

# From serotonin to Zika: Evolution and beyond

Guillermo Reales

I<sup>N</sup>aGeMP  
INAGEMP



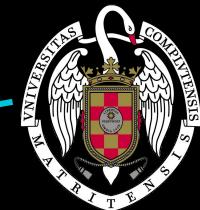
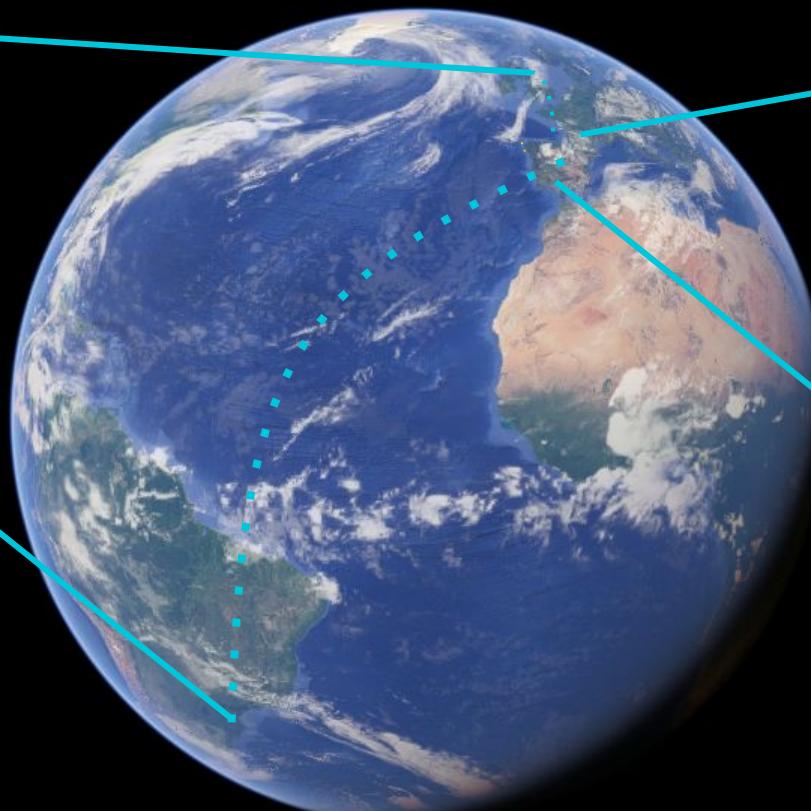
# *My journey so far....*



*University College London*  
Short stage for CANDELA  
project - 2013



*Universidade Federal  
do Rio Grande do Sul*  
PhD Genetics and  
Molecular Evolution  
2014 - 2018  
Postdoctoral researcher  
2018 - 2019  
*(In progress)*



*Universidad Complutense  
de Madrid*  
MSc. Physical Anthropology  
2012 - 2013



*Universidad de Granada*  
BSc. Biology  
2006 - 2012

# Outline

## Part I

Serotonin, behaviour, and natural selection in New World Monkeys

## Part II

The making of a receptor: An evolutionary scenario for 5-HT3 receptor in Chordata

## Part III

Searching for relevant host genetic variants in Congenital Zika Syndrome outcome



# Part I

## Serotonin, behaviour, and natural selection in New World Monkeys

JOURNAL OF Evolutionary Biology



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### Serotonin, behavior, and natural selection in New World monkeys

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GISLENE L. GONÇALVES\*, ALCIDES PISSINATTI§, FRANCISCO M. SALZANO\* &  
MARIA CÁTIRA BORTOLINI\*

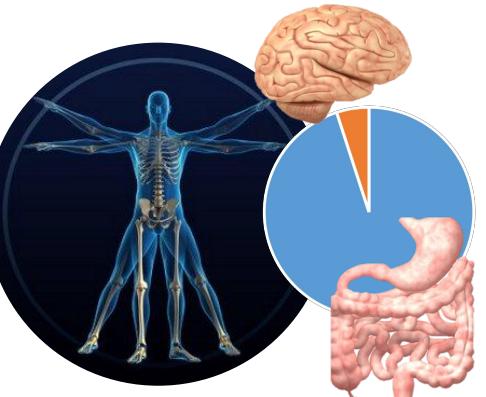
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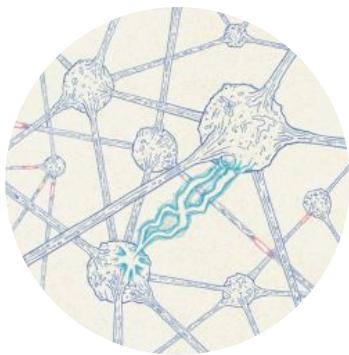
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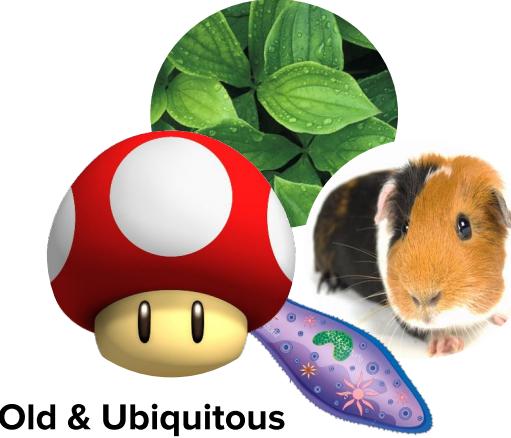
# Serotonin in a nutshell



Every organ



Neurotransmitter



Old & Ubiquitous



Many, many functions!

