

The liver fluke *Opisthorchis felineus*: molecular biology and carcinogenic potential

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Epidemiologically important fish-borne liver trematodes

- Family Opisthorchiidae: *Opisthorchis felineus* (Rivolta, 1884), *O. viverrini* (Poirier, 1886), and *Clonorchis sinensis* (Loos, 1907)
- These liver flukes are known to cause serious human diseases affecting bile ducts and the gall bladder – opisthorchiasis and clonorchiasis
- According to the Food and Agriculture Organization and World Health Organization, liver flukes family Opisthorchiidae are the 8th in the overall global list of 24 food-borne parasites
- Liver fluke infection is recognized as the major risk factor of **cholangiocarcinoma**



*Opisthorchis
viverrini*



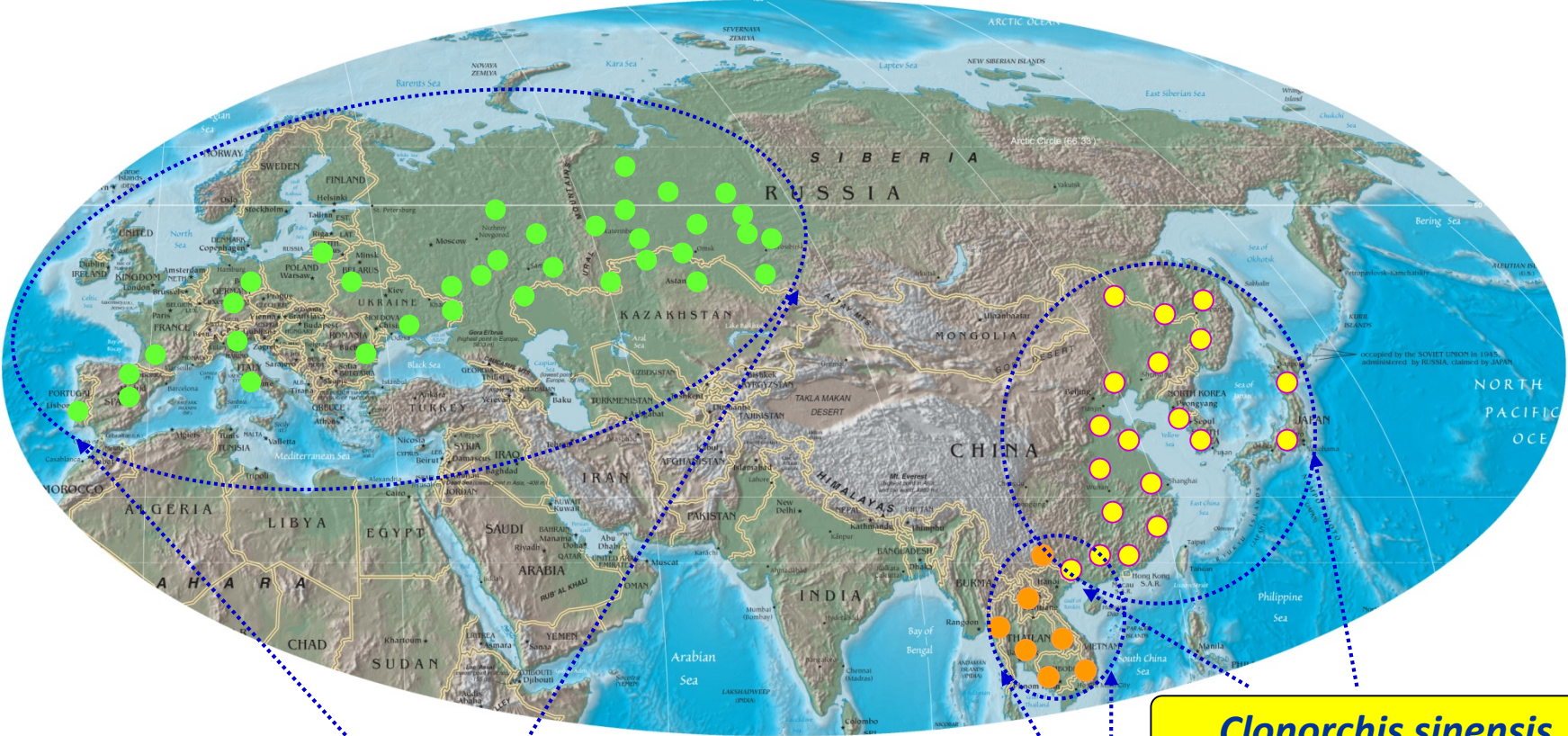
*Opisthorchis
felineus*



*Clonorchis
sinensis*

- An estimated 12.5, 67.3 and 601 million people are currently at risk for infection with *O. felineus*, *O. viverrini* and *C. sinensis*, respectively

Geographical range of Opisthorchiidae liver flukes



Opisthorchis felinus

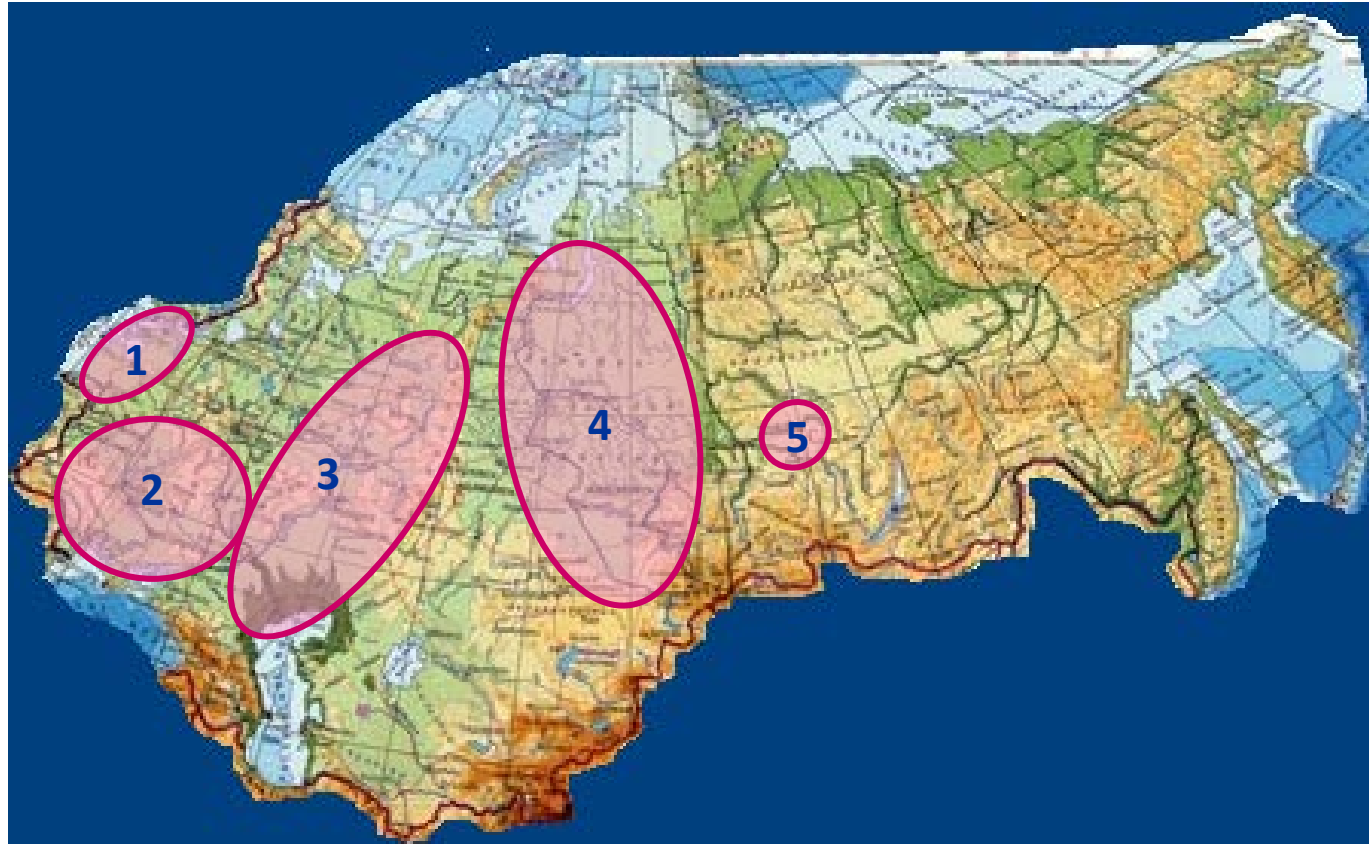
Opisthorchis viverrini

Clonorchis sinensis

Brief history of *Opisthorchis felineus*

- 1884 S. Rivolta, an Italian scientist, described *Distomum felineum* (sin. *Opisthorchis felineus*), a new helminth species parasitizing the bile ducts of cat's liver
- 1891 In humans, this helminth species was discovered by K. Vinogradov, a professor of the Tomsk University (Russia)
- 1895 R. Blanchard determined the taxonomic position of *O. felineus*
- 1900 M. Askanazy published information about parasite infection caused by *O.*
1904 *felineus* at cats and dogs in Italy, France, Holland, Germany and Russia.
- 1919 Academician K. Skryabin organized system work on identification and the
1959 description of the helminthiasis loci in the territory of the USSR, gave the description of the Opisthorchiasis loci, created the term "biohelminthiasis"

Foci of opisthorchiasis in former USSR



- 1 - The basin of the Baltic Sea, the rivers Neman and Zapadnaya Dvina
- 2 - The basin of the Black Sea, the rivers Dnepr, Don and Dnestr
- 3 - The basin of the Caspian Sea, the rivers Volga and Ural
- 4 - The Ob–Irtysch basin (The Arctic Basin), the rivers Ob and Irtysch
- 5 - The Arctic Basin, the river Birjusa

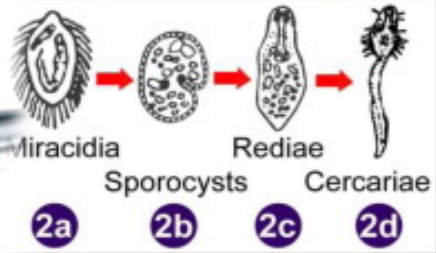
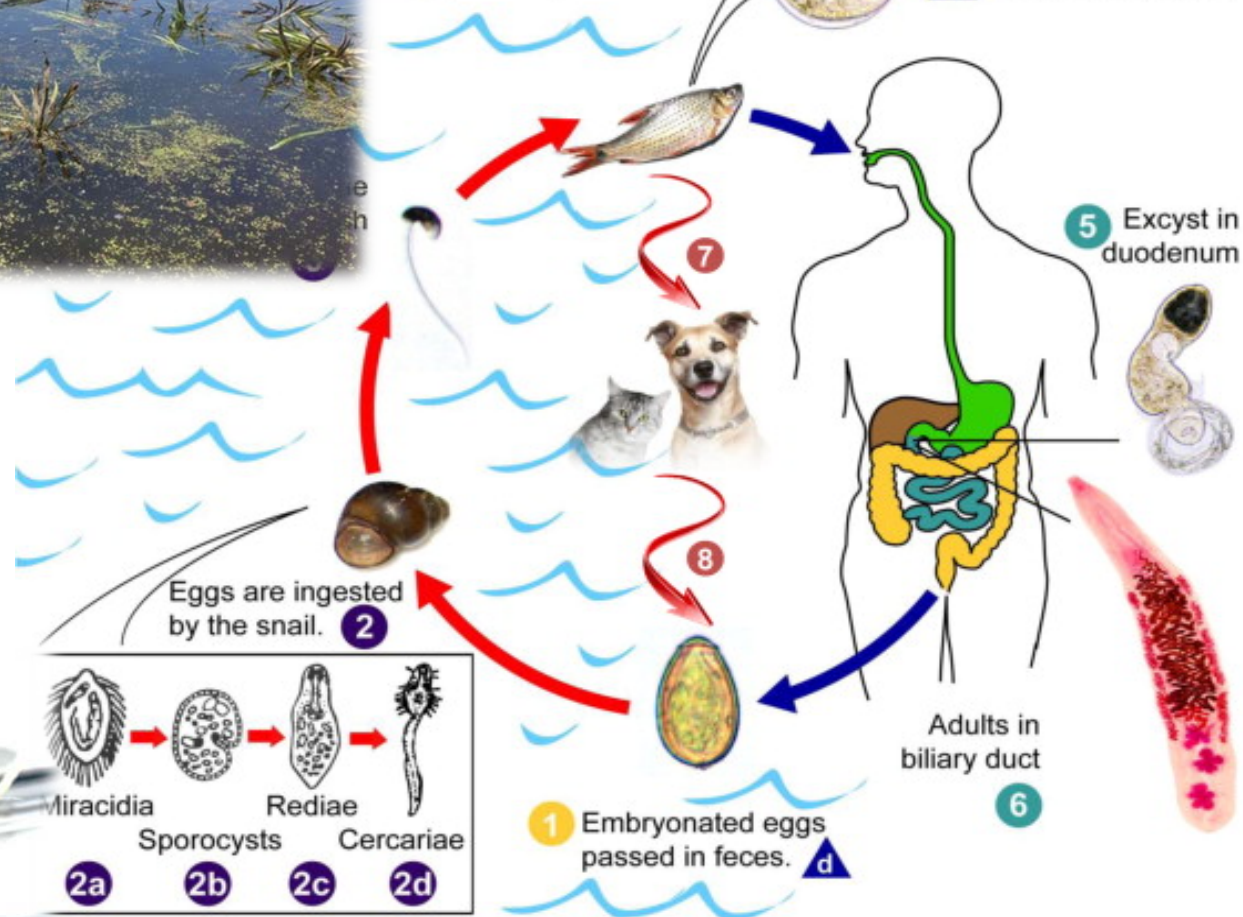
Opisthorchis felineus: life cycle stages



