and 'fusions' of dermal bone elements. The developmental basis for these abnormalities has long remained mysterious. To better understand how early ossification may impact on the adult cranial phenotype, we employed an intra-individual live-staining approach coupled with comprehensive longitudinal fluorescence imaging. We discovered that cave-dwelling forms develop secondary ossification centers that periodically fuse with – or remain distinct from - the larger growing bony element. In the adult, these distinct fragments resemble the same ultrastructure as a normal bone-to-bone boundary. Since the positions of sensory structures can be influenced by bony margins, we next evaluated whether superficial neuromasts may "mirror" the patterns observed in an asymmetric circumorbital bones. We quantified the position of neuromasts and used a novel method to determine the degree of similarity in their patterns, across the left-right axis. We discovered that cave-dwelling forms show significantly less symmetric neuromast distributions, reflecting their robust asymmetry in cranial form. Taken together, this work illuminates the developmental basis for cranial bone asymmetry and provides the opportunity to explore if fragmentation evolves neutrally, or whether it may be linked to the adaptive process of expanding non-visual sensation in complete darkness.

RECUPERATION OF VISION IN A BLIND CAVEFISH POPULATION.

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The entrance of Caballo Moro Cave (CM) is a karst window: A habitat within cave systems that are exposed to light resulting from cave ceiling collapse. The cave's entrance pit is 4 km away from the nearest surface fish locality. The entrance pit leads to a 90m stream pool. Light only reaches half of the pool, while the other half remains in darkness. The distribution of fish is biased, with blind fish in the dark area and eyed fish in the illuminated area. What is the origin of the eyed Caballo Moro cave fish? Are they simply surface fish that swam across kilometers of underground passages or are they direct descendants of cave fish that have been selected to re-acquire eyes?

Whole genome DNA-sequencing of 8 eyeless CM, 7 eyed CM and 6 surface fish, showed that the eyed CM fish are more similar to eyeless CM fish (Fst=0.137) than to surface fish (Fst=0.179). Results also showed that eyed CM retain portions of surface genome which are absent in the eyeless CM genome. Abundance of contiguous surface SNP's on contigs were tabulated. Very few long surface genome fragments of 10 contiguous SNP's were found (0.1%) while on the contrary small fragments of single isolated surface SNP's abound (35.0%). This suggests an old evolutionary time of isolation from surface fish and selection for specific surface portions of the genome maintained in the standing genetic variation of Caballo Moro fish.

In line with the genomic data, we found that eyed CM share a host of characters with eyeless cavefish that include: large eggs, small liver lobules, reduced pigmentation of the peritoneum, large fat deposits, suborbital bones with the signature of belonging to a cavefish that has recuperated the eyes, and VAB.

Contrary to surface fish, eyed CM attack less other epigeomorphic fish (39.6% +/-36.0 stdev) and instead prefer to attack troglomorphic fish, potentially generating a reproductive behavioral barrier, promoting the possibility of long term sympatric speciation in these populations.

A YEAR IN THE LIFE OF TWO POPULATIONS OF *ASTYANAX MEXICANUS*.

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Has evolution actually been observed as it happens by anyone? The answer is yes. One famous example is the Darwin finches. The Grants spent twenty years in the Galapagos to show that after a severe drought, the struggle to survive favored larger birds with strong beaks for opening hard seeds.

However, has anyone seen evolution occur in nature, in a charismatic vertebrate, in the cave environment? While most DNA and EvoDevo studies support evolution, these studies have analyzed *ex post facto* events of evolution. Their data is typically based on characters that resulted from evolution, after it has taken place. The purpose of this research was to follow a population of cavefish where change in heritable traits are tracked over real physical time.

Río Subterráneo Cave contains a wide array of intermediate, hybrid phenotypes living alongside surface fish. This is due to seasonal migration of surface fish into the cave after flooding and subsequent introgression in the existing cave population. The population was tracked throughout an entire year, with field trips every two months. Condition factor, as an estimate of fish health, and environmental conditions such as carbon content of the debris were tracked. As the dry season progressed, food is limiting and characters that give an advantage for survival were selected for. At the end of a year-long study, it is expected that we will be able to document selection in situ through demographic analyses of the variations in phenotypes (troglomorphic or non troglomorphic) along the year and according to seasonal environmental variations in a population of *Astyanax*.

A similar year-long study from Pachón cave showed that fry are efficient predators of aquatic micro-crustaceans. Food regimes change between post-larval and adult stages to become more dependent on partially decomposed material and guano. Surprisingly, the environment appears to be less “food poor” for juvenile cavefish during the dry season than in the rainy season.

PHYLOGEOGRAPHICAL CONVERGENCE BETWEEN TWO TROGLOBITIC ORGANISMS IN THE SIERRA DE EL ABRA

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Many authors assume that the multiple populations of *Astyanax mexicanus* from Sierra de El Abra and Sierra de Guatemala derived from several independent colonizations, despite both Sierras being a continuous karstic area. They have inherently assumed there are effective barriers that prevented troglobite migration between caves, generating separate biogeographical areas.

Previous studies using the 16S rRNA and its calibrated molecular clock have shown that *Anelpistina quinterensis*, a troglomorphic silverfish insect, was able to migrate underground to reach both mountain ranges within the last 12,000 years. Thus, the Servilleta canyon that separates both Sierras has not been an effective biological barrier that prevented underground migration of troglobites. In the current study we have sequenced the DNA of an aquatic troglobite, the mysid shrimp *Speleomysis quinterensis*, whose biogeography overlaps with that of *Astyanax*. Specimens were collected from Sierra de Guatemala (Caballo Moro cave) and from Sierra de El Abra (Pachon, Tinaja, and "Chiquitita" caves). Chiquitita cave is a new and previously unpublished locality with *Astyanax*. This new locality becomes the southernmost locality of the El Abra area with *Astyanax mexicanus*, and the 32nd cave with troglobitic *Astyanax*.