# Serotonin receptors in human

13 RECEPTORS | 7 TYPES

## Slow

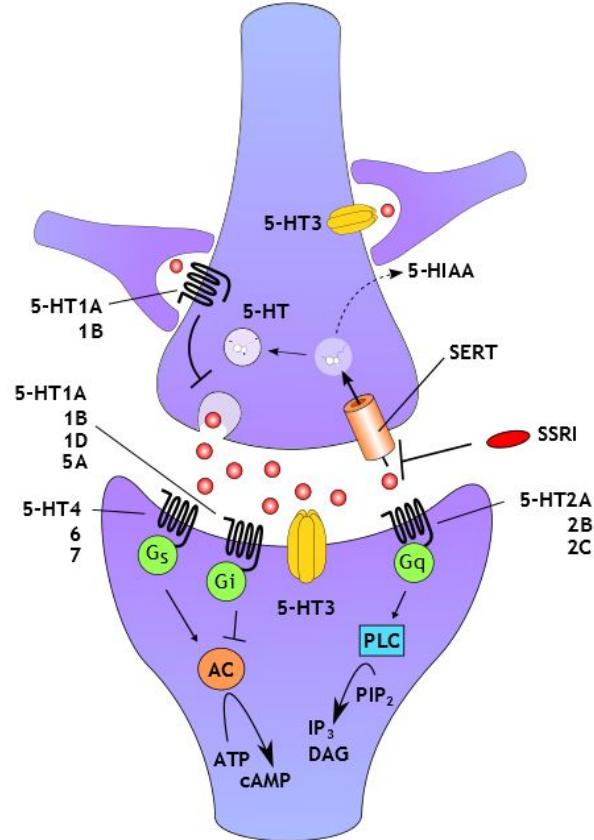
**GPCRs** (G-protein coupled receptors)

- Inhibitory: 1A, 1B, 1D, 1E, 1F & 5A
- Excitatory: 2A, 2B, 2C, 4, 6 & 7

## Fast

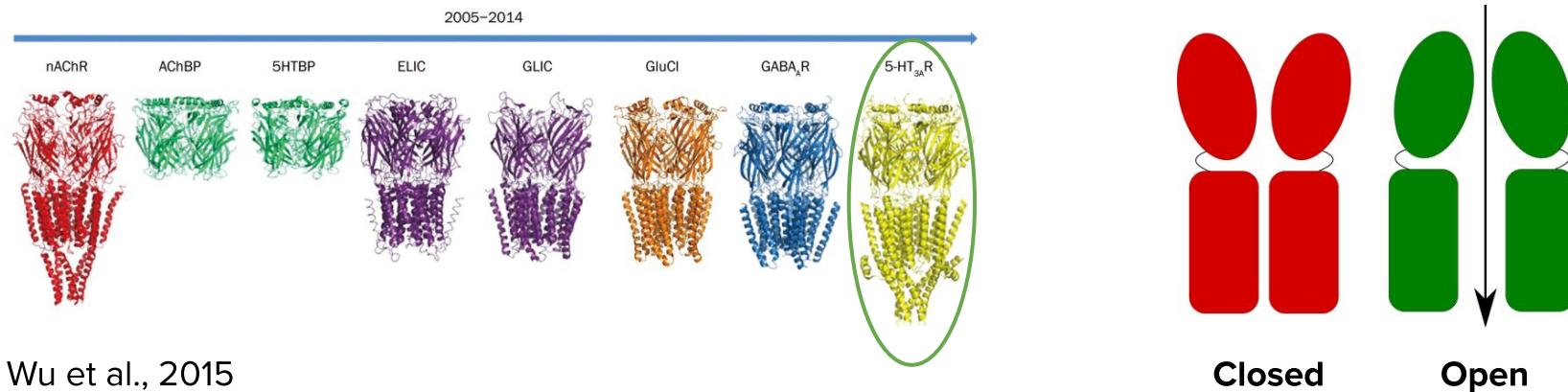
**LGIC** (Ligand-gated ion channel)

- 5-HT3 receptor - 5 genes: *HTR3A-E*
- $Na^+$ ,  $K^+$   $Ca^{2+}$



# pLGICs (pentameric ligand gated ion channels)

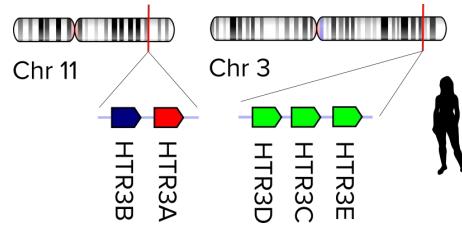
- Very old neurotransmitter receptor superfamily. All domains of life. More than 40 genes in vertebrates.
- Five subunits form a pore that opens upon activation.
- Cationic – Excitatory (nAChRs, 5-HT3R, ZAC). Anionic – Inhibitory (GABAAR, GlyR).



# The 5-HT3 receptor

X-ray structure of 5-HT3 receptor, as deposited by Hassaine et al., 2014 at PDB. Reconstructed using ChimeraX