4 Metacercariae in flesh or skin of fresh water fish are ingested by human host.



i = Infective Stage
d = Diagnostic Stage



5 Excyst in duodenum



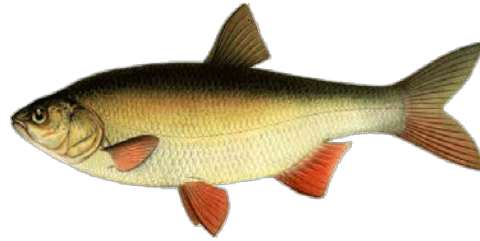
Adults in biliary duct

6

1 Embryonated eggs passed in feces. **d**



Infected fish is the only source of *Opisthorchis felineus* infection



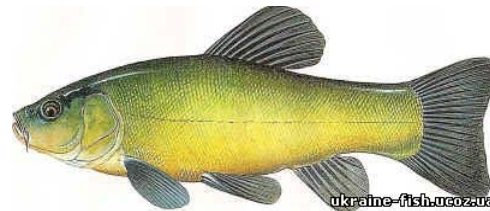
Ide (*Leuciscus idus*)



Roach (*Rutilus rutilus*)



Dace (*Leuciscus leuciscus*)



Tench (*Tinca tinca*)



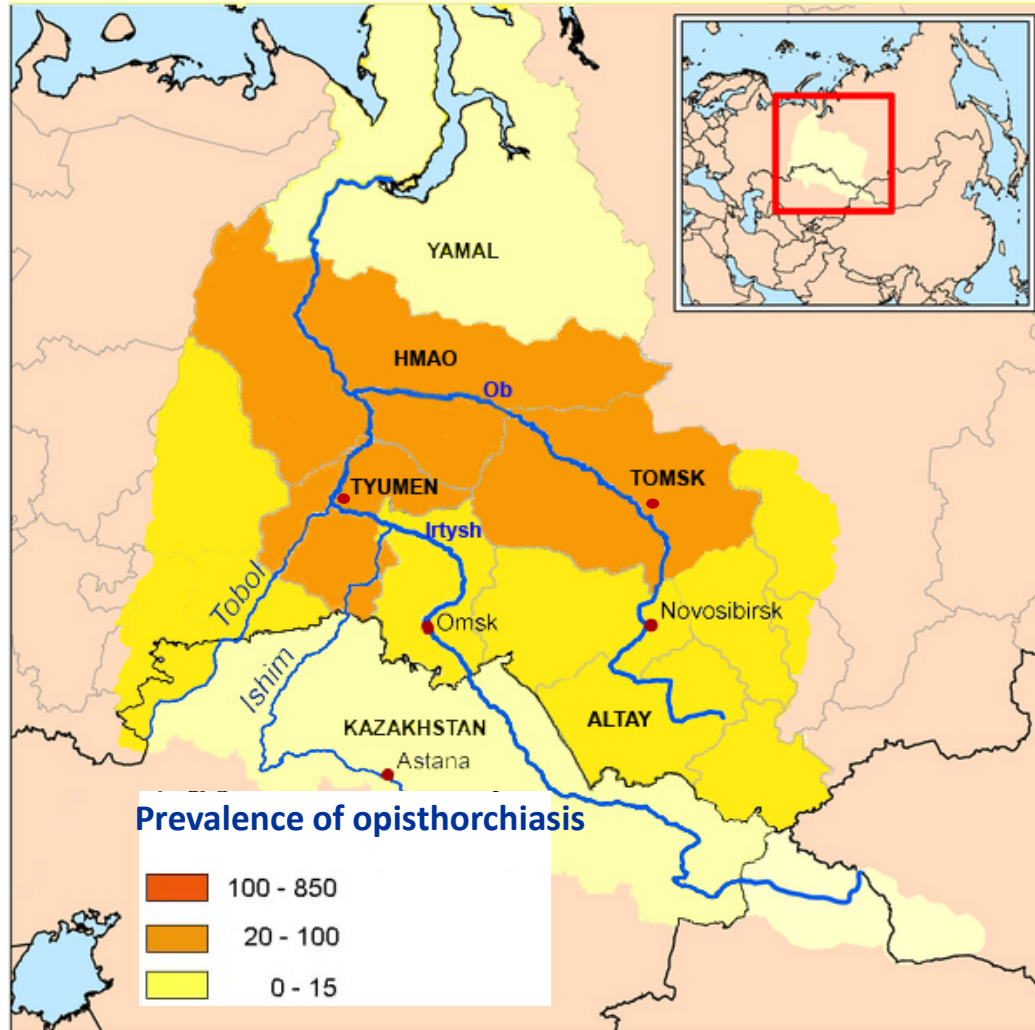
Spelding (dried fish)



Frozen sliced raw fish
("stroganina")

The liver fluke *Opisthorchis felineus*: the parasite presenting a serious public health threat in Western Siberia

Official statistical data (cases/100000)



Published data (Russian medical journals)

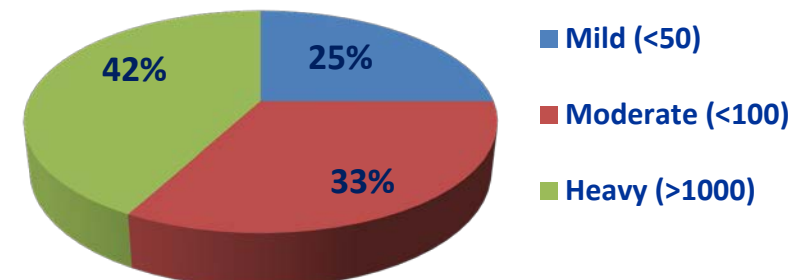
Prevalence (%)

OMSK	20,8 ± 3,7% (1997 – 1998 years)
	19,6 ± 1,4% (2006 – 2008 years)
HMAO	16,3% (1966 – 1975 years)

Autopsy

TYUMEN	46% (1966 – 1987 years)
TOMSK	13-18% (1985 – 1997 years)

Intensity of *O. felineus* infection



Laboratory of Molecular Mechanisms of Pathological Processes

ICG SB RAS

The main lines of investigations

- Comparative studies of molecular biology of epidemiologically important liver flukes
- Molecular mechanisms of pathogenesis of liver fluke infection

Plan of presentation

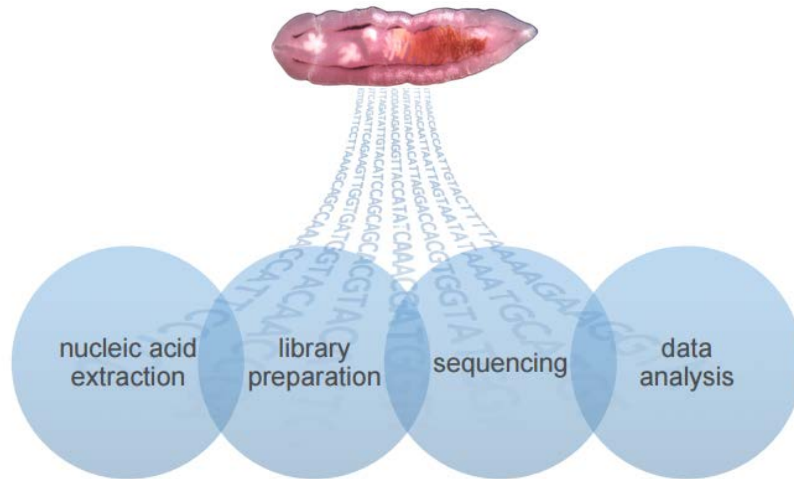
- Functional genomics of Opisthorchiidae liver flukes
- Carcinogenic potential of liver fluke *Opisthorchis felineus*
- Search of potential molecular targets for new anthelmintic agents and combinatorial treatment of liver fluke infection

***Opisthorchis felineus* Genome Project**

***O. viverrini* and *C. sinensis* but not *O. felineus* have been recently characterized at the levels of genome and transcriptome. To address this knowledge gap, we have sequenced the *O. felineus* genome and used the *de novo* assembled draft genome to gain new insights into genetic features of the liver flukes**

**Nikita I. Ershov et al. BMC Genomics. 2019.
DOI: [10.1186/s12864-019-5752-8](https://doi.org/10.1186/s12864-019-5752-8).**

Opisthorchis felineus genome sequencing



Type	Insert size	Reads, 10 ⁶	Coverage to assembly	
			reads	frags
PE	180 bp	564,0	71	86
PE	260 bp	266,7	31	66
MP	2 Kbp	12,4	2.0	23
MP	4 Kbp	16,0	2.5	42
MP	6 Kbp	14,9	2.3	54
MP	8,5 Kbp	10,6	1.6	50



Illumina HiSeq 1500

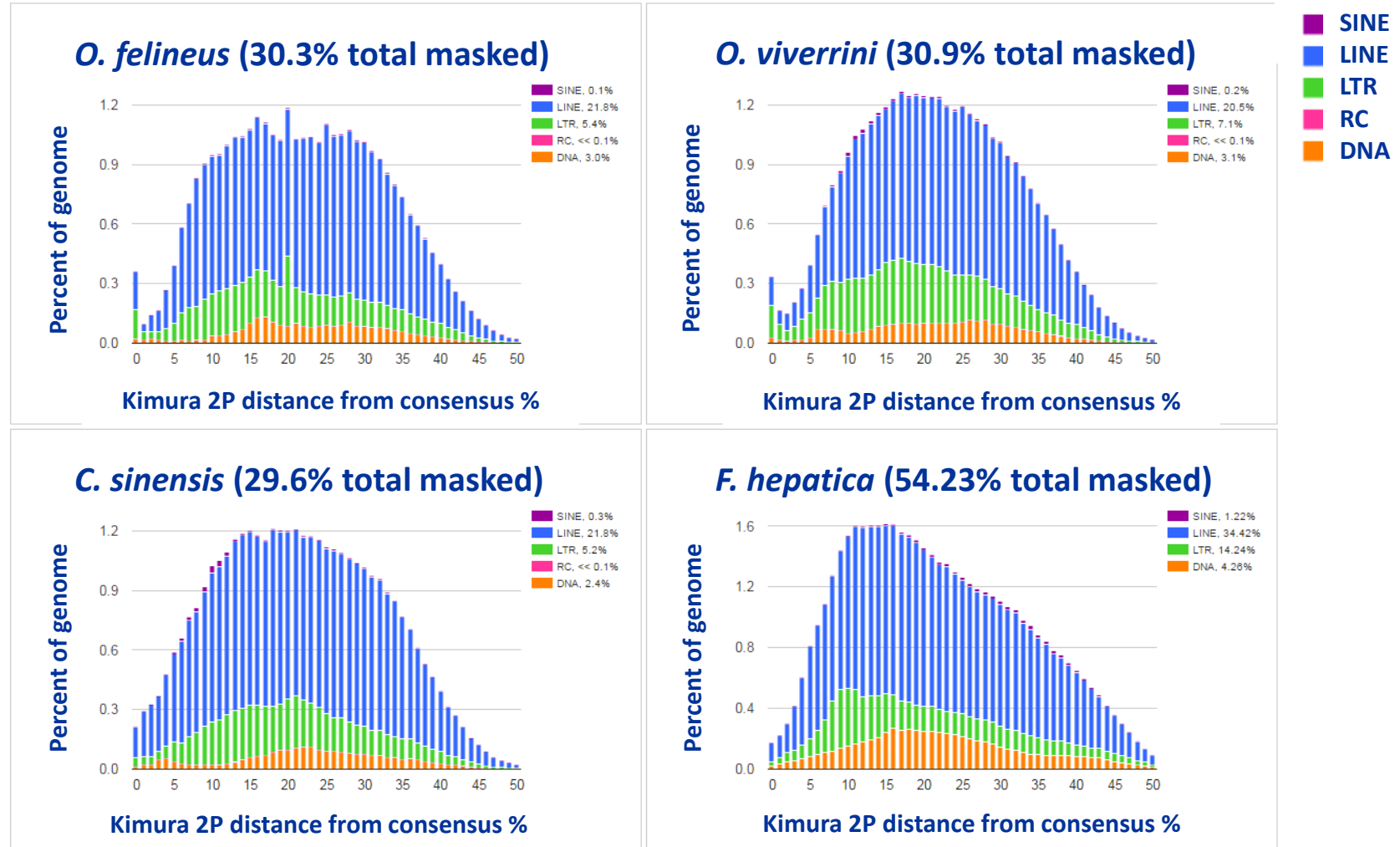


Computing cluster

Characteristics of genomes: five species of trematodes

	Genome sizes	Number of genes	Repetitive elements in genome
<i>O. felineus</i>	680.0 Mbp	11 317	30.3%
<i>C. sinensis</i> [Wang, 2011]	516 Mbp	16 000	29.6%
<i>O. viverrini</i> [Young, 2014]	634.5 Mbp	16 379	30.9%
<i>S. mansoni</i> [Berriman, 2009]	364.5 Mbp	11 809	40%
<i>F. hepatica</i> [Cwiklinski, 2015]	1.3 Gb	11 700	54.2%

Repetitive elements in *O. felinus*, *C. sinensis*, *O. viverrini* and *F. hepatica* genomes

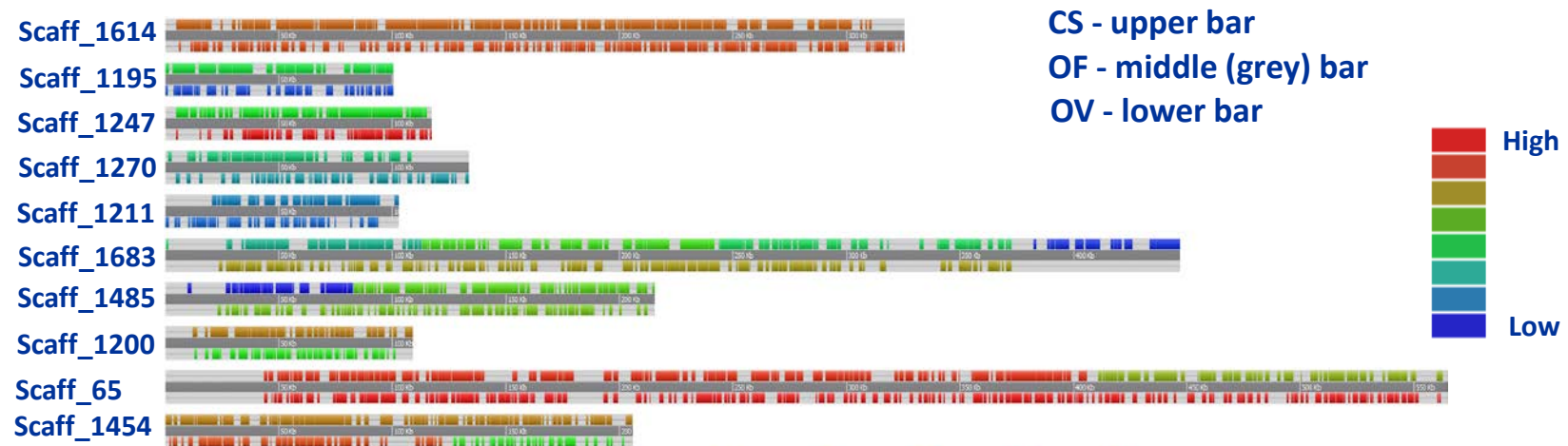


The most part (90.1%) of the repeats in *O. felinus* genome are retrotransposons (LTR, LINE and SINE elements), while remaining 9.9% are comprised by DNA transposons. Overall repeat landscape of *O. felinus* genome correspond to *C. sinensis* and *O. viverrini* landscapes.

