

Follow-up meeting 2 GENBAS

Location: Liège

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04/11/2014

Present: Maarten Van Steenberge, Loic Kéver, Eric Parmentier, Jeroen Van Houdt, Erik Verheyen, Sofie Derycke

Excused: Jos Snoeks, Pascal Poncin

Participants arrived between 11.50 – 12.30h, informal lunch from 12h-13.30h

Start meeting: 13.45h

1/ Overview of the work already performed, problems encountered and actions to be taken for each WP

WP1: Behavioural and acoustic experiments (presented by Loic)

Task 1.1: Installation of the tanks, experimental set up.

Task 1.2: Recording and characterisation of the behaviour during con- and heterospecific encounters.

Task 1.3: Recording and characterisation of acoustic communication during con- and heterospecific encounters.

Task 1.4: Data analysis behaviour and acoustics.

Overview:

Aquaria are up and running and ready to receive the live specimens from the three species

Four fish (1 males and 3 females) of *O. ventralis* have been kept in one aquarium for five months, courtship behavior has been observed once; mouthbrooding female was set apart, but no eggs could be retrieved the day after. They were probably eaten by the female.

Four kinds of sounds have been recorded in the male when a female is present: spasm sound, head sound, flickering sound, chase sound. The spasm sound is linked to courtship, the three others not sure yet.

Interesting fact: none of these sounds are produced by pharyngeal teeth or involve the swim bladder. The four sounds were related to different movements of the body (e.g. pectoral fin flickering and jaw protrusion). It suggests *Ophthalmotilapia* sounds are produced by different mechanisms.

Problems:

1. it takes a long time to initiate courting; the new sounds were not recorded previously, which indicates that it may take a long time before they start to make the sound
=> It would also be interesting to see which sounds are produced when more than one male is present (Erik)

2. One big pump responsible for the three compartments => risk: if something goes wrong, all fish will be lost at once

Actions to be taken:

1. Follow the fish during 12h instead of half a day (Loic)
2. Test whether aquarium is ready by adding a few specimens of 'sensitive' cichlids
3. Maintain some "backup" fish

WP2 : Specimen and tissue collection

Task 2.1: Purchase, import and determination of live fishes.

Task 2.1: DNA extraction samples genotyping by sequencing.

Task 2.2: Brain dissection and RNA extraction samples.

Overview:

Siegfried Loose provided 2 females and 11 males from *O. nasuta*, no mating behavior observed yet, they will be brought to a bigger tank. One specimen died after finclipping.

Other species have been ordered, they now arrived in the aquarium shop in Germany D-loop of the 2010 samples are ready to be sent for sequencing (ca 30 specimens from 13 localities were collected in 2010)

Most of the 2014 samples are also ready to be sequenced, additional specimens are expected to arrive any day

Preparatory phase of DNA extraction has started: high quality DNA is obtained from the three species, and from all four campaigns

RNA extraction of *Tilapia* results in enough RNA to do RNA seq, even from a single part of the brain

Problems:

1. DNA extraction: shearing was also observed, may be caused by filters of the extraction kit;
2. digestion pattern of the restriction enzymes differs after two hours even within the same species

Actions to be taken:

1. fish will be picked up in the aquarium shop in the week of 10th November (Maarten)

2. Genotype all fish upon arrival in Liège to verify their origin => take ca 5mm of the dorsal or anal fin (definitely not the pelvic fins!). No need to recognise all the fish at this moment, we will assume that the traider is an honest man (Loic and Maarten)
3. Prepare D loop of *Cyathopharynx* (Maarten)
4. Make a NJ tree of all D-loop sequences (study from Nevado and the new samples) (Maarten)
5. Optional: spent one week at Siegfried Loose to record sounds in *O. nasuta* if they do not start courtship behaviour after being located to a bigger tank
6. DNA extraction for GBS (Sofie):
 - a. mechanical shearing does not affect GBS (Jeroen) -> test protocol with phase separation from Bart Hellemans on a few samples and include them in the pilot run
 - b. use both ApeKI and PstI in the pilot
 - c. test digestion with both enzymes overnight to see whether profiles become more similar
7. RNA extraction: test on 6 parts of the brain that are as fresh as possible; dissect the head more to the front, olfactory bulbs go to the nose and can often be seen as two white 'nerve' like structures (Sofie)
8. Contact follow-up committee to discuss RNAseq experiments and parts of the brain that are important for mate recognition (Sofie): diencephalon regulates hormone production, but these signals may already be too far downstream the behaviour pattern

WP3: NGS

Task 3.1: Genotyping by sequencing

Task 3.2: RNA sequencing

Overview:

Nothing to report at this point

WP4: Genomics data analysis

Task 4.1: Preparatory data analysis for genotyping by sequencing.

Task 4.2: Data analysis genotyping by sequencing.

Task 4.3: Data analysis RNA sequences.

Overview:

Collections are being sorted in Tervuren; samples scattered in RBINS and Tervuren will be brought together and stored back in RBINS (Maarten)

Actions to be taken:

1. Preparatory phase GBS investigate cichlid genomes in silico to determine restriction enzyme (*burtoni* is closest related available genome)

WP5: data integration

Task 5.1: Integration of the behaviour and acoustics with the gene expression.

Task 5.2: Integration of behaviour and acoustics with introgression patterns.

Task 5.3: Integration of the gene expression analysis with the genotyping by sequencing.

Overview:

Nothing to report at this point

WP6: Project coordination

Task 6.1: Coordination of the project.

Task 6.2: Reporting and administration.

Task 6.3 Communication with the follow up committee.

Task 6.4 Dissemination of the results. (All)

Overview

Website for the project has been created: www.genbas.be

New dropbox folder has been created

Technical bulletin has been submitted

Two abstracts for poster presentations during the Zoology 2014 conference have been approved

Actions to be taken:

1. provide information on results/problems/solutions for the year report (**Maarten, Loïc and Sofie**)
2. Provide information for the news section on the website (just information for the general public, like arrival of fishes, movie of a female laying an egg, map of all localities where we have samples from, etc) (**all**)
3. Create doodle for next meeting in beginning of February at the Genomics Core facility (**Sofie**)

The meeting was ended at 16.45h, and was followed by a visit to the aquaria.