Histone DNA sequences from Pachon and Chiquitita troglobitic mysid shrimps are identical, supporting that aquatic troglobites dispersed between the northernmost and the southernmost of Sierra de El Abra in recent geological times. But there is also evidence that Tinaja mysid shrimps derived from an independent phyletic line, implying two separate evolutionary histories. The phylogeography of the mysids is in agreement and overlaps with the mitochondrial phylogeography of *Astyanax* cavefish. This convergence supports that caves in the central area of Sierra de El Abra, where Tinaja cave is located, had effective barriers that modulated evolutionary histories across multiple aquatic troglobitic species.

ASTROBLEPUS PHOLETER: CONVERGENT REGRESSIVE EVOLUTION OF THE MC1R GENE IN CAVEFISH SPECIES

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Jumandi cave is home to Ecuador's most charismatic troglobite, the catfish *Astroblepus pholeter*. It was defined as "essentially unpigmented" and published photos show yellow-white specimens. However, specimens we collected in 2016 do show pigmentation throughout the body, unlike the previously published photos. It is unknown if previous authors took photos with high contrast to create an enhanced image, collected a particularly unpigmented specimen, or if there has been introgression from surface fish.

Since *A. pholeter* is not albino, we sequenced the MC1R gene. In mammals, MC1R variants result in red hair with light skin color due to low concentrations of eumelanin. Two mutations were found in ultra-conserved regions. Interestingly, the first locus has been discussed in the literature regarding pigmentation in felids, suggesting a functional consequence for the *A. pholeter* mutation. MC1R has also been the target of mutations in the regressive evolution of *Astyanax* cavefish. We propose convergent regressive evolution of the MC1R gene across cavefish species.

The species was also portrayed with an unusual mechanosensory cave adaptation. The authors reported an increase in number of mechanosensory skin denticles along the dorsal surface of the head and trunk that could detect the direction of water flow and the proximity to the substrate by changes in fluid velocity. However, comparisons to the local surface loricarioid catfish show that *A. pholeter* actually has fewer denticles throughout its head and body than the local surface species.

Behaviorally, *A. pholeter* can detect light and presents nyctophilia, a preference for darkness. Histology of the eye showed that despite reduction in overall size and the eye being embedded under the skin, *A. pholeter* has conserved all of the major components of the eye; lens, optic nerve, and all retinal layers. While dermal layers may blur images, the eyes are most likely functional.

THE MEXICAN BLINDCAT PROJECT: NEW DISCOVERIES AND FUTURE EFFORTS

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The endangered Mexican blindcat (*Prietella phreatophila*, Carranza 1954) is one of only four stygobitic ictalurid catfish in North America. Members of two monotypic genera (*Satan eurystomus* and *Trogloglanis pattersoni*) are known from the Edwards Aquifer in Texas and, until recently, *Prietella* (represented by *P. lundbergi* and *P. phreatophila*) was only known to occur in Mexico (northern Coahuila to southern Tamaulipas). The recent discovery of *P. phreatophila* in a cave on the Amistad National Recreation Area in Val Verde County, Texas is the result of decades of sporadic effort on both sides of the US/Mexican border and has stimulated a renewed effort to investigate the distribution, ecology, evolutionary history, and conservation status of this species. Collaborative efforts among The San Antonio Zoo, The University of Texas at Austin, Zara Environmental and The National Park Service are currently focused on habitat surveys in Texas as well as captive husbandry and propagation. Future efforts will include collaborators from the Comisión Nacional de Áreas Naturales Protegidas, Área de Protección de Recursos Naturales Sabinas, and the Laboratorio de Genética para la Conservación, Centro de Investigaciones Biológicas del Noroeste, La Paz to conduct expanded fieldwork in Mexico, hydrogeologic studies, and surveys using environmental DNA.

BEAUTY UNDERGROUND: CAVEFISHES IN CHINA

Yahui Zhao[1], Danté Fenolio[2], Daphne Soares[3], Andrew Gluesenkamp[2], Matthew L. Niemiller[4], Prosanta Chakrabarty[5]

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Cavefishes are a distinct group of vertebrates restricted to subterranean environments for at least part of their lives. Based on their troglomorphy, which includes physiological, behavioral, and morphological adaptations to subterranean habitats, such as the reduction or loss of eyes and pigmentation, cavefishes can be divided to two types: stygobionts (troglomorphic) and stygophiles (non-troglomorphic). China is home to the greatest diversity of cavefishes in the world, with more than 60 stygobiotic species. The Chinese Cavefish Working Group (CCWG) is conducting fieldwork, and new endemic species are being discovered and described. With the exception of *Onychostoma macrolepis* in northern China, all species are found in southwestern China, mostly in the karst of the Yunnan-Guizhou Plateau. *Sinocyclocheilus*, the largest cavefish genus, has experienced rapid evolution and diversification, with some species possessing horns and humpbacks (i.e., horn-like structures and hyper-development of a dorsal protuberance similar to a humpback), probably resulting from parallel evolution. Sympatric distributions among Chinese cavefishes is common, and sometimes more than two species are found in the same cave or subterranean river. Caves and karst are very fragile environments, and cavefishes are extremely sensitive to environmental perturbations. Threats from rapid economic growth have increased the need for conservation efforts for cave-dwelling communities in recent decades. Conservation of Chinese cavefishes is an urgent issue. The CCWG is a collaborative team composed by researchers from China and the United States, with a primary goal to document the distribution and diversity of Chinese cavefishes and gather vital data on their status and threats to wild populations. This information can be used to make sound conservation and management decisions. Research efforts are not limited to surveys and collection but also include taxonomy, phylogenetics, biomechanics, ecology, population biology, and conservation science.