Genome synteny *O. felineus*, *O. viverrini* and *Clonorchis sinensis*

Genome alignment statistics	Coverage
<i>O. felineus</i> vs <i>C. sinensis</i>	493213134 (0.42)
<i>O. felineus</i> vs <i>O. viverrini</i>	161445964 (0.26)

Alignment of the top 10 *O. felineus* contigs with the highest coverage by *O. viverrini* and *C. sinensis* genomic sequences

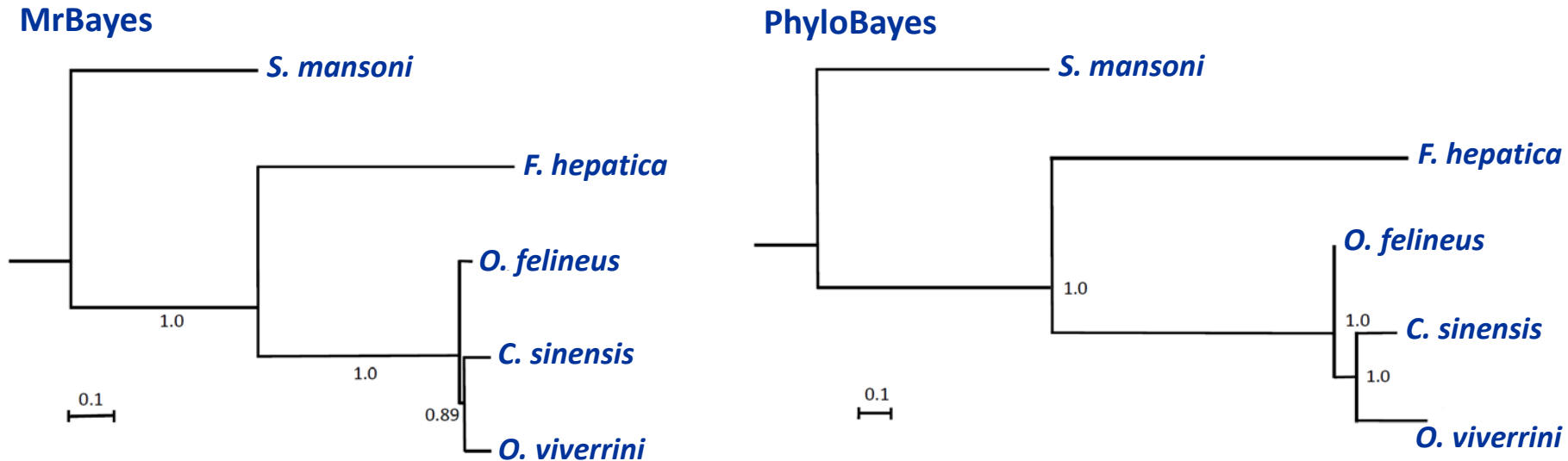


The *O. felineus* contig sequence is represented by middle grey bar. Alignment of the *C. sinensis* (CS) genomic sequences is shown by upper bar, alignment of the *O. viverrini* (OV) genomic sequences is shown by lower bar. Aligned sequences from the same contig have the same color.

Genome-wide synteny between *O. felineus*, *O. viverrini*, and *C. sinensis*

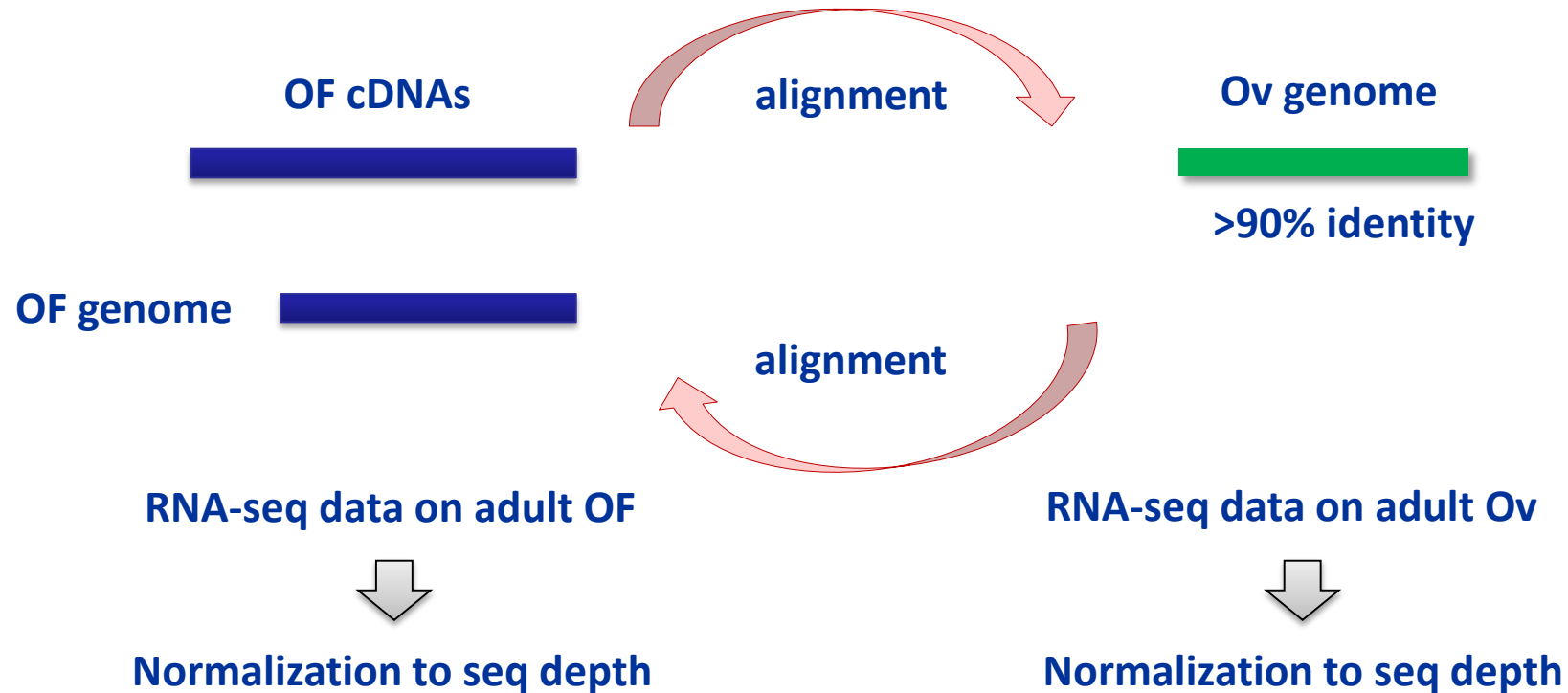
- Considerable variation in the liver fluke genomes
- Structural similarity between the *O. felineus* and *C. sinensis* genomes is higher as compared with that of *O. viverrini* to *O. felineus* and to *C. sinensis*
- These data match well the results of chromosome analysis: *O. felineus* and *C. sinensis* have seven pairs of chromosomes versus *O. viverrini* carrying six chromosome pairs

Phylogenetic relationships



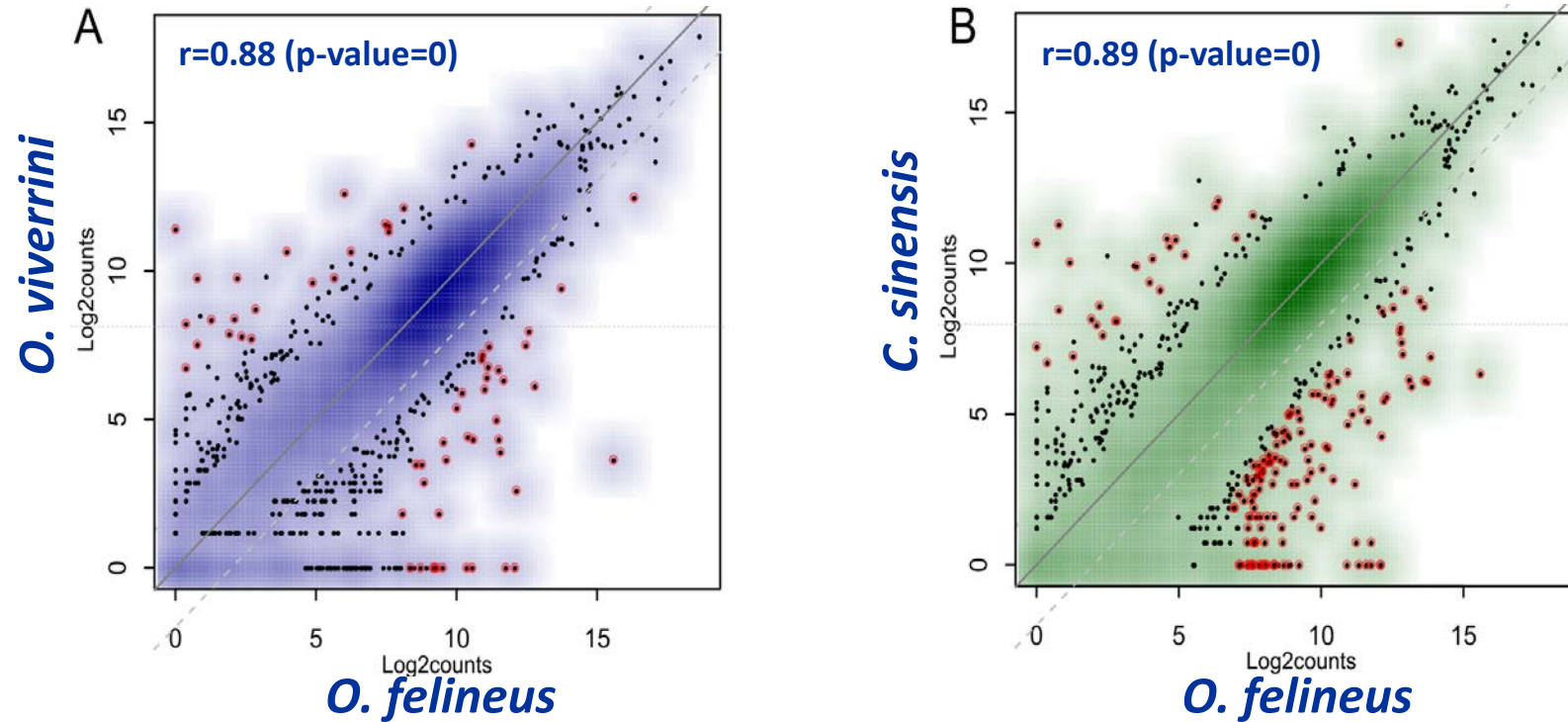
- *C. sinensis* and *O. viverrini* diverged almost immediately after *O. felineus* separated from the common ancestor of these three liver fluke species
- A comparison of the phylogenetic trees for the three studied opisthorchiids and the data on their synteny suggests that the *O. viverrini* genome was structurally remodeled after it had diverged from its common ancestor with *C. sinensis*
- Taken together, results of analysis of the synteny between three opisthorchiid species and of their phylogenetic relationships demonstrate that *O. felineus* and *C. sinensis* are closely related and do not support separation of *C. sinensis* from the genus *Opisthorchis*
- Presumably, *C. sinensis* occupies an intermediate position between *O. felineus* and *O. viverrini*

Comparative transcriptomics



- The predicted cDNAs of *O. felineus* were aligned to the *O. viverrini* and *C. sinensis* genomes using Spaln2 splice-aware aligner
- The best found alignments were mapped back to the *O. felineus* genome
- Only reciprocal-best pairs that cover each other by >90% were retained
- The described workflow allowed for identification of nearly-identical 'orthologous' coding sequences for 9952 (87%) and 10 077 (88%) genes for the comparisons to *O. viverrini* and *C. sinensis*, respectively