5 monomer subtypes (A – E)  
A in every functional receptor

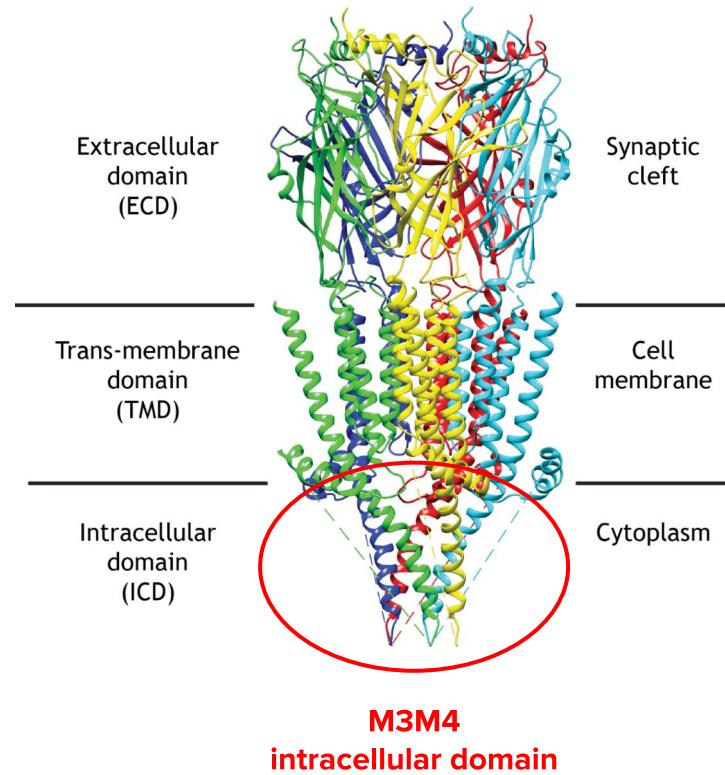


Different stoichiometry  
- different properties

Homopentamer A → <1 pS  
Heteropentamer AB → 9 – 17 pS

CNS, PNS and GI tract

Cortical regions, amygdala,  
striatum and hippocampus



# Genes & Behaviour & NWMs

**Evolutionary pattern in the OXT-OXTR system in primates: Coevolution and positive selection footprints**

Vargas-Pinilla et al., 2015. *PNAS*

Oxytocin and arginine vasopressin receptor evolution: implications for adaptive novelties in placental mammals

Paré et al., 2016. *Genet. Mol. Biol*

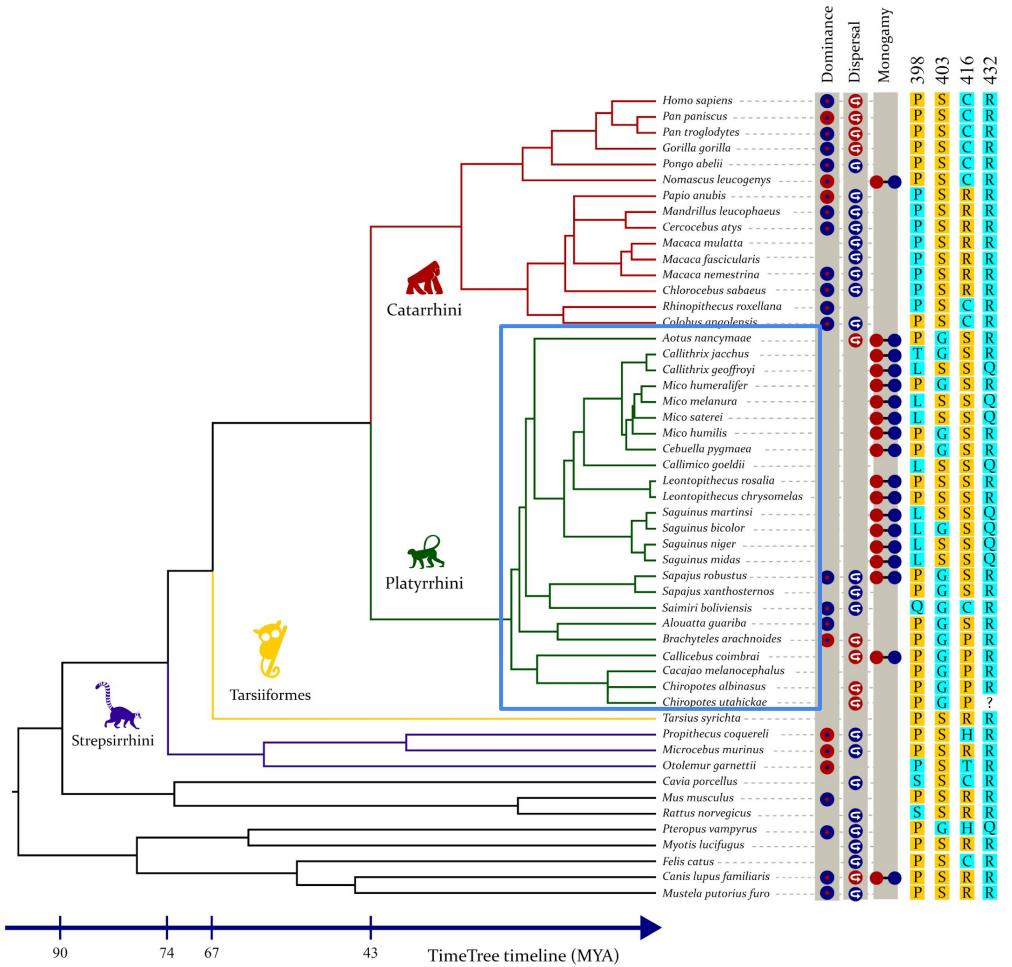
**Functional New World monkey oxytocin forms elicit an altered signaling profile and promotes parental care in rats**

Parreiras-e-Silva et al., 2017. *PNAS*

**AVPR1b variation and the emergence of adaptive phenotypes in Platyrrhini primates**

Fam et al., 2019. Accepted at *Am. J. Primatol.*





1 Sequence HTR3A M3M4 fragment  
(22 NWM sp.) + Data mining (31 sp.)



2 Molecular Evolutionary analyses

$$\omega = dN/dS \quad \text{Site \& Branch-Site Models}$$

3 Site-trait association analyses



Superwise      Phylogenetic Latent Liability Model

4 Linear motif analyses



# Molecular evolution analysis

## Site Model tests

Allow  $\omega$  to vary among sites

**M8a** (Neutral) vs. **M8** (Positive selection):  $\omega_{10} = 6.460$ ; LRT **P =  $5.87 \times 10^{-4}$**

Sites under positive selection (Bayes Empirical Bayes, pp > 0.99) = **416**

## Branch-Site Model tests

Allow  $\omega$  to vary among sites and branches, a foreground branch must be specified