PARALLEL EVOLUTION IN NEOTROPICAL CRATER LAKE CICHLIDS

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Evolution sometimes repeats itself producing the same phenotypic adaptations in independent lineages exposed to similar environmental conditions. These striking examples of parallel evolution emphasize the central role of natural selection in speciation, but also support the notion of a deterministic, predictability to evolution. A classic textbook example of parallel evolution are cichlid fishes of the East African Great Lakes Tanganyika and Malawi, where remarkably similar trophic morphologies and coloration types have evolved from different founder lineages within a few million years. Here we report a striking case of parallel speciation in small volcanic crater lakes in Nicaragua, Central America, over a period of no more than just a few thousand years. Several ecologically and morphologically equivalent species of Midas cichlids (*Amphilophus* spp.) have evolved independently in several Nicaraguan crater lakes. Moreover, the speciation events follow the exact same succession in independent radiations, adaptations to alternative lake regions occurring first and specializations to alternative trophic resources within habitats happening only later. Natural selection through ecological specialization to newly available niches is the likely for driving sequential parallel ecological speciation in crater lake cichlid fishes.

Abstracts for Poster Presentation

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P2 **Andrew Box:** IMMUNOLOGY WITHOUT ANTIBODIES: STUDYING FISH HEMATOPOIETIC TISSUE USING INTRINSIC CELL SIGNALS, IMAGE CYTOMETRY AND ADVANCED ANALYSIS METHODS

P3 **Nicolas Cetraro:** HOUSING PROTOCOL AND EXPRESSION OF SELECTED AUTISM GENES IN ASTYANAX MEXICANUS.

P4 **Emily Collins:** DIVERGENT EVOLUTIONARY PATHWAYS FOR AGGRESSION AND TERRITORIALITY IN *ASTYANAX* CAVE FISH

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P6 **Shane Kaplan:** UTILIZING LIVE-FLUORESCENT STAINING TO VISUALIZE BONE GROWTH THROUGHOUT DEVELOPMENT IN *ASTYANAX MEXICANUS*
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P8 **Li Ma:** PARALOGOUS CYSTATHIONINE ß SYNTHASE GENES IN ASTYANAX MEXICANUS: FUNCTIONAL DIVERSIFICATION AND EVOLUTION OF CAVEFISH EYE DEGENERATION

P9 **Patricia Ornelas García:** EVOLUTIONARY HISTORY OF VISUAL PIGMENTS IN ASTYANAX GENUS

P10 **Courtney Passow:** ORGAN-SPECIFIC PATTERNS OF GENE EXPRESSION AMONGEXTREMOPHILE FISH POPULATIONS EXPOSED TO TOXIC HYDROGEN SULFIDE AND PERMANENT DARKNESS

P11 **Constance Pierre:** FUNCTIONAL AND BEHAVIORAL CONSEQUENCES OF THE MUTATION IN MAO IN THE BLIND CAVEFISH *ASTYANAX MEXICANUS*

P12 **Misty Riddle:** WHAT'S IN YOUR WATER? PATHOGEN LANDSCAPE ANALYSIS OF LAB-RAISED *ASTYANAX MEXICANU*S; PRACTICAL GUIDELINES TO INCREASE BIOSECURITY

P13 **Noah Simon:** EXTRA-OCULAR *OPSIN* EXPRESSION OF *ASTYANAX MEXICANUS* CAVE AND SURFACE MORPHS

P14 **Víctor Manuel Sosa Jiménez:** LITTLE CREATURES REVEAL GREAT STORIES: GUT MICROBIOME OF THE CAVEFISH ASTYANAX MEXICANUS (DE FILIPPI, 1853)

P15 **Jenny Sung:** USING CRISPR / CAS9 TO FUNCTIONALLY VALIDATE CAVEFISH CANDIDATE GENES IN ZEBRAFISH

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P1

NEUROGENESIS IN THE CAVEFISH OLFACTORY EPITHELIUM

Maryline Blin, Eugène Tine, Sylvie Rétaux

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The idea of a sensory compensation for absence of vision in animals living in the dark was proposed by several authors. It has long been hypothesized that the olfactory system might have evolved to provide a mechanism for food- or sexual partner-searching in troglomorphic *Astyanax* populations.

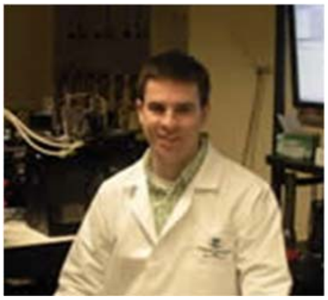
Recently, we showed on our laboratory Pachón population that the olfactory placode and the olfactory epithelium are continuously enlarged in cavefish at the expense of a reduction of the lens placode and mass, when compared to surface fish. Furthermore, using behavior tests we showed that cavefish are able to respond to 10^{-5}-fold lower concentrations of amino acids than surface fish (Hinaux et al., Development (2016) 143, 4521-4532).