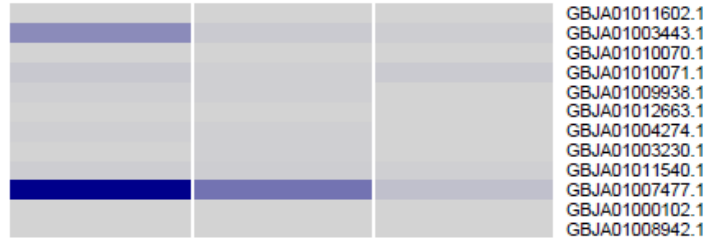
Differences in gene expression of *O. felineus*, *O. viverrini*, and *C. sinensis*



- Expression of most genes of these three opisthorchiid species is at almost the same level independently of the sources of RNA-seq data, obtained by different laboratories
- Some genes have a significantly different level of expression. In total, 61 such genes were recorded for the pair *O. viverrini*–*O. felineus* and 160, for *O. felineus*–*C. sinensis*. The genes with expression values differing more than fourfold ($p < 0.01$) are colored red
- Products of majority of the differentially expressed genes contain domains characteristic for helminth-secreted proteins

O. felineus Glutathione S-transferase sigma (prostaglandin D synthase)

mRNA abundance of genes coding for excretory/secretory products



O. felineus *O. viverrini* *C. sinensis*

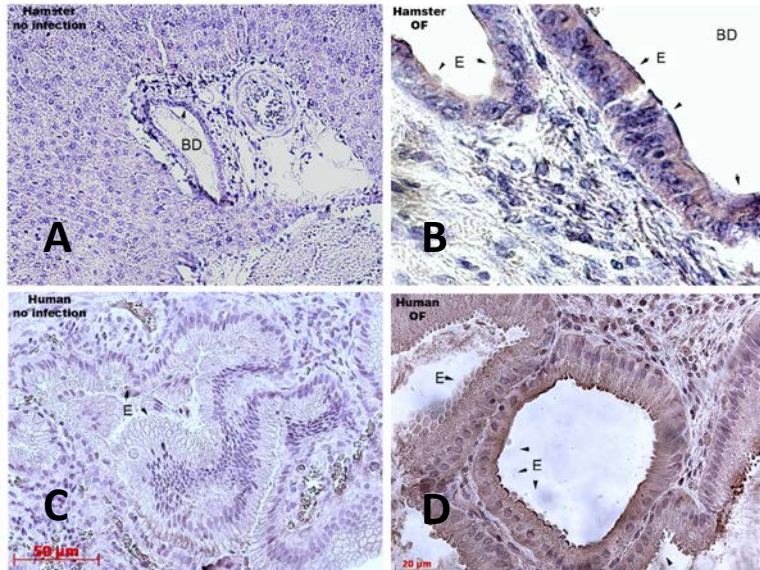
Glutathione Peroxidase

Glutathione S-transferase (GST)

Thioredoxin Peroxidase

GBJA01011602.1
GBJA01003443.1
GBJA01010070.1
GBJA01010071.1
GBJA01009938.1
GBJA01012663.1
GBJA01004274.1
GBJA01003230.1
GBJA01011540.1
GBJA01007477.1
GBJA01000102.1
GBJA01008942.1

Immunostaining of GST sigma *O. felineus*



A Hamster sample: Control

B Hamster sample: *O. felineus* infection

C Human sample: *O. felineus* negative

D Human sample: *O. felineus* positive

E: epithelium of bile ducts; BD: bile ducts. Epithelium cells are indicated with arrows.

Ethacrynic acid inhibits the enzymatic activity *O. felineus* GST sigma in a dose-dependent manner

Incubation media					
Inhibitor	Protein quantity, μg	ΔA340 nm	Inhibitor concentration, μM	Average, activity ± SD, u/mg	%, inh
-	25	0.11	0	0.18 ± 0.02	0
-	50	0.21	0	0.19 ± 0.03	0
-	100	0.34	0	0.16 ± 0.03	0
EA	25	0.09	6	0.16 ± 0.01	9.69
EA	25	0.051	10	0.12 ± 0.02	36.10 *
EA	25	0.047	100	0.088 ± 0.003	49.18 **
Worm Lysates					
Inhibitor	Protein quantity, μg	ΔA340 nm	Inhibitor concentration, μM	Average, activity, u/mg	%, inh
-	12.3	0.18	0	1.25 ± 0.18	0
EA	12.3	0.14	20	0.80 ± 0.10	36.12 **
EA	12.3	0.06	100	0.32 ± 0.04	74.1 ***
EA	12.3	0.05	200	0.31 ± 0.01	75 **

The reaction is measured by observing the conjugation of 1-chloro, 2,4-dinitrobenzene with reduced glutathione. Ethacrynic acid was used as an inhibitor for GST sigma. *p < 0.05; **p < 0.01; ***p < 0.005. SD: standard deviation; EA: Ethacrynic acid. The calculated IC₅₀ was 60.8 μM.

Summary

- The draft *O. felineus* genome size is approximately 684 Mbp, being slightly longer as compared with *C. sinensis* and almost the same as the *O. viverrini* genome; and all three genomes have very similar content and diversity of repetitive elements
- Expression levels of most genes are practically the same in *O. felineus*, *O. viverrini* and *C. sinensis*. This suggests a high similarity of all biological processes in adult liver flukes that colonize the bile ducts of mammals
- Our data can be used for study of genetic mechanisms underlying a complex life cycle of liver flukes and the adaptation of parasites to environmental factors in different climatic conditions and to different host species

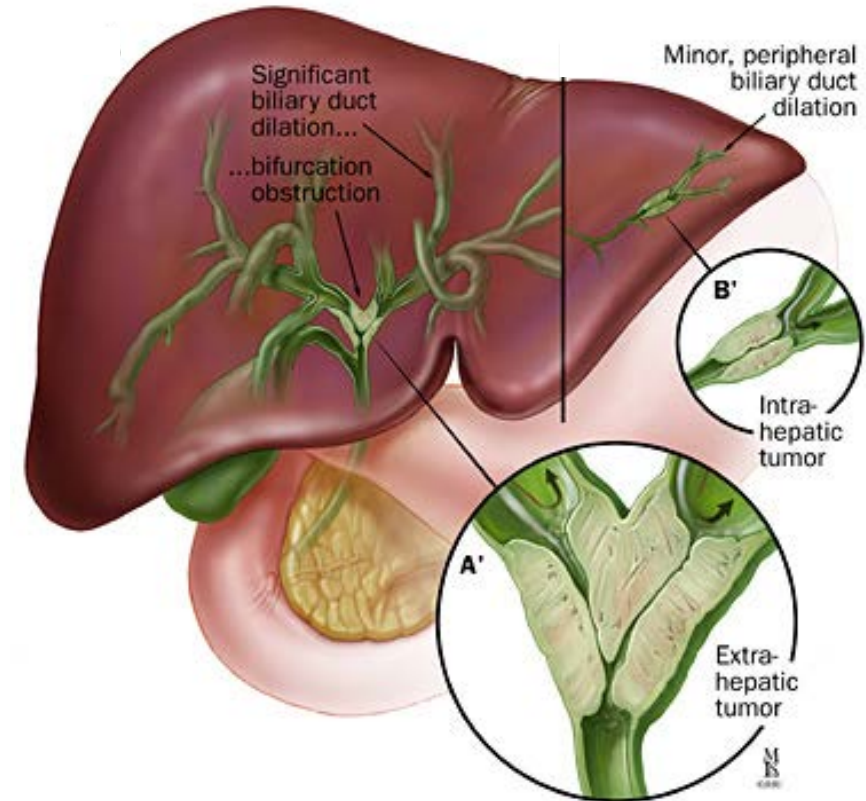
Carcinogenic potential of liver fluke
Opisthorchis felineus

Liver flukes infection is strongly associated with cholangiocarcinoma

International Agency for
Research on Cancer:
O. viverrini and *C. sinensis* were
both classified as “**carcinogenic
to humans**” (Group 1)

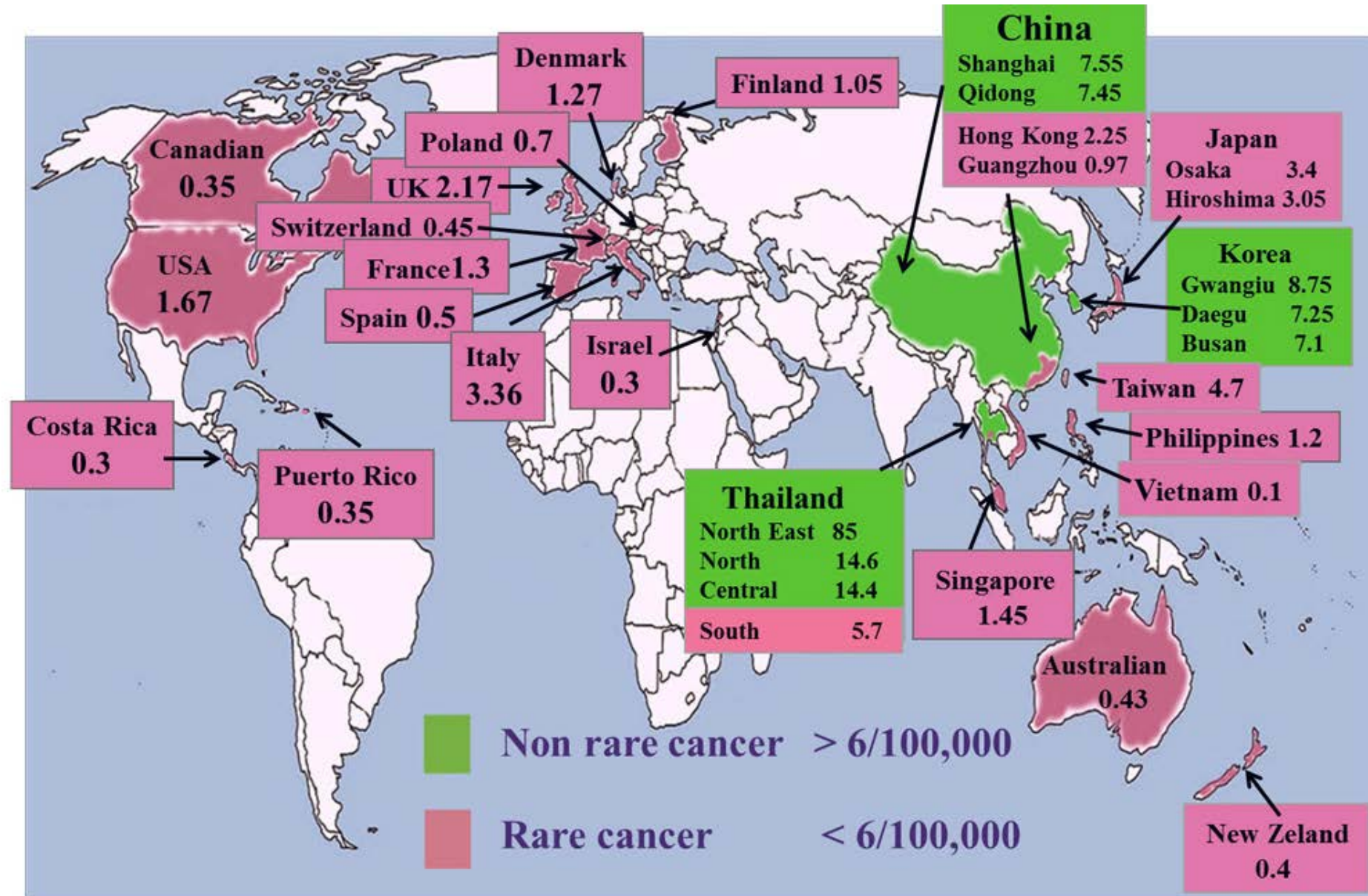
www.thelancet.com/oncology Vol 10 April 2009

**Carcinogenic potential
of *O. felineus* is not studied**

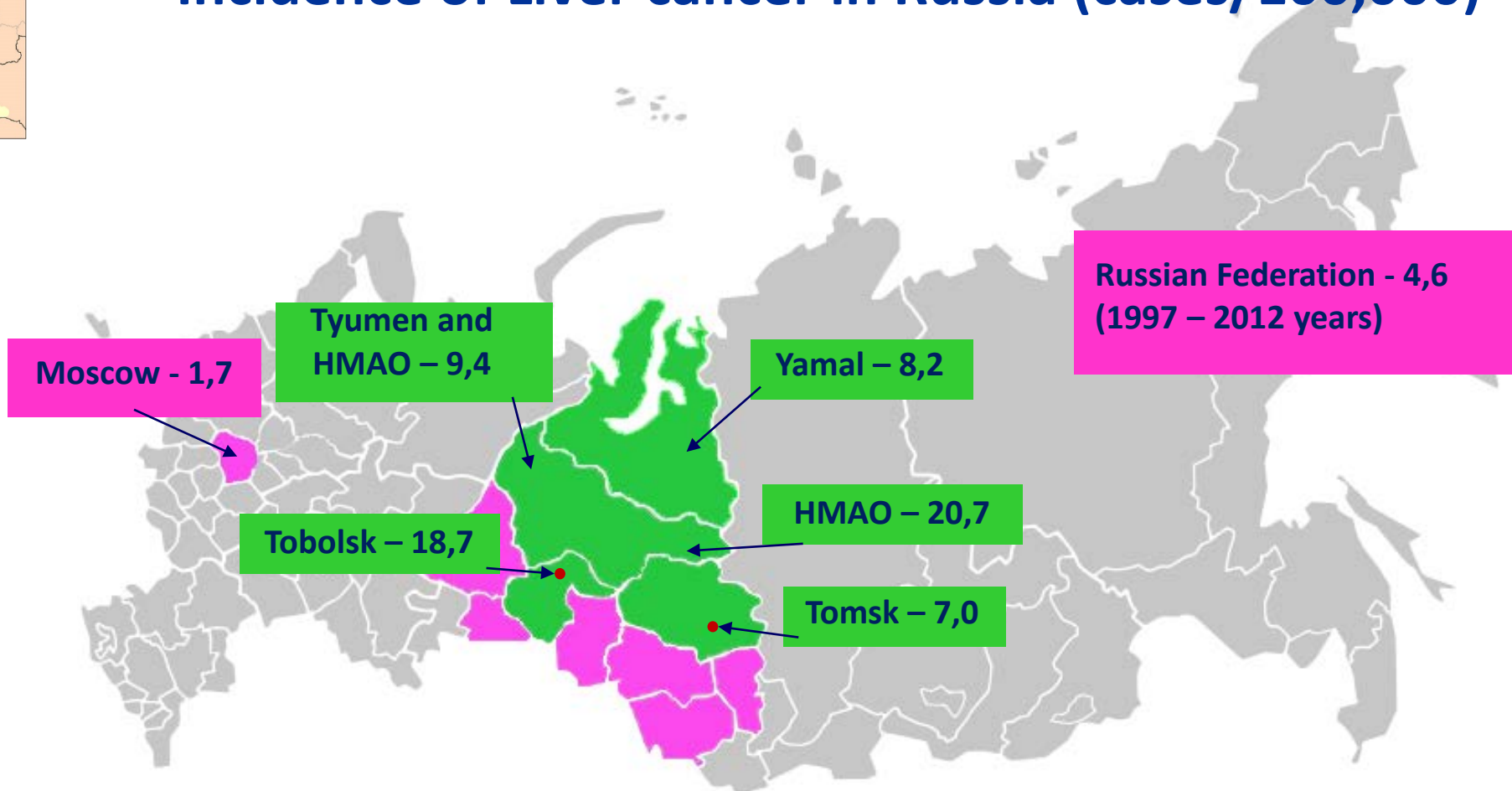


Cholangiocarcinoma (CCA)