**M2a\_rel** (Neutral) vs. **CladeC** (Positive selection)

$\omega_{FG}$  (Callitrichinae) = 1.445; LRT **P = 0.024**

Sites under positive selection (Bayes Empirical Bayes, pp > 0.99) = **398, 403, 432**

# Classical association analyses

Fisher's Exact &  
Kruskal-Wallis tests

## Superwise package

GRealesM/superwise



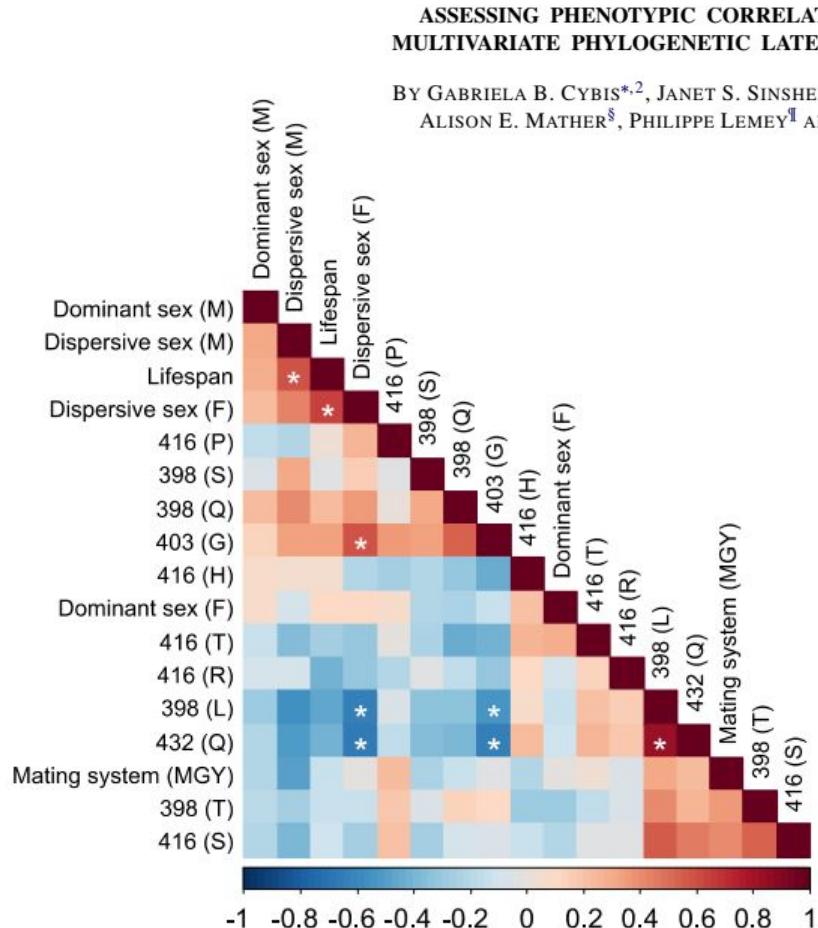
Fisher's Exact test		
Trait (Category)	Site (aa)	Adjusted P-value
Mating system (MGY)	416 (S)	$1.67 \times 10^{-4}$
Dispersive sex (No bias)	416 (S)	0.002
Dominant sex (None)	416 (S)	0.021
Dispersive sex (No bias)	398 (L)	0.046

P-values were adjusted by Bonferroni correction

**416(S)** appears predominantly in NWMs, and is associated with social monogamy, among other traits

# Phylogenetic Latent Liability Model

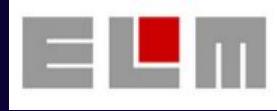
Calculates correlations between phenotypic traits while taking into account shared evolutionary history



Prof. Gabriela Cybis

Female dispersal was significantly positively correlated with **403(G)**, and negatively with **398(L)** and **432(Q)**.

**416(S)** – Social Monogamy correlation was positive, yet not significant ( $\text{cor} = 0.42$ ,  $\text{pp} = 0.94$ )

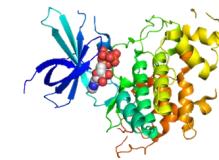
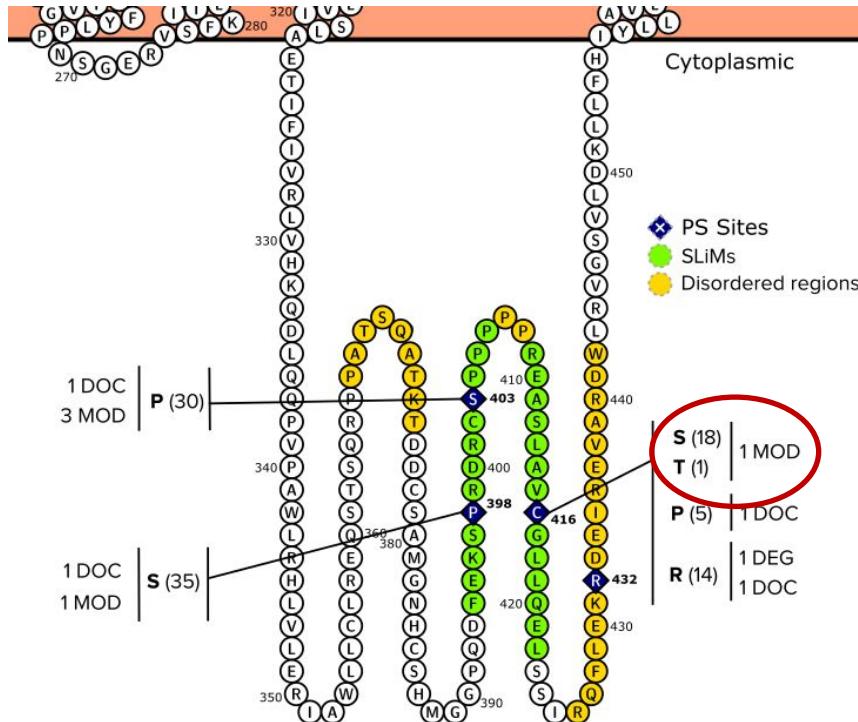