We therefore compared the olfactory sensory system in *Astyanax* cavefish (Pachón) and surface fish from a neuroanatomical point of view. As in other teleost fish species, the *Astyanax* olfactory system consists of a sensory part in the nostril, the olfactory epithelium (OE), which contains the olfactory sensory neurons. These neurons express olfactory receptors and are activated upon binding of odorant molecules. They project axons to the olfactory bulb, the forebrain region that processes olfactory information.
As it is clear from embryo to adult stages that the OE is larger in cavefish than in surface fish we investigated cellular biology aspects of this sensory organ: we determined the amount of proliferation at several developmental and larval stages. We calculated the neuronal renewal rates, quantified the neuronal apoptosis and estimated the lifespan of olfactory neurons. We analyzed neuroanatomy using specific antibodies.
The main differences we observed between the two morphs are (i) more neurons are produced in the cavefish OE than in the surface fish OE and (ii) a part of these supernumerary neurons are microvillus-type neurons, specialized in food research.
The potential outcomes of such an increased neurogenesis and modified neuronal composition in the cavefish OE will be discussed.

Work supported by FRM grant [Neural bases of sensory compensation and behavioral adaptation in a blind fish]

P2

IMMUNOLOGY WITHOUT ANTIBODIES: STUDYING FISH HEMATOPOIETIC TISSUE USING INTRINSIC CELL SIGNALS, IMAGE CYTOMETRY AND ADVANCED ANALYSIS METHODS



Andrew C. Box· *Robert Peuß, Jeffrey Haug and Nicolas Rohner*

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The of study fish immunology by flow and image cytometry is often plagued by a lack of commercially available antibodies or other labeling reagents. Consequently, such work benefits from leveraging intrinsic information from brightfield, light scatter and non-species-specific dyes such as DNA dyes for identifying and enumerating cells types and cell states. However, traditional approached to analysis employing manual gating to identify and score populations from multiparameter data is necessarily error prone and biased by user input, thus confounding efforts to study heterogeneous cell populations such as those present in most primary blood or tissue samples in an unbiased manner.

Image cytometry on intrinsic cell features and DNA labeling with t-SNE dimensionality reduction performed in R or FCS Express, or other unsupervised clustering methods, allows identification and study of populations based on multiparameter intrinsic cell features by reducing information from multiparameter data to a number of dimensions more easily interpreted by the analyst, thus aiding in the study of emerging model systems.

Here we present data illustrating that identification of several discrete cell populations in the cavefish *Astyanax mexicanus* hematopoietic tissue can be accomplished using image cytometry and tSNE transformation and that such an approach can be employed to assist the researcher in understanding how experimental manipulations affect multiple cell types within a sample set despite a lack of commercially available antibodies and other reagents.

P3

HOUSING PROTOCOL AND EXPRESSION OF SELECTED AUTISM GENES IN *ASTYANAX MEXICANUS*.

Nicolas Cetraro, Noah Simon, Masato Yoshizawa

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Astyanax mexicanus is a model fish with sighted surface-dwelling (surface fish) and blind cave-dwelling (cavefish) forms. Since *A. Mexicanus* stated to be recognized as a biomedical model too, we are urged to develop a standard protocol for its housing. Here we present a simple way to build the system and, based on it, preliminary data for autism gene expressions. The key features required for the system are: (1) water-flow, connecting multiple tanks into one, allowing pheromones from females to stimulate other fish to increase the ratio of spawning, (2) tight temperature control by a heater and a chiller, due to the fish are sensitive enough of the changes as low as a 1˚C shift, (3) filtered water from a reverse-osmosis system treated with Seachem Neutral Regulator and Reef Crystal Sea Salt to condition water at pH~7 and conductivity at 700 µS, and (4) rough surface at the bottom of an aquarium to initiate spawning, perhaps due to a females preference to avoid embryos being eaten by conspecific or others. We are currently successful to make them spawn 15 per our 20 tanks setup every other week. Based on this system, we performed qPCR in the risk genes of autism spectrum disease (ASD). Cavefish is a proposed to look into multigenic features of ASD-like behaviors. There are a number of ASD gene homologs that changed expression level at 72hpf, and under the intervals of quantitative trait loci (QTL), including *shank3a, shank3b, grin2ab, adnp, pogza, suv420h1, kmt5b, scn2a,* and *ptena.* qPCR performed on brain RNAs from 1month, 2 month, 4 month and 1 year old showed general expression trend: lower expression in the earlier stage but no difference in the young adult between cave and surface fish, suggesting these genes may alter the proper developmental process in the nervous system as suggested in human. We will soon investigate further details of gene expressions by RNAseq method.

P4

DIVERGENT EVOLUTIONARY PATHWAYS FOR AGGRESSION AND TERRITORIALITY IN *ASTYANAX* CAVE FISH

Emily Collins[1]*, Jennifer Rutkowski*[2]*, Patricia Ornelas García*[3]*,*
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Sierra de El Abra cave populations of *Astyanax* lack aggressive behavior, territorial behavior, and have increased food searching behavior compared to surface populations. This docile nature of El Abra cave populations compared to their aggressive surface counterparts is part of a "cavefish behavioral syndrome" and is thought to be evolutionarily advantageous in low-nutrient cave environments. However, we report here that *Astyanax* cave fish from the Sierra de Guatemala region have aggression levels and territoriality comparable to surface fish, yet retain enhanced feeding behavior.