Worldwide incidence of Cholangiocarcinoma (cases/100,000)



Incidence of Liver cancer in Russia (cases/100,000)



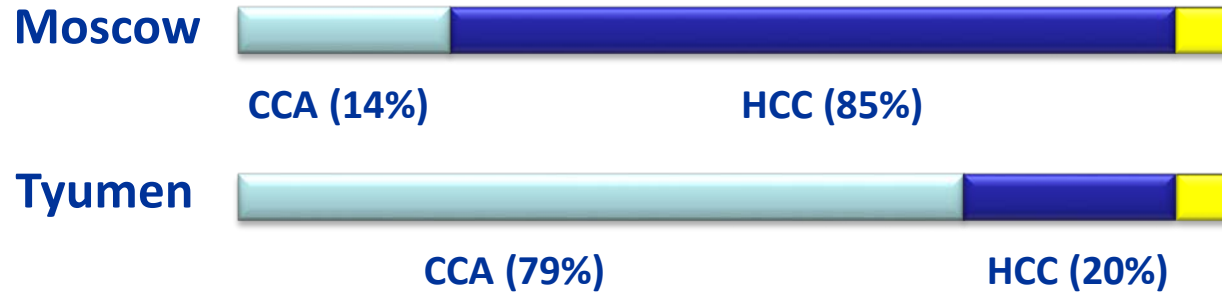
Russian Federation 2012
 Cancer - 525931 cases
 Liver cancer - 6287
Cases/100,000 - 4,6
The 14th position (1,5%)

Tyumen and HMAO
 1962-1971 years
 Liver cancer - 1225
Cases/100,000 - 9,4

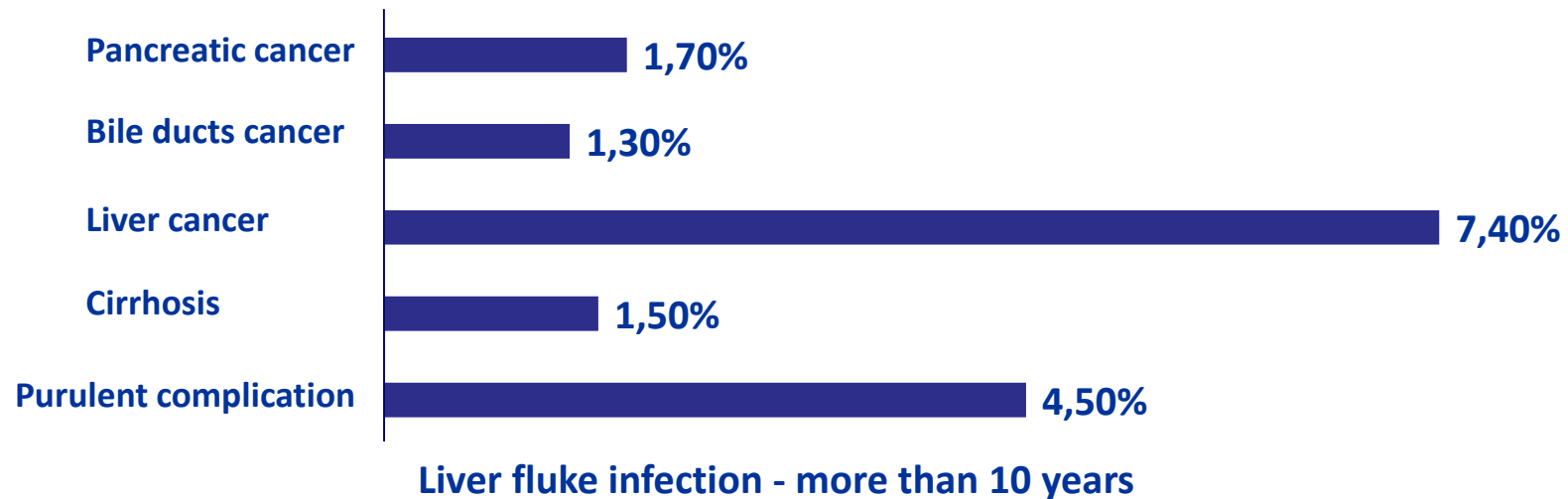
■ Non rare cancer > 6/100,000 cases
■ Rare cancer < 6/100,000 cases

Liver and bile duct cancer in Russia

Percentage of liver cancer types

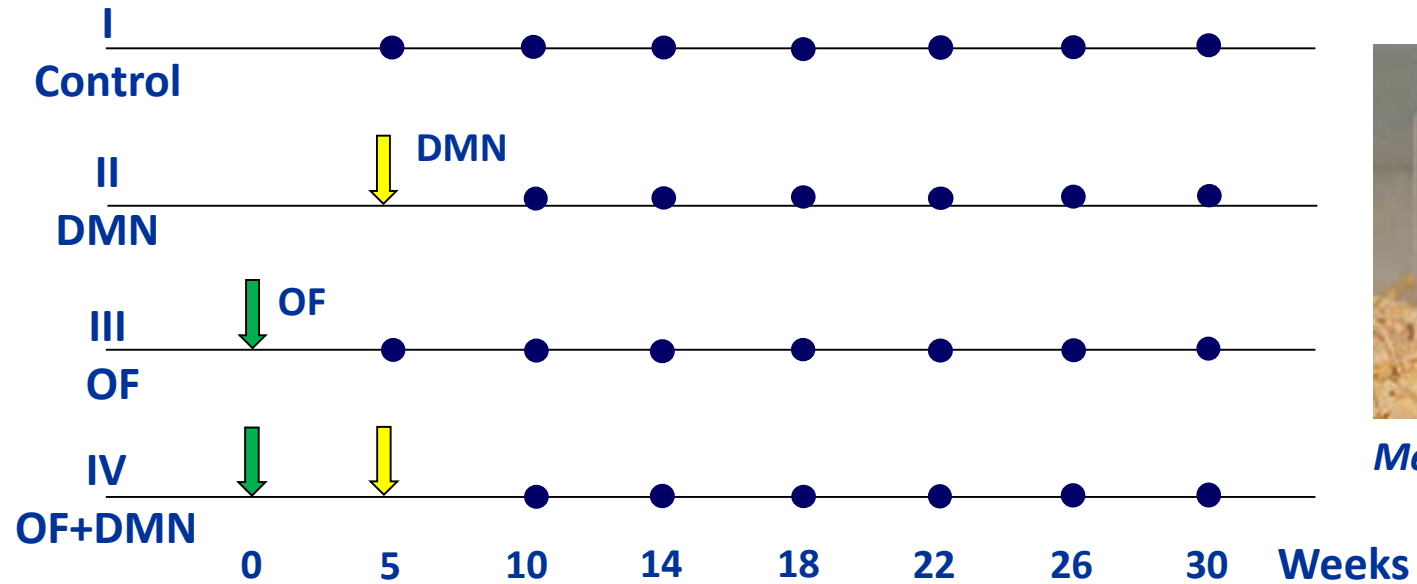


Chronic Opisthorchiasis (Tobolsk Mortuary, autopsy data, 1950-1987)



Two-step model of cholangiocarcinogenesis

Scheme of the experiment



Mesocricetus auratus

↓ – infection with *O. felineus* (OF)

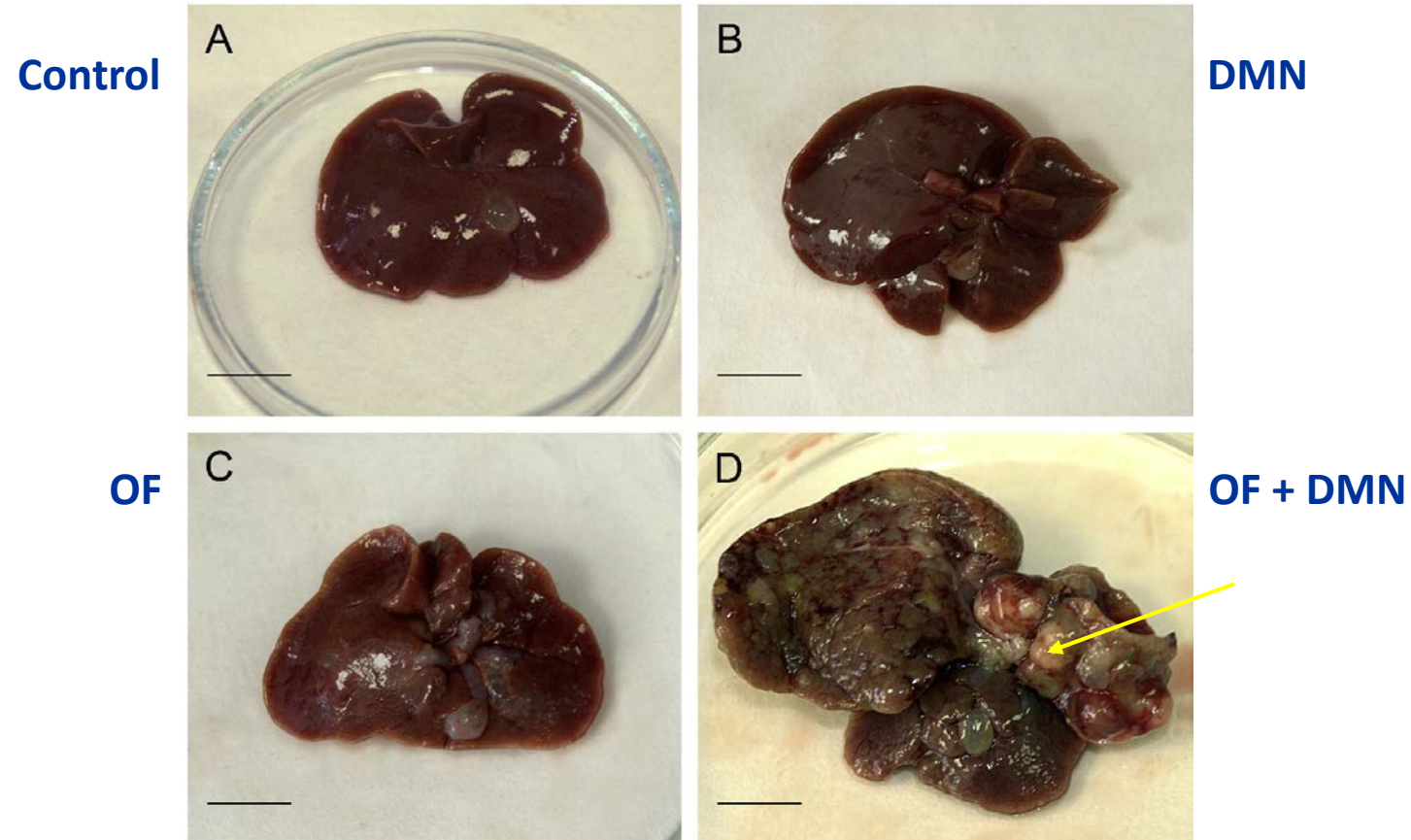
50 metacercariae

↓ – treatment with dimethylnitrosamine (DMN)