elm.eu.org

## Linear motif analysis

In order to find potential functionally relevant variants

PONDR® (VL-XT) → 49% M3-M4 disordered



GSK3

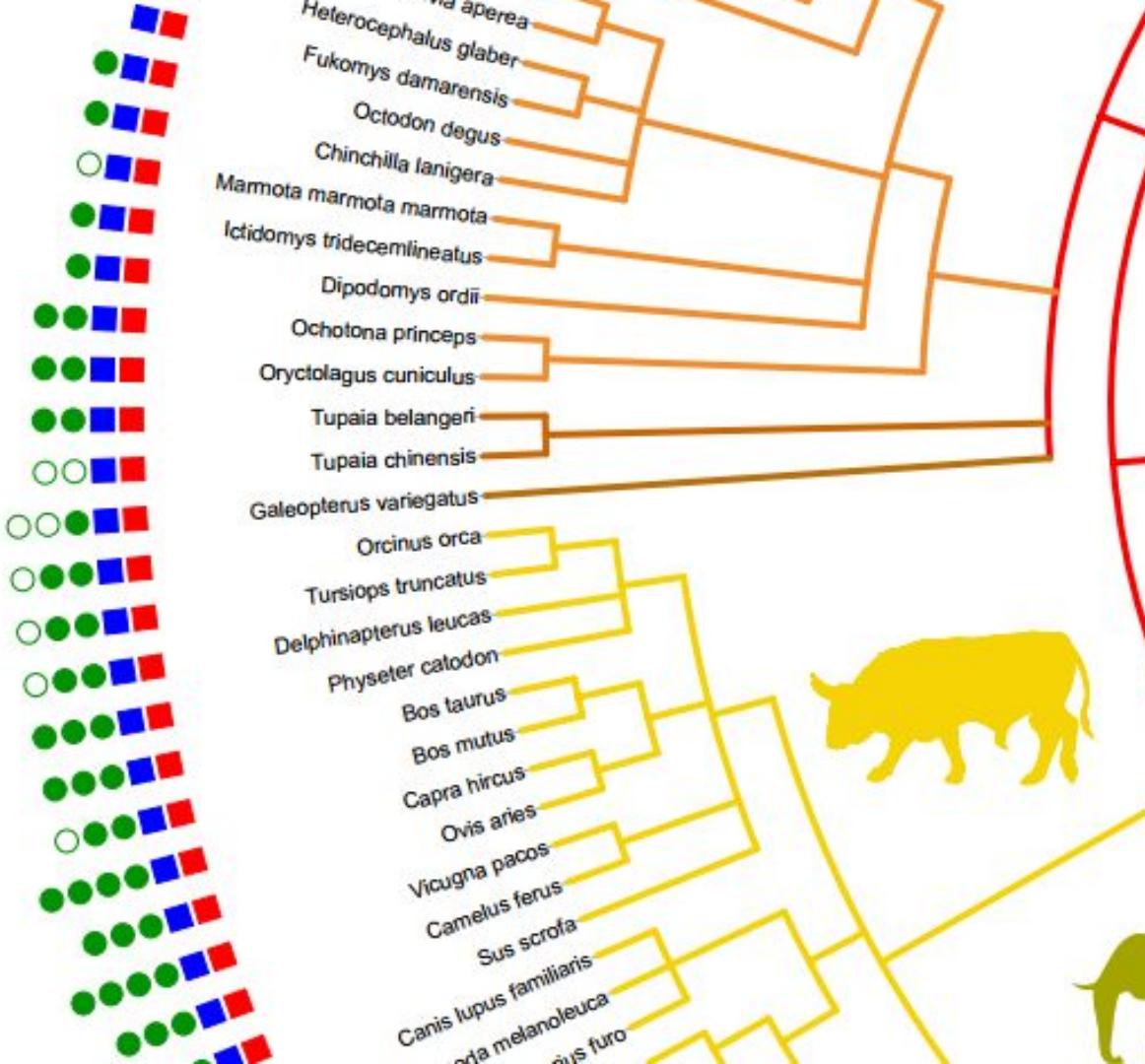
- Kinase with 500+ known substrates
- Essential for CNS development and function
- Inhibited by serotonin via signaling cascade
- Known to interact with 5-HT1B, and with other ionotropic receptors (e.g. AMPA, NMDA and GABA-A)

# Results highlights and discussion

- 4 sites under **positive selection** (**398, 403, 416, 432**) in the HTR3A M3M4 domain, three of them in the Callitrichinae branch.
- **416S**, predominant among NWMs was associated with social monogamy (classical), although positive correlation was not significant (PLLM).
- A SLiM for GSK3 at **416S/T**, previously shown to interact with other serotonin and ionotropic receptors.
- Our results suggest putative functional relevance of these variants in NWMs (although further experimental evidence is required).