In our behavioral studies, El Abra cave fish (4 Pachon cave specimens) only exhibited an average of 0.83 (+/- 0.98 SD; N=6) aggressive attacking behaviors during a 5 min period, while Molino and Caballo Moro cave populations, from Sierra de Guatemala, and surface fish exhibited respectively 22.1 (+/- 7.9 SD; N=6), 26.5 (+/- 14.4 SD; N=6) and 52.1 (+/-34.8 SD; N=6) attacks. Sierra de El Abra cavefish did not form territoriality, which was evident in both Sierra de Guatemala cave fish and in surface fish. Feeding behavior studies showed that contrary to the above, Sierra de Guatemala were more similar to El Abra cavefish: Surface fish were satiated after being fed 1.8% (+/- 0.6%; N=13) of their body weight, Pachon cave fish after 3.6% (+/- 0.4%; N=8), and Sierra de Guatemala cave fish after 6.5% (±1.6%; N=10).

Sequencing data revealed a P106L mutation in the MAO gene in El Abra cave fish that is not present in surface fish or Sierra de Guatemala cavefish. MAO is a neurotransmitter degrading enzyme, thus it may enhance food searching behavior while reducing aggression and territoriality. Tests with deprenyl, a MAO inhibitor, reduced aggression in Molino (58.4% ± 70.9) and Caballo Moro specimens (74.8% ± 21.0) as well as in their territoriality and food satiation. This evidence suggests divergent evolutionary pathways of the cavefish behavioral syndrome.

P5

PARALLEL EVOLUTION OF BODY SHAPE IN *ASTYANAX* (CHARACIDAE) MORPHOTYPES

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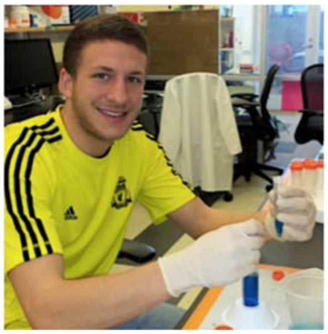
Divergent selection is usually associated with contrasting morphologies in the speciation process. Here we investigate the patterns of body shape variation among sympatric *Astyanax* (Characidae) morphotypes from three lake systems (Lake Catemaco, Mexico and Lakes Managua and Nicaragua, Nicaragua). Previous phylogenetic studies have shown that each lake correspond to an independent lineage in the *Astyanax* evolutionary history, being the most southern populations the oldest ones (Nicaraguan Lakes), while the Lake Catemaco population correspond to the most recent lineage. Using geometric morphometric analysis, we developed a MANCOVA model to evaluate the effect in body shape of: adaptive divergence (morphotypes), size (centroid), habitat (lake system factor), variation of size between morphotypes (interaction morphotype*centroid) and variation of body shape of morphotypes between lakes (interaction morphotype*lake). Lake system was the most important factor explaining the morphological variation (showing a strong effect of habitat on body shape) followed by morphotype. Both morphotypes from Catemaco Lake showed the body mass concentrated at the anterior part compared to body mass equally distributed on body in Nicaraguan specimens. Related to adaptive divergence by trophic morphology, *Bramocharax* morphotype from the three lakes showed a component of parallel evolution of body shape (longer head-snout and a slender body), which is consistent with the theoretical expectation for fish adapted to high sustained swimming speeds and related to an openwater environment. Also as predicted, lineage age was correlated with morphological divergence: the older Nicaraguan morphotypes (about 6.9 m.y. old) showed a higher morphological divergence. Additionally, we performed a phylogenetic reconstruction including specimens of *Bramocharax* morphotype from Managua Lake and Costa Rican Rivers, in order to clarify its relationships with other *Bramocharax* morphotype populations.



P6

UTILIZING LIVE-FLUORESCENT STAINING TO VISUALIZE BONE GROWTH THROUGHOUT DEVELOPMENT IN *ASTYANAX MEXICANUS*

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Astyanax mexicanus cavefish have evolved several extreme morphologies as a consequence of life in total darkness. In the skull, adult cavefish exhibit cranial aberrations that impact the circumorbital bones encircling the collapsed eye orbit. These include dermal bone fragmentation, which is marked by the incomplete fusion of multiple smaller bony pieces. In contrast, only single, intact bones are present in the extant surface-dwelling fish. Yamamoto *et. al.* (2003) demonstrated that juveniles of both morphotypes commence bone formation from a single condensation of mesenchymal cells, i.e., the primary ossification center. In cavefish, however, they observed multiple secondary ossification centers based on analyses of fixative-preserved specimens. We hypothesized that these secondary "ectopic" ossification centers may persist into adulthood as mature "fragments". To evaluate this notion, we performed an intra-individual live-staining procedure involving the bone-specific fluorescent stain, calcein. This enabled us to document dynamic features of bone growth, reabsorption, and remodeling over the first year (52 weeks) of cave and surface fish life. We quantified differences in the size (area growth over time), shape, and presence/absence of ossification centers within individuals to compare developmental patterns between the morphotypes. We confirmed that cavefish develop spontaneous secondary ossification centers, some of which persist as fragments, while others resorb back into the larger SO3 element. Surface fish SO3 bones developed uniformly from the primary ossification center in the anterior-posterior direction, while cavefish bones developed from a number of ossification centers, leading to highly irregular shapes across development. This work underscores developmental differences in bone development between morphotypes, and provides the opportunity to understand the molecular, genetic and developmental basis for these unusual cranial phenotypes.