12,5 ppm

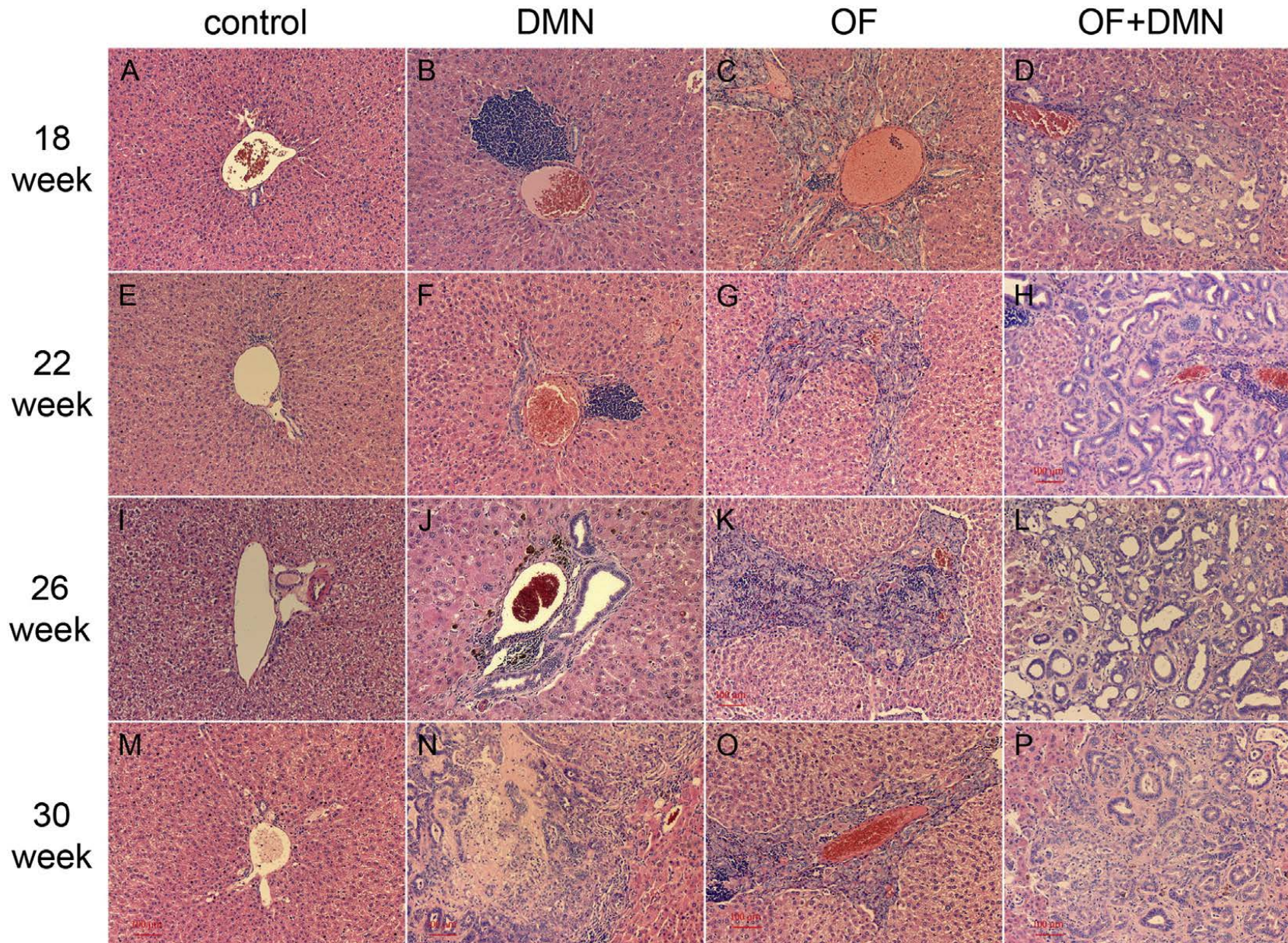
● – points of experiment

Two-step model of cholangiocarcinogenesis



The gross appearance of the liver, gallbladder, and extrahepatic bile ducts at 30 weeks post-infection: A, group I (control); B, group II (dimethylnitrosamine [DMN]); C, group III (infection with *Opisthorchis felinus*); and D, group IV (*O. felinus* + DMN). The scale bar is 1 cm. The arrow indicates small whitish yellow neoplasms on the liver surface.

Development of bile duct cancer in a hamster model



O. felineus + DMN

- CCA in the liver of the hamsters after 18 weeks p.i.
- After 30 weeks p.i., CCA was detected in all animals of this group

Hepatobiliary histopathological features of the hamster liver. Hematoxylin and eosin (H&E) staining, ×100 magnification.

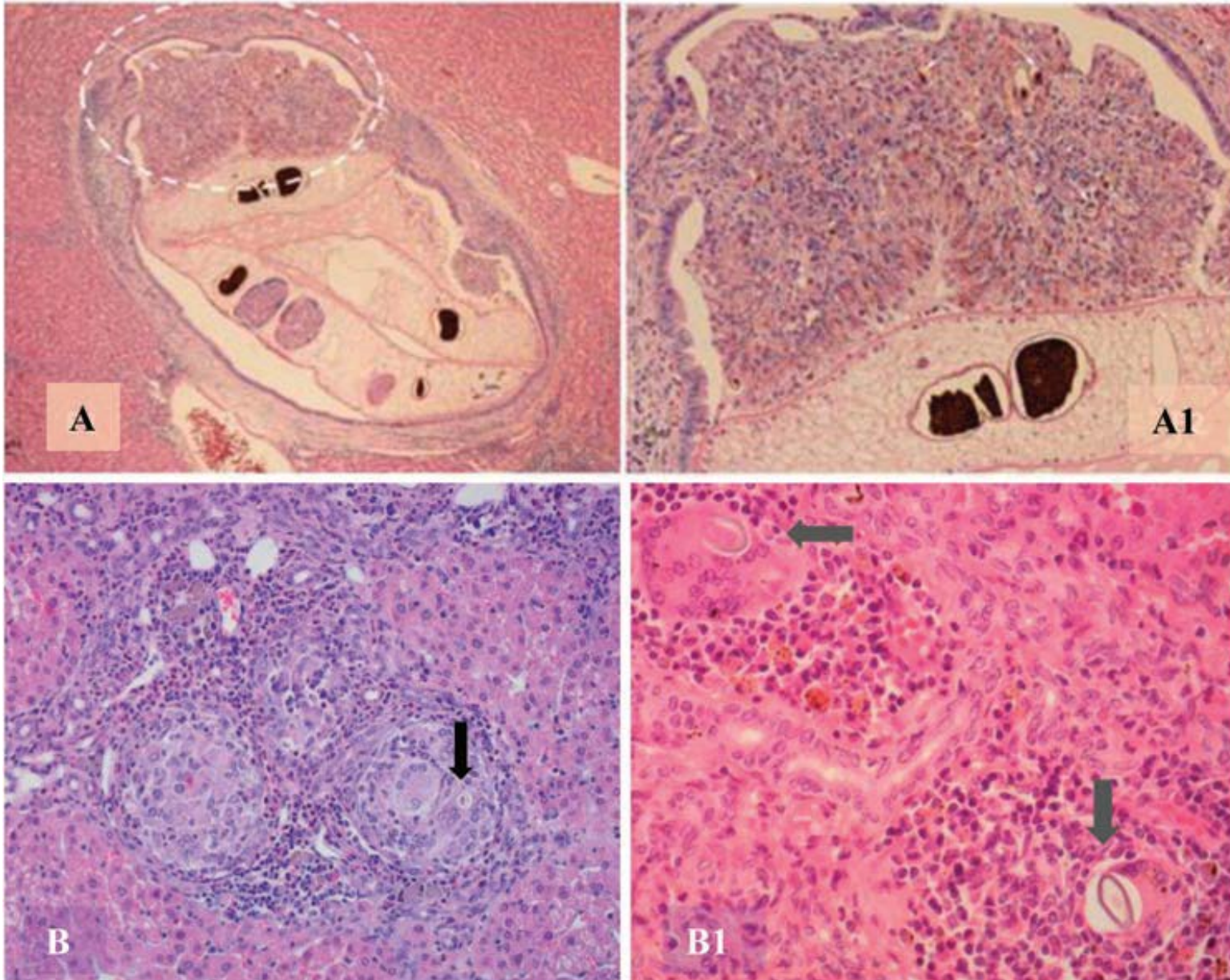
Conclusion

- **Two-step model of cholangiocarcinogenesis: *O. felineus* infection promotes formation of CCA in hamster model**

Question

- **How can one evaluate histopathological changes in hamsters experimentally infected with *O. felineus*?**

Granulomatous inflammation



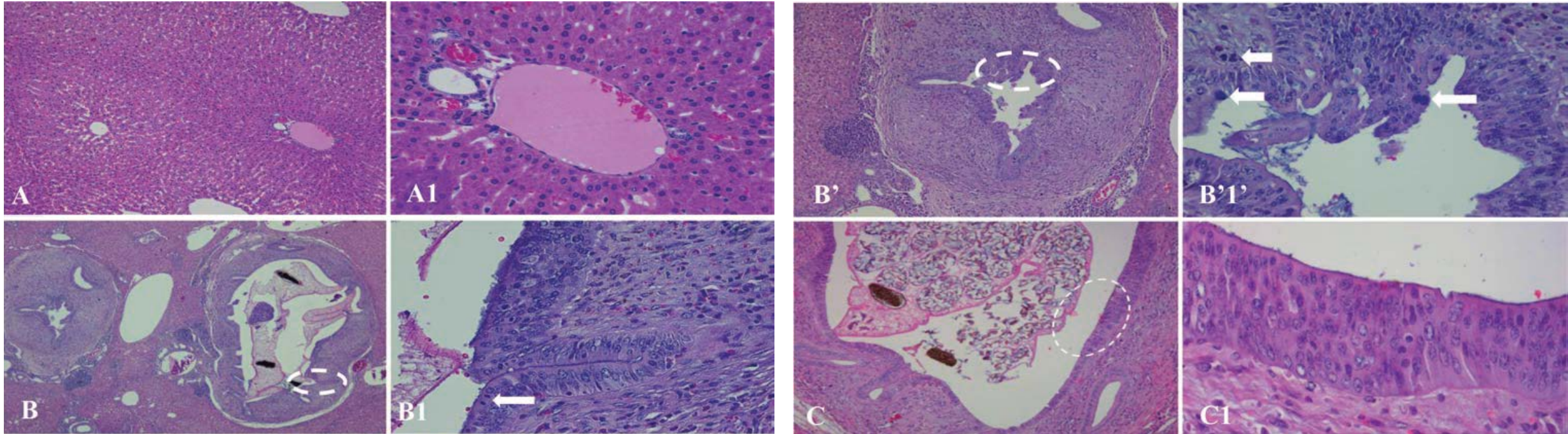
A. Epithelioid granuloma with multinucleated giant cells, lymphocytes, and eosinophils in portal area, occasionally surroundings eggs. Biliary duct obstruction caused the presence of an adult *O. felinus* liver fluke (magnification $\times 40$)

A1. Dashed line defines magnified area. Evidence of egg granulomata identified by white arrows surrounding by inflammatory cells (magnification $\times 100$)

B. Granulomas with multinucleated giant cell (magnification $\times 100$)

B1. Mononuclear and eosinophilic cell infiltration in portal regions and multinucleated giant cells surroundings eggs (arrow) (magnification $\times 400$)

O. felinus infection induces Biliary Intraepithelial Neoplasia



A: normal portal unit with bile duct, hepatic arteriole, portal venule, and a clearly defined limiting plate (magn $\times 200$). The smaller or interlobular bile ducts are lined by cuboidal or low columnar epithelium. No evidence of inflammation (H&E staining).

A1: defines magnified area (magnification $\times 400$) of normal portal unit.

B: biliary obstruction caused by the *O. felinus* worm with portal area enlargement (H&E staining, magnification $\times 100$).

B1: dashed line defines magnified area (magnification $\times 100$).

B' and B'1': biliary obstruction caused by *O. felinus*. Bile ducts were lined by enlarged nuclei, with pseudo-stratification, hyperchromatism and some loss of polarity, nuclear crowding, mitotic figures and low-to moderate-grade of dysplasia (**BillN**).

C: epithelium lining a large intrahepatic bile duct displays flat hyperplasia with dysplastic changes (**BillN1/2**).

C1: increased cellularity, modestly increased pseudo-stratification, and nuclear irregularities including variation in size and polarity, cytologic atypia including presence of nucleoli and loss of polarity (**BillN2**).

Conclusion

- Biliary Intraepithelial Neoplasia is not cancer, but it is associated with higher risk for developing cancer in future
- It is a precancerous state

Question

- What molecular mechanisms are involved in the development of a precancerous condition induced by *O. felineus*?