## Part II

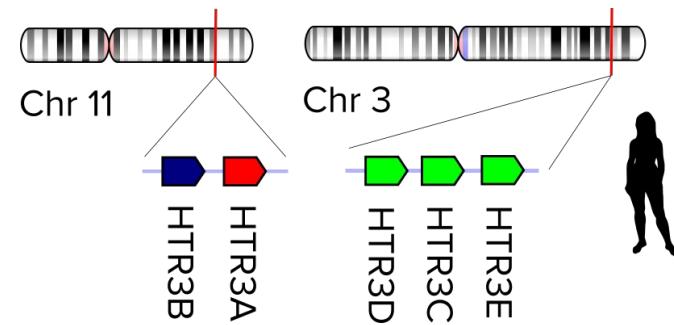
The making of a receptor:  
An evolutionary scenario  
for 5-HT<sub>3</sub> receptor in  
Chordata



# Introduction

- What is the origin of the 5-HT3 receptor family?
- How did the 5-HT3 receptor and their subunits evolved across Chordate evolutionary history?
- Did some subunits get lost or duplicated in evolution? In which groups?
- Did some subunits evolved faster than others?

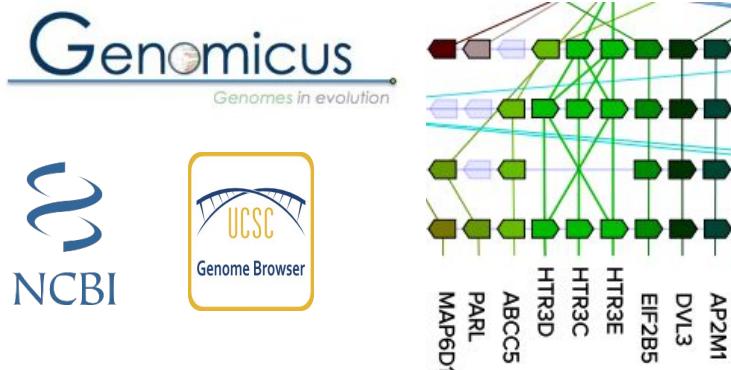
Answering these questions will provide insights into receptor functionality within an evolutionary context





Data Mining  
(1210 sequences, 211 species)

## Synteny Analysis



Phylogenetic Analyses  
(Maximum likelihood & Bayesian)



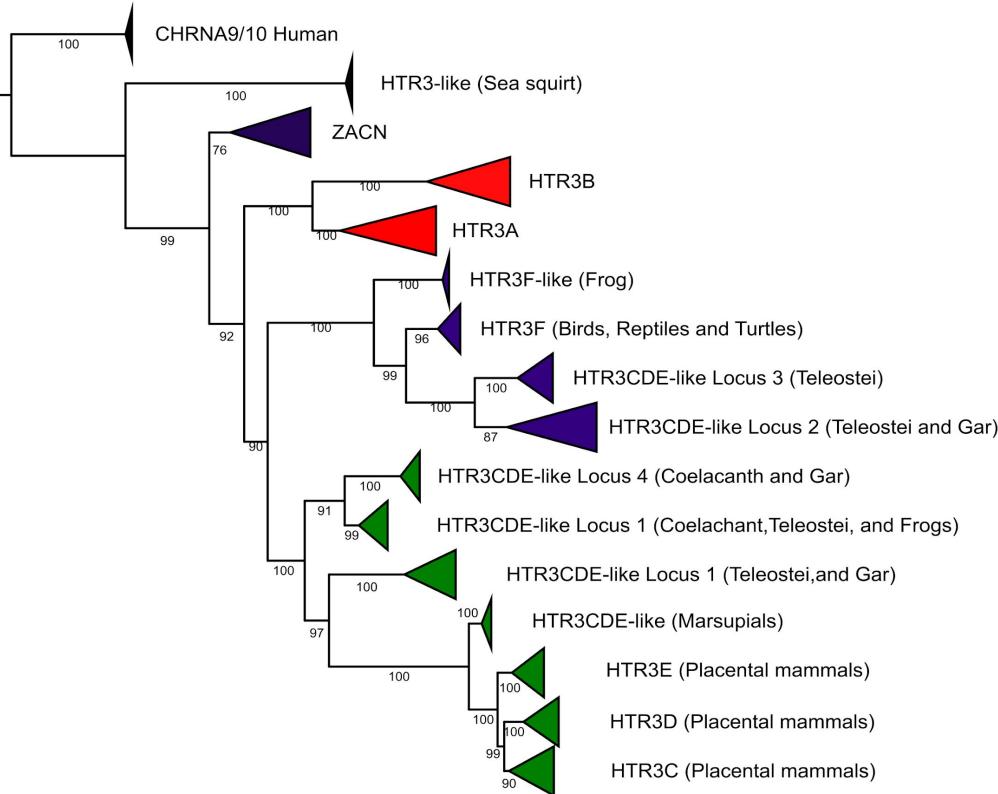
## Domain Analysis

## Modeller

Program for Comparative Protein Structure Modelling by Satisfaction of Spatial Restraints

## Molecular Modelling

# Highlights



**HTR3A** present in all species.  
Consistent with its essentiality

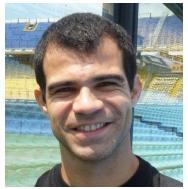
**HTR3B** was lost in birds and some  
reptiles (but not in turtles and  
crocodiles!)

**HTR3C-E** a more complicated  
history (Up to 8 different loci!)

**HTR3F** A novel 5-HT3 subunit?

Maximum Likelihood tree with UFBoot  
support values. 31 species. 202 DNA  
sequences.