P7
REGULATORY EVOLUTION IN CAVEFISH METABOLISM

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The two morphs of *Astyanax mexicanus* – the surface and the cave forms – have adapted to contrasting habitats during evolution. Cave environments offer limited or seasonally restricted food supply and consequently, the cave-dwelling populations had to drastically adjust their metabolism to withstand long starvation periods. To that end, they have adapted themselves to store large amounts of fat and tolerate diabetes-like symptoms albeit without any negative effects such as fatty liver and glucose intolerance. Much of these adaptive traits gained during evolution can be attributed to sequence-level changes in the genome. These changes can be the result of both coding and non-coding changes. While coding mutations can bring about changes directly to the structure and/or function of the protein, non-coding mutations can result in changes in expression level, spatial and/or temporal patterns of gene expression leading to varied phenotypes without pleiotropic effects. In this study, we focus to unravel the non-coding mutations that li ma
y be helping cavefish survive adverse living conditions and remain healthy. We have performed ATAC-seq and ChIP-seq for histone modifications and p300 on livers of Surface, Pachón and Tinaja to narrow down to differential regulatory regions like enhancers and promoters. Studying these differentially active regulatory regions will help us understand how metabolic adaptation has evolved at the gene regulatory level in cavefish.

P8

" PARALOGOUS CYSTATHIONINE BETA SYNTHASE GENES IN ASTYANAX MEXICANUS: FUNCTIONAL DIVERSIFICATION AND EVOLUTION OF CAVEFISH EYE DEGENERATION

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The cystathionine ß synthase a (cbsa) gene is located in an eye QTL and may be one of the multiple genes responsible for cavefish eye loss. The coding region of the surface fish and cavefish cbsa genes differ by several nucleotide substitutions and a short indel that does not change the reading frame, but these changes are unlikely to affect CBSA protein function. Evidence for a cis-acting mutation was obtained by F1 hybrid expression analysis. ATAC sequencing and open chromatin analysis suggested that the best candidate for a cis-acting mutation is a sequence change in a large cbsa intron. The Astyanax genome also contains the paralogous cbsb gene, which is not downregulated in cavefish or located in a known eye QTL. The cbsa and cbsb genes have overlapping but distinct embryonic expression patterns. The cbsb gene was expressed in most tissues of surface fish and cavefish embryos, and the expression levels were higher in early cavefish compared to surface fish embryos. In contrast, the cbsa gene showed enhanced levels of expression in the surface fish retina, lens, and brain, but was strongly downregulated in these regions in cavefish embryos. Later in cavefish larval development, cbsa was upregulated in the pancreas and liver. The cbsa and cbsb genes were also found to have overlapping but distinct morphant phenotypes: cbsa knockdown induced a smaller eye and loss of the ventral retina, a condition resembling the cavefish eye phenotype, whereas cbsb knockdown resulted in absence or extreme reduction of the entire eye. Comparison of sequenced teleost genomes indicated that most species exhibit a single cbs gene, which duplicated into cbsa and cbsb before the divergence of Astyanax and Danio. Therefore, gene duplication and functional diversification in the ostariophysian lineage may have allowed cbsa to contribute to eye loss without consequences on overall fitness during the evolution of cavefish eye degeneration.

P9

EVOLUTIONARY HISTORY OF VISUAL PIGMENTS IN ASTYANAX GENUS

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Astyanax mexicanus correspond to a unique model system, due to other aspects, because it gives the opportunity to study regressive evolution. In this respect one of the most conspicuous characteristics of troglobitic Astyanax correspond to vision loss, and together with this regression other constructive characteristic have arise. In order to understand the mechanisms that underlies complex structures evolution under extreme environmental conditions, we characterized the evolutionary history of the visual pigment rhodopsin, a relatively conserved photoreceptor, for 16 Astyanax cavefish populations. Our results suggest the SPNs for the new lineage populations (Molino, Caballo Moro and Subterraneo), while the Escondido population showed an 300 bp indel. El Abra region presented several common SNPs among its populations. In comparison with surface populations, several cavefish shown truncated rhodopsin sequences, being the most extreme example the Sótano Escondido with a large indel in the protein. Further analyses are needed in order to understand the role of other visual pigments in the Astyanax model system, and in particular in its role in eye degeneration.

P10

ORGAN-SPECIFIC PATTERNS OF GENE EXPRESSION AMONG EXTREMOPHILE FISH POPULATIONS EXPOSED TO TOXIC HYDROGEN SULFIDE AND PERMANENT DARKNESS

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Elucidating the physiological mechanisms of adaptation to different environmental condition remains a major task, especially in non-model organisms. High-throughput sequencing technologies have transformed the quantification of genome-wide gene expression patterns in natural systems through the use of RNA-seq. Here, we performed an RNA-seq study on three different organs of fish adapted to H_2S rich environments and caves to quantify patterns of gene expression. We specifically asked: (1) How do gene expression and functional responses vary among populations and organs? (2) Is there evidence of shared expression responses in populations exposed to the same environments, and how do shared responses vary among levels of organization? (3) What inferences can analyses of differential expression provide about potential molecular mechanisms underlying adaptation? To address this, we assembled and annotated transcriptomes of *Poecilia mexicana* based on transcripts from 16 wild-caught individuals with four individuals and three tissue types per ecotype. We mapped all reads to the *Poecilia mexicana* genome and identified variation in gene expression patterns between ecotypes and tissues. We uncovered rampant variation in gene expression. Organ type was the major driver of expression among samples illuminating the importance of choosing focal organs in the study of gene expression in nature. We also identified variation in the amount of shared differentially expressed genes, where shared responses increased with level of biological organization. Nonetheless, shared responses were more common in the sulfidic habitats, highlighting the effects of H_2S on gene regulation. Overall, our analyses provided insights into transcriptional variation in a unique system with coinciding sources of selection, and functional annotation of differentially expressed genes provide a springboard for investigating physiological modifications associated with adaptation to extreme environments.