Oxidative stress markers accumulation in time-dependent manner

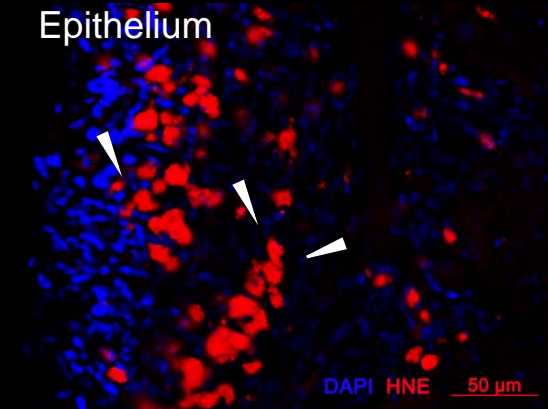
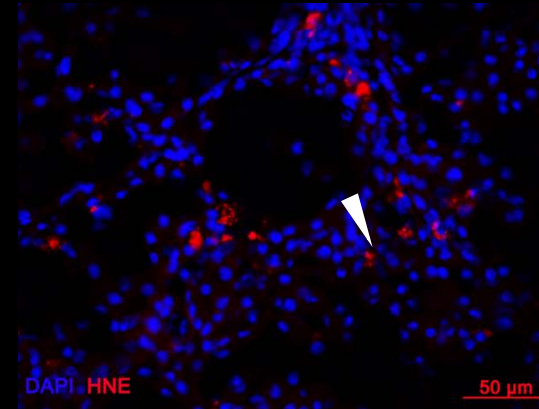
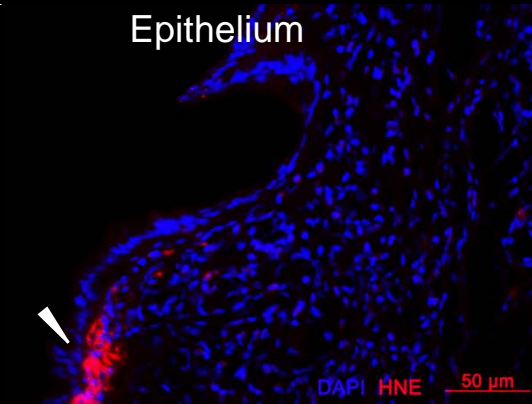
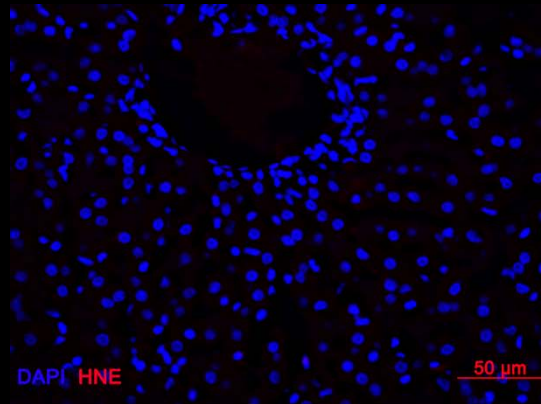
No infection

1 month

3 months

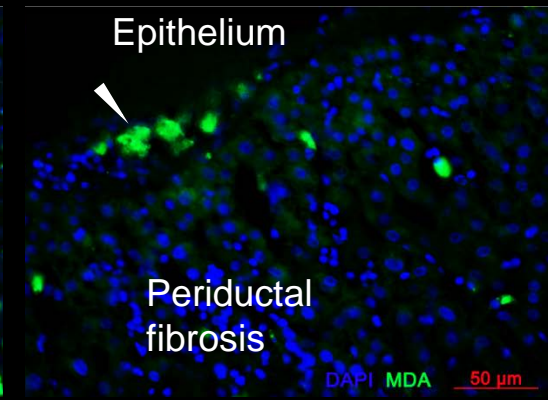
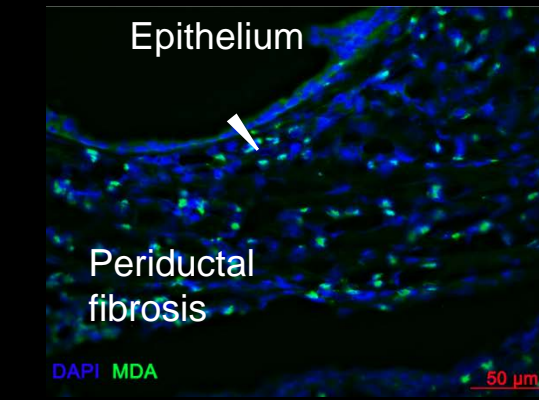
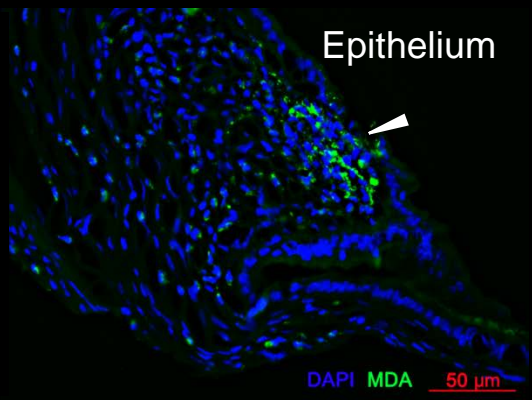
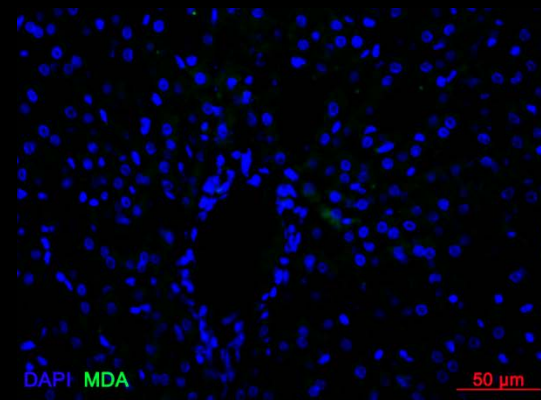
18 months

HNE
4-
Hydroxy
nonenal



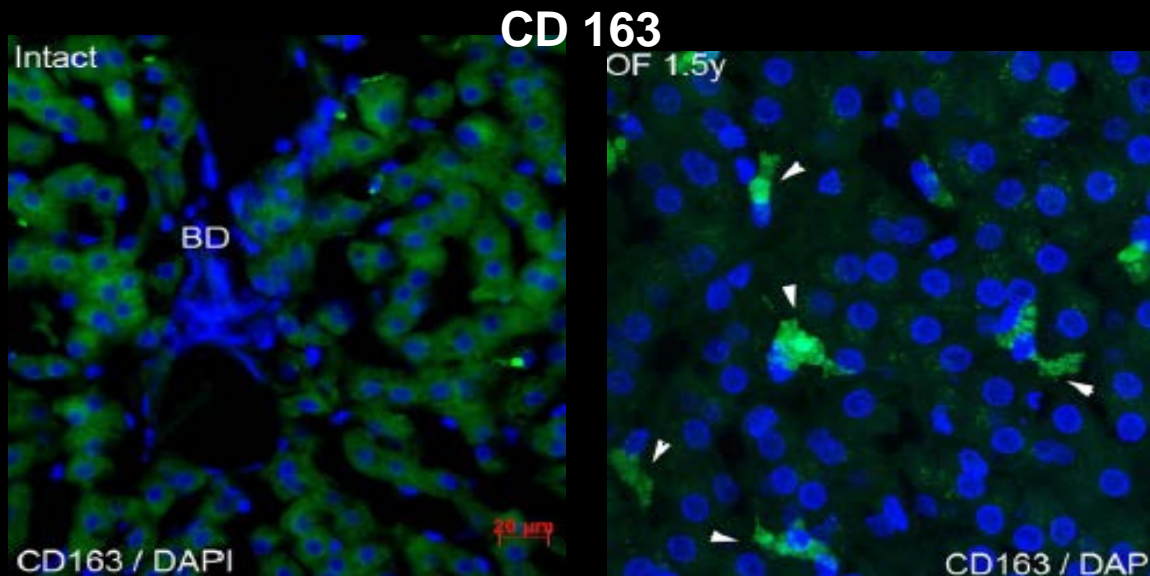
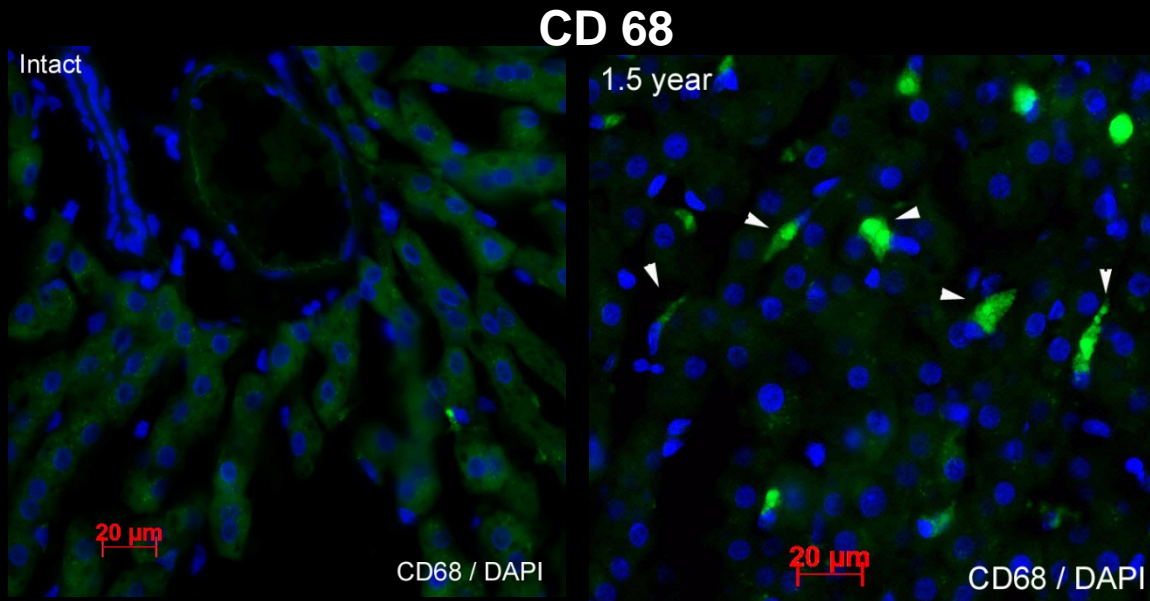
MDA

Malondi
aldehyde



Lipid peroxidation byproducts 4-hydroxynonenal and malondialdehyde were upregulated; these changes in general correlate with the dynamics of hepatic histopathological changes.

Inflammation markers are upregulated in the liver of infected hamsters



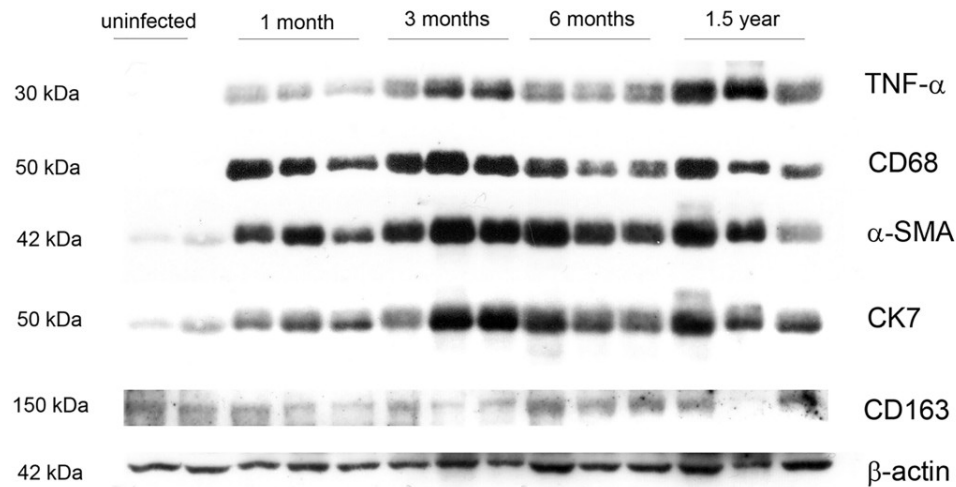
Expression of CD68 and CD163 demonstrated by immunohistochemistry.

CD68 – marker of monocyte lineage and tissue macrophages.

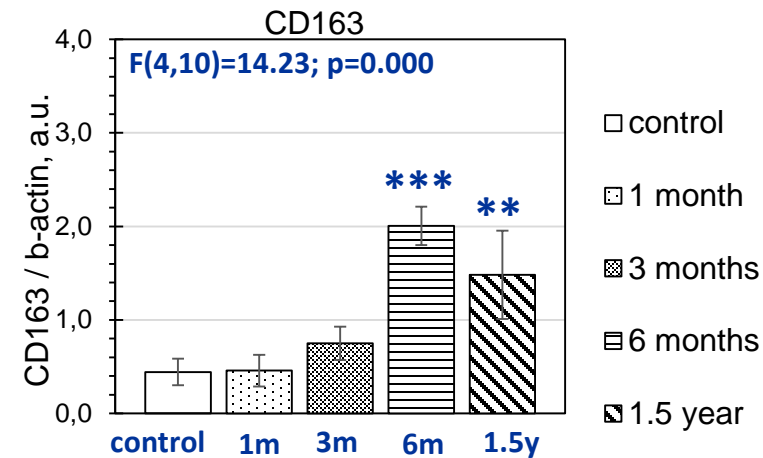
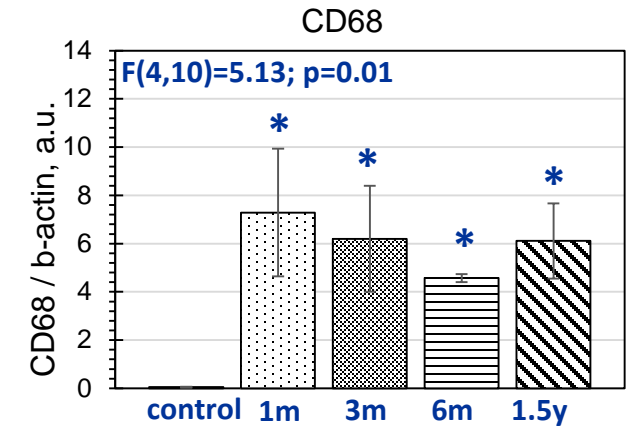
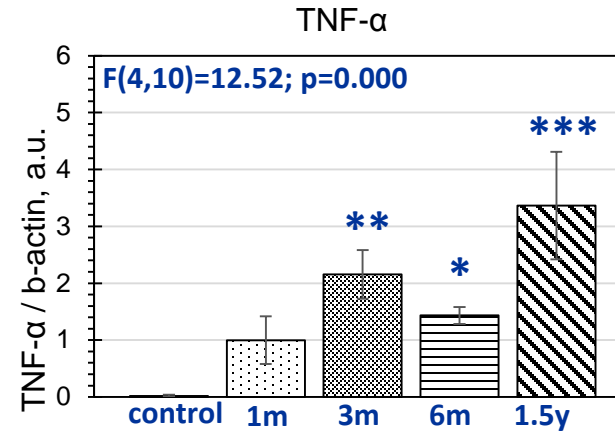
CD163 – marker of macrophages with alternative activated phenotype

In the liver of uninfected animals, both CD68 and CD163 proteins were hardly noticeable. Cells expressing both proteins CD68 were found in the inflammatory infiltrates, but also in the liver parenchyma of the infected animals. As long as the infection lasted, the number of cells CD163+ grew