# Future Steps



- Estimation of main evolutionary events using calibrated trees

Prof. Fagundes

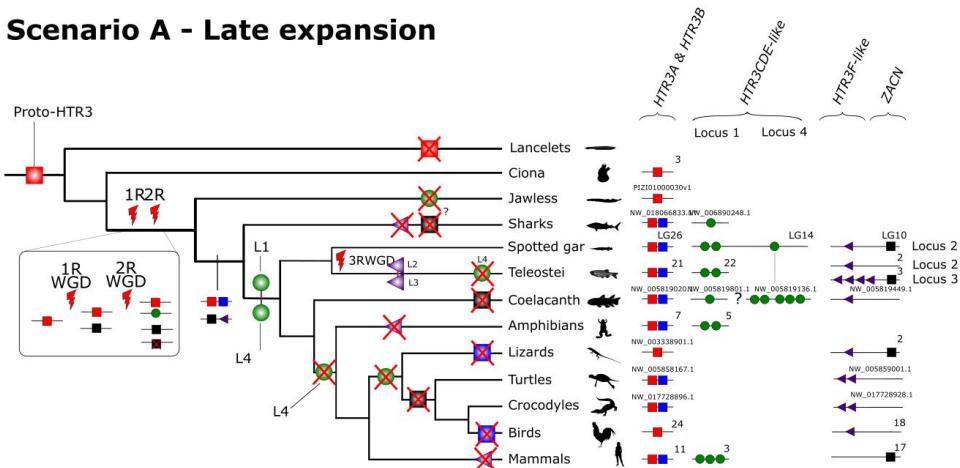


- Molecular modelling of avian *HTR3F*

MSc. Martiela Freitas

- Manuscript to be finished and submitted by late July
- Presentation of main results at **SMBE Congress** (20-25th July)

## Scenario A - Late expansion

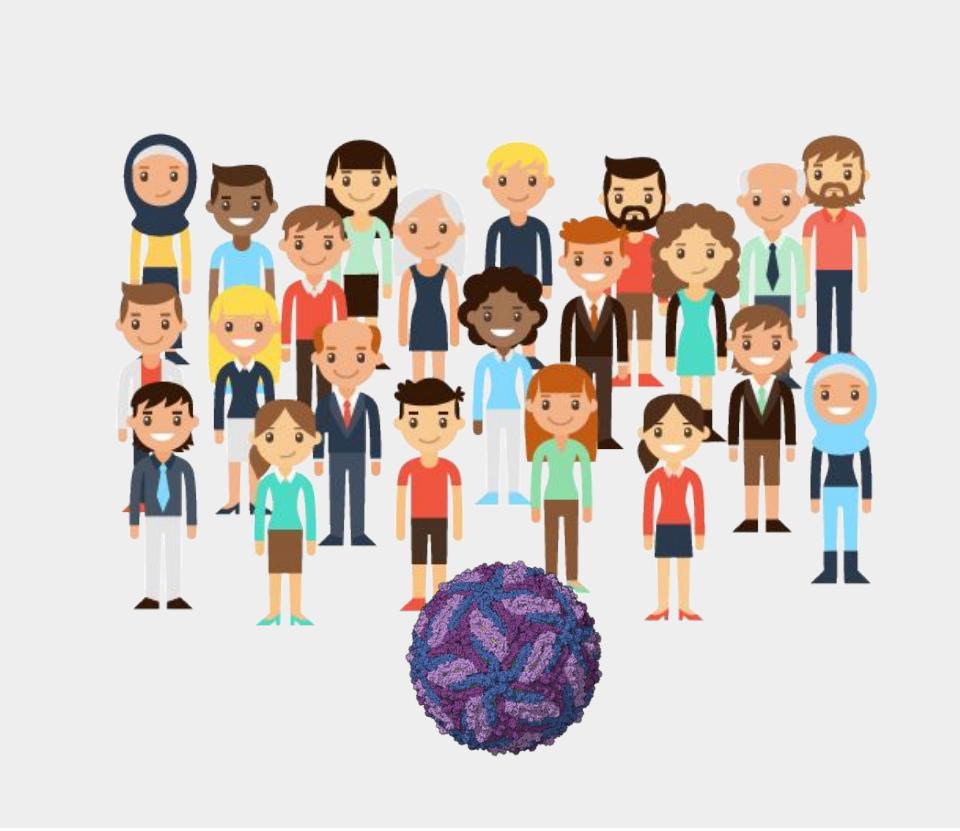


## Part III

### Searching for relevant host genetic variants in Congenital Zika Syndrome



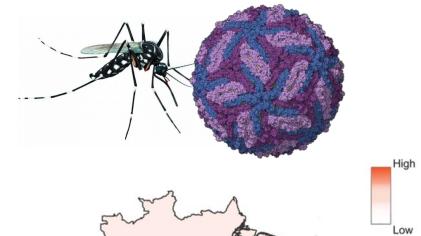
Prof. Schuler-Faccini's Lab



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# Introduction

- Zika virus (ZIKV), a mosquito-borne and sexually-transmitted virus.
- Mild to no symptoms. Risk of Congenital Zika Syndrome (CZS) in pregnant women.
- 2.952 confirmed cases of CZS in Brazil (2015-2018, PAHO), especially in the northeast.
- ~400 of affected and unaffected children to mothers infected by Zika virus during pregnancy, from different locations (collection in progress).



Differential susceptibility among **human populations**, and among **species**.