P11

FUNCTIONAL AND BEHAVIORAL CONSEQUENCES OF THE MUTATION IN MAO IN THE BLIND CAVEFISH *ASTYANAX MEXICANUS*

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Cavefish (CF) present complex behavioral changes: increased locomotor activity, loss of aggressive behavior, schooling and sleep. This corresponds to the "cavefish behavioral syndrome". We aim at understanding the genetic and neural bases of the behavioral adaptation of *A.mexicanus* to the cave environment.

Our group has previously described a point mutation in the Pachón CF gene encoding Monoamine Oxidase(MAO), the serotonin degrading enzyme. This mutation causes a two-fold reduction of the MAO activity, and increased brain serotonin level. As accumulated evidences suggest that changes in neuro-modulatory systems generate significant variations in complex behaviors, we address the following question: what are the consequences of this MAO mutation at the level of the serotonergic system and the behavior in CF?

We therefore compared the behaviour and brain serotonin levels of:

1. Normal SF
2. Normal CF
3. CF without the mutation obtained by crosses (the mutation is not fixed in Pachón)

Monoamine levels were measured in the brain of 5 month-old fish by HPLC. The level of serotonin in the brain of the CF without the mutation was the same as in SF brain. Thus, the difference in the levels of serotonin between CF and SF is only due to the MAO mutation.

We analyzed fish locomotion. In 5 month-old fish, locomotor activity of CF without the mutation is lower than in the mutated CF but still higher than in the SF, indicating that the difference in locomotion between CF and SF is partially due to MAO mutation. However, at 1month of age, locomotor activity is the same in CF with or without the mutation. This suggests that chronic inactivation of MAO leads to progressive changes during brain development.

The MAO mutation is found in 4 different independently-evolved cave populations. To generate a phylogeographic map of MAO mutated alleles, we genotyped 133CF and 178SF from various caves and rivers. The results suggest that the mutation is probably selected in the cave environment.

P12

WHAT'S IN YOUR WATER? PATHOGEN LANDSCAPE ANALYSIS OF LAB-RAISED *ASTYANAX MEXICANU*S; PRACTICAL GUIDELINES TO INCREASE BIOSECURITY

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We know surprisingly little about the pathogens in lab-raised *Astyanax mexicanus* considering their influence on fish behavior, fertility, and physiology. We performed a comprehensive disease screen on fish from two independent recirculating systems containing a mixture of Surface, Pachon, Tinaja, and Molino populations. Microscopic examination of detritus and water revealed midge fly larvae, monogenean flukes, polychaetes, nematodes, and ciliates. While these are not all parasitic, they could influence water quality and/or be secondary hosts for fish pathogens. We treated one recirculating system with Praziquantel (¼ tsp per 25 gallons) and cleaned each component with 0.5% bleach. One week later, sixty-five adult fish were randomly sampled from each system. Skin scrapes and gill clips revealed two species of flukes (*Dactylogyrus sp. And Gyrodactylus sp.*) at low numbers in most fish from the treated and untreated system. Virus isolation using pooled fish tissues revealed no cytopathic viruses and similarly no bacteria of interest were isolated by culture. However, we identified six *Mycobacterium* species by PCR screening. Mycobacteria are common in fish facilities and nearly impossible to eradicate as they can be vertically transmitted. Consistent with the molecular identification of *Mycobacterium*, histopathology revealed granuloma formation in the visceral organs of some fish, a common feature of infection. Mycobacteria cause disease in humans; contact with water or fish without gloves should be avoided particularly if you have any breaks in the skin. To manage *Mycobacterium* infection, regularly disinfect surfaces using 70% ethanol, euthanize older fish, and avoid transferring water or fish between tanks and systems. We found that routine detritus removal was the most effective way to decrease fluke, nematode, and midge fly larvae abundance. Knowing and managing what's in your water will give you confidence in maintaining the health of your colony and producing reliable data.

P13

EXTRA-OCULAR *OPSIN* EXPRESSION OF *ASTYANAX MEXICANUS* CAVE AND SURFACE MORPHS

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Opsins are proteins classically known for forming the key light-sensing molecules responsible for the function of binocular and pineal eyes. Recent studies, however, showed *opsin* expression in deep-brain and skin of fish, suggesting light-sensing function in these tissues. Interestingly, changes in light conditions induced activity shifts in the blind *Astyanax* cavefish--calmer in the dark and more active in the light--, suggesting extraocular opsin activity. In zebrafish 42 opsin genes in 20 classes (*rh1*, *rh2*, *lws*, *exorh*, etc.) have been identified and are expressed in many tissues including: brain, eye, gut, heart, liver, muscle, pineal, skin and testis. Functional roles for some extraocular photoreceptors have been demonstrated in zebrafish deep-brain (phototaxis in darkened environment) and tilapia skin (pigmentation shift in response to light); the pineal gland also plays a role in light-dependent behavior (shadow response) in cavefish larvae. Here, we aimed to determine the opsin profile of *A. mexicanus* and explore the expression differences between cave and surface morphs in different tissues: telencephalon, tectum, pineal, basal brain, eye, and skin. A bioinformatics survey of the *A. mexicanus* genome revealed 29 orthologs of the 42 zebrafish opsins, representing 16 opsin classes thus, majority of teleost opsin classes are covered in the current genebuild. Tissue samples from the retina, pineal, telencephalon, tectum, cerebellum, other deep-brain area and skin, were dissected from adult surface and cave fish. Using qPCR, we detected high expression levels of multiple opsins in the cavefish pineal, implicating the pineal as a key tissue in cavefish light-dependent activity. We plan to perform CRISPR/Cas9 and/or pinealectomy to test the involvement of opsin genes and pineal for the light-dependent activity observed in cavefish.