Densitometric analysis of CD68, TNF- α , and CD163 protein levels in the liver of uninfected and infected hamsters



There was direct time-dependent elevation of TNF- α ($R = 0.79$; $p < 0.001$) and CD163 protein levels ($R = 0.58$; $p = 0.022$)

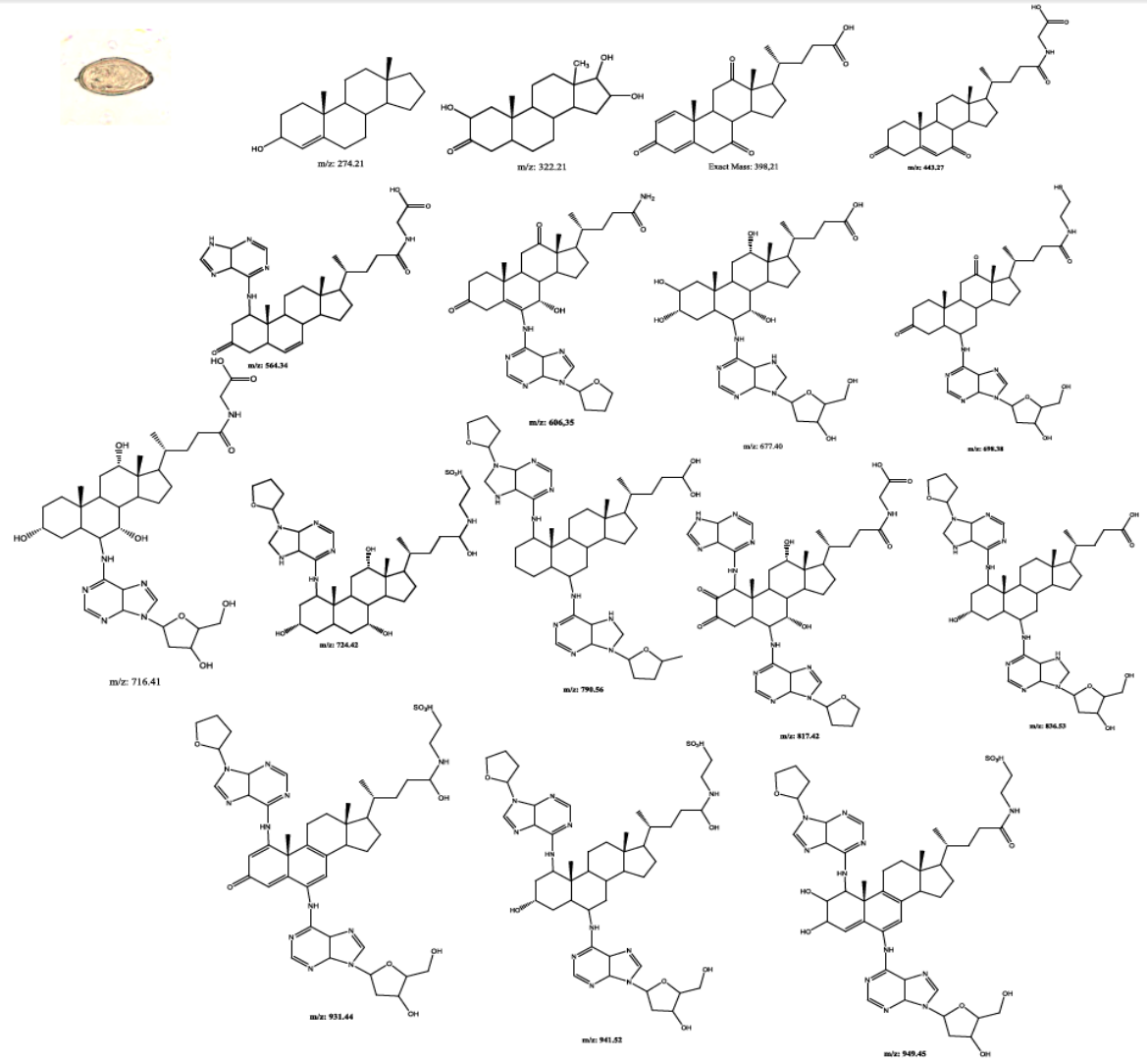
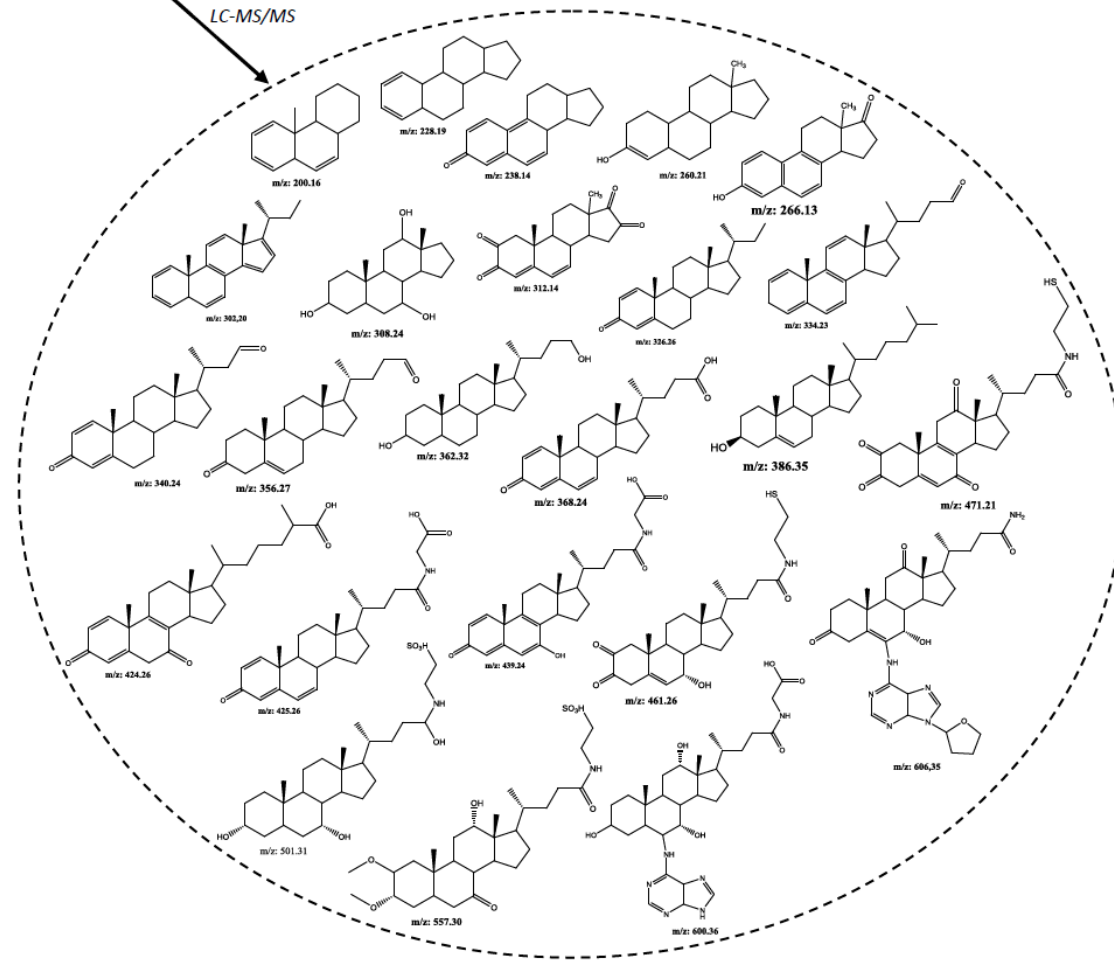


Oxysterol-like molecules in developmental stages of *O. felinus*



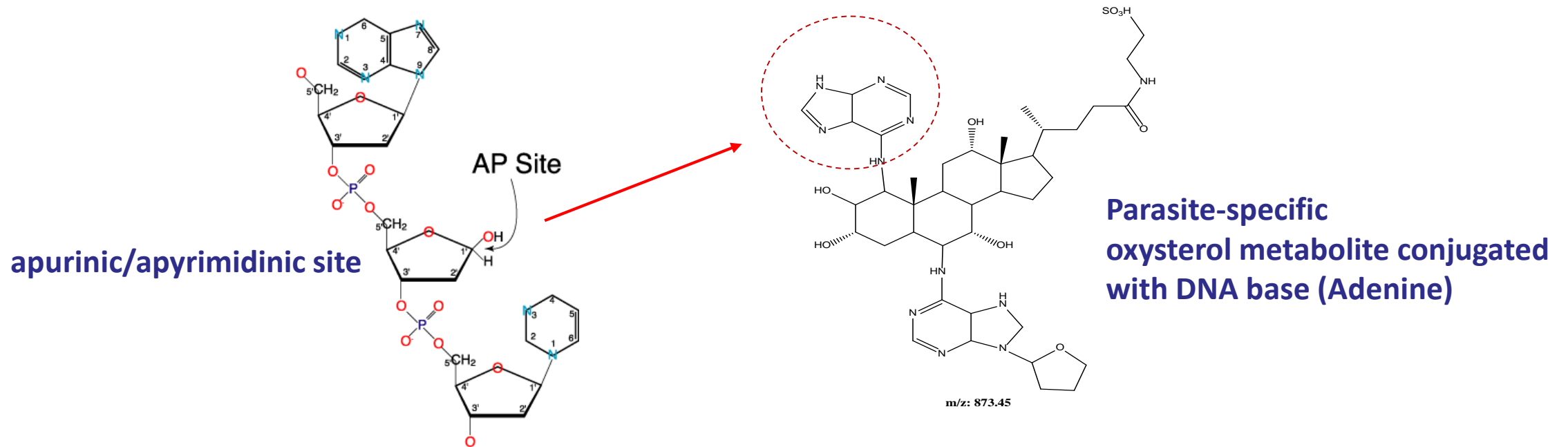
O. felinus

LC-MS/MS

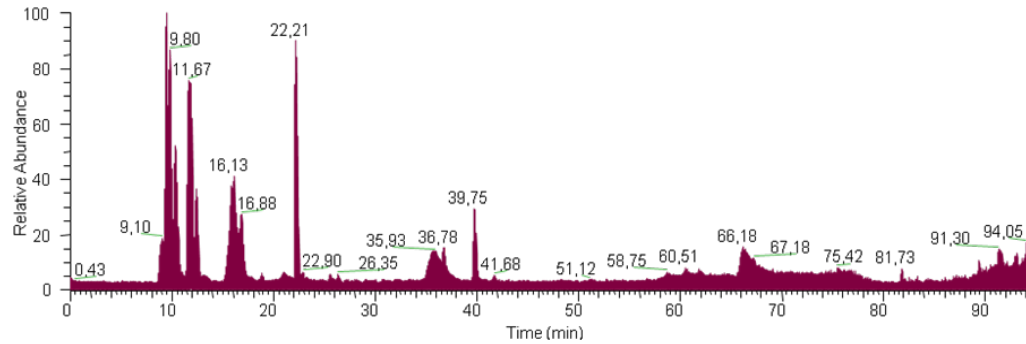


Oxysterols

- Products of oxidation of cholesterol that arise through enzymatic or non-enzymatic processes
- Oxysterols display mutagenic, genotoxic, pro-oxidative and pro-inflammatory properties that can contribute to malignancy
- Associations between oxysterols and the development and progression of cancer of colon, lung, breast and bile ducts have been proposed



Parasite oxysterol-like molecules in biological fluids during infection



Biofluids of *Opisthorchis felineus*-infected hamster

Bile

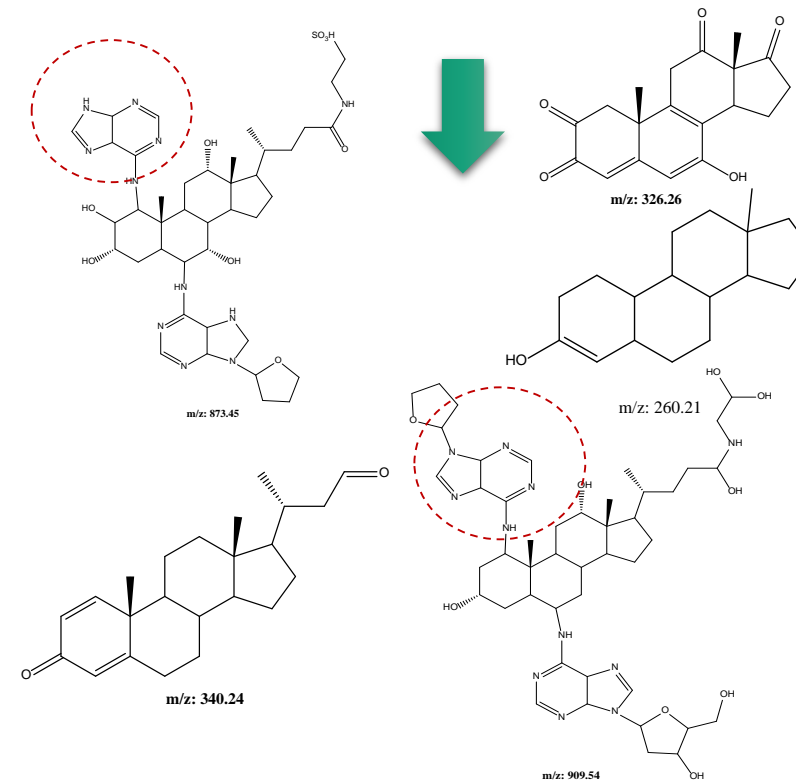
Metabolite (m/z)	(from <i>O. felineus</i>)	(from <i>O. felineus</i> and after metabolization)
339		117, 226, 257, 430, 483, 617, 872, 908

Sera