Baby with Typical Head Size



Baby with Microcephaly



Baby with Severe Microcephaly

# 269 candidate genes

48 from the literature

Cell entry mechanism  
Cell machinery  
Innate immunity

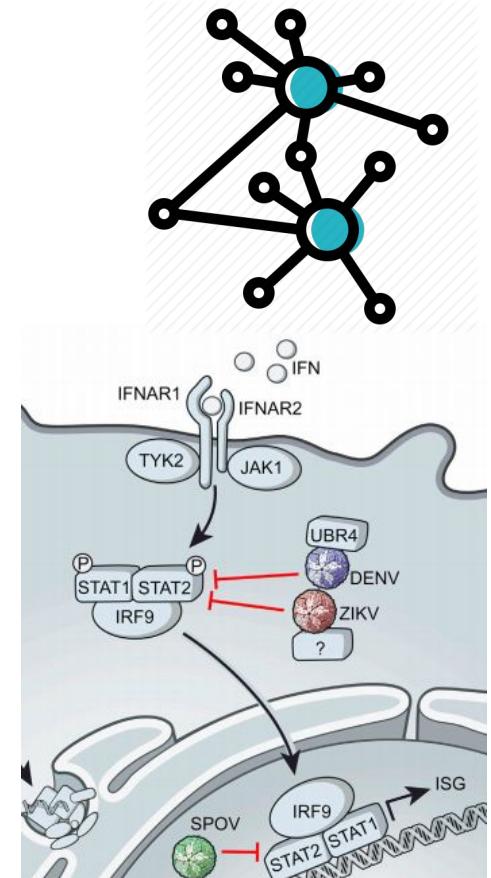
3 recent interactome studies

8 common genes (3 out of 3)  
221 common genes (2 out of 3)

## STAT2 as an example

Transcription factor, essential to type-I interferon signaling pathway.

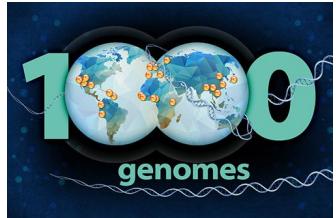
ZIKV promotes its degradation in humans yet not in mice!



Grant et al., 2016. *Cell Host & Microbe*

# Populational Approach

How do these candidate genes vary in world and Brazilian populations?



2504 individuals, 26 pops  
84.4M variants



ABRaOm

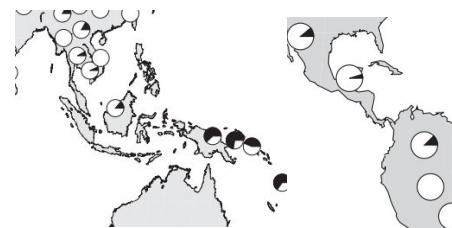
609 Brazilian inds  
1.4M exome variants



340 Brazilian inds  
906k microarray variants

A Haplotype at *STAT2* Introgressed from Neanderthals and Serves as a Candidate of Positive Selection in Papua New Guinea

Fernando L. Mendez,<sup>1,2</sup> Joseph C. Watkins,<sup>3</sup> and Michael F. Hammer<sup>1,2,\*</sup>

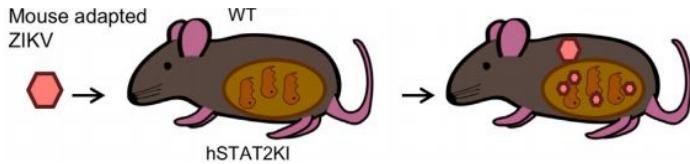


Could variants selected in the past have relevance on Zika virus epidemics?

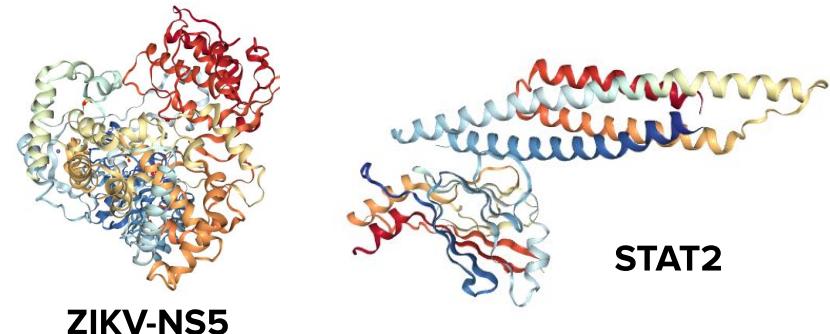
# Comparative genomics and modelling approach

Differential Zika virus infection outcome among species (eg. humans and mice). Why?

- Search for variants with potential functional relevance among species (Molecular evolution).
- If **STAT2** makes a difference, can we **model** it?



Immunocompetent mice with knocked-in hSTAT2 develop fetal infection  
(Gorman et al., 2018)



# Other works in population genetics and evolution

## ARTICLE

Received 4 Nov 2014 | Accepted 14 May 2015 | Published 24 Jun 2015

DOI: [10.1038/ncomms8500](https://doi.org/10.1038/ncomms8500)

OPEN

A genome-wide association study identifies multiple loci for variation in human ear morphology

Adhikari, et al. 2015. *Nat. Comm.*

**A tale of agriculturalists and hunter-gatherers: Exploring the thrifty genotype hypothesis in native South Americans**

Reales, et al. 2017. *Am. J. Phys. Anthropol.*

**Measuring the impact of European colonization on Native American populations in Southern Brazil and Uruguay: Evidence from mtDNA**

Tavares, et al. 2019. *Am. J. Hum. Biol.*

# Thanks to



Prof.  
Bortolini



Prof.  
Schuler-Faccini



Prof.  
Paixão-Côrtes



Prof. Cybis



Prof. Fagundes



MSc. Martiela  
Freitas



MSc. Marcelo  
Bragatte