P14

LITTLE CREATURES REVEAL GREAT STORIES: GUT MICROBIOME OF THE CAVEFISH ASTYANAX MEXICANUS (DE FILIPPI, 1853)

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Microbiome studies have revealed a great diversity of microorganisms inhabiting vertebrates, being them a good indicator of environmental niche. Both vertebrates and microorganisms developed a close association as a unique biological entity, which can carry out specific biological processes. Several studies have supported the stomach microbiomes as a differentiated microenvironment, being useful for local adaptation studies, particularly in those coevolutionary processes related to adaptation to extreme environments, like caves. Some factors that have been related to stomach microbiome are: species lineages, developmental stage, sex, trophic level (diet and trophic habits), and extrinsic factors (environment). Astyanax mexicanus model gives a unique opportunity to study microbiome-environment coevolution since stomach microbiomes could shed light on microorganisms role during the adaptational process to the troglodyte life. Our main objective was to characterized the stomach microbiomes from independently diverged cavefish lineages of A. mexicanus from two cave populations (Pachon and Río Subterraneo) and one surface population (Río Rascon). We characterized 130 operational taxonomic units (OTUs) at the level of family, belonging to 16 bacterial Phyla. Proteobacteria (γ-Proteobacteria) was the most abundant and OTUs richest group, including the following families: Enterobacteriaceae, Aeromonadaceae and Moraxellaceae. Other abundant groups were Fusobacteria, Bacteroidetes, Firmicutes and Actinobacteria that not only were abundant, but also rich in OTUs number. Interestingly Cianobacteria was found in both surface and cavefish metagenomes, while we only expected them in surface populations. Most of groups were shared among surface and cavefish populations, suggesting geographic´s region connectivity, although some chemosynthetic groups were specific to cavefish. Also some unique OTUs were associated to certain populations or gender differences were detected.

P15

USING CRISPR / CAS9 TO FUNCTIONALLY VALIDATE CAVEFISH CANDIDATE GENES IN ZEBRAFISH

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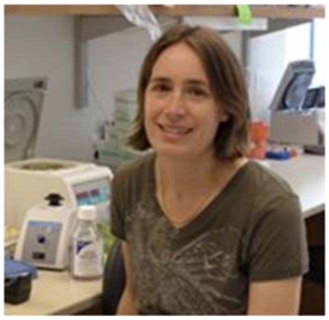
The CRISPR / Cas9 system has proven to be a powerful gene editing tool in creating specific mutations in various organisms. We explore the use of CRISPR / Cas9 to create homologous SNP replacements, knockouts, and whole-exon exchanges in the zebrafish to test cavefish-specific mutations. We have established a high-throughput protocol for designing the CRISPR construct and evaluating the efficiency of mutagenesis and heritability. The injected fish are genotyped for mutation rate and germline transmission and then maintained as stock populations. We see a range of success rates, with the knockout mutations generated by non-homologous end joining proving to be the most efficient at 100% success in generating mutations and 92.3% in germline transmission. However, for the SNP replacements, only 36.4% of our constructs have successfully created mutants, and within these lines, there is an average of 22.5% mutation rate, and 13.6% show successful germline transmission. The whole exon exchanges show a 27.9% mutation rate, but we have yet to observe successful germline transmission. To build on our work in zebrafish, we use this as a pipeline for introducing cavefish morphotype mutations into the surface morphs of *Astyanax mexicanus*. Currently, we inject at night during the natural breeding hours of the *A. mexicanus*, but we are also putting in lightboxes to alter their circadian rhythm for a more convenient microinjection time.

P16



EFFECT OF INSULIN RECEPTOR MUTATION P211L ON INSULIN RESISTANCE IN CAVEFISH

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Nourished Astyanax mexicanus cavefish possess larger body fat and glycogen stores than their surface fish relatives. To make optimal use of their high-energy reserves during seasons of food shortage, cavefish have adapted their metabolism. Key players in glucose homeostasis are insulin and its receptor. It was found that cavefish are insulin-resistant, that they become hypoglycemic after two weeks of starvation and that they tolerate drastic changes in blood glucose levels much better than surface fish. Mexican cavefish carry two insulin receptor genes for insulin receptors a (IRa) and b (IRb). Both IRa and IRb genes were shown to be expressed at similar levels in the fish muscle and liver. In muscle, their expression was slightly reduced (~20%) in Tinaja and Pachon cavefish compared to surface fish. Sequencing of the cavefish IRa gene revealed a coding change in a conserved amino acid from proline to leucine (P211L), a mutation that was also identified in a patient with severe insulin resistance. Genotyping showed that the P211L mutation was selected for independently in multiple cavefish populations. Homology modeling of the Tinaja cavefish IRa protein on the insulin-binding α-subunit of the human insulin receptor did not indicate any gross structural variations. However, the mutation was predicted computationally to have a slight destabilizing effect on the α-subunit and to slow protein folding by about 2-fold. To further explore the mutation's effect on receptor function in vitro, we used HEK293T cells transiently expressing either wild-type surface or mutant Tinaja cavefish IRa. Flow cytometry did not show any significant differences in the binding of fluorescently labeled insulin to the two receptors. The downstream insulin response mediated by the receptor-insulin interaction was measured by Western blotting using primary antibodies against Akt and Phospho-Akt. Preliminary quantification indicated a reduced activity of the Tinaja IRa. We are currently in the process of generating stable cell lines to replicate these in-vitro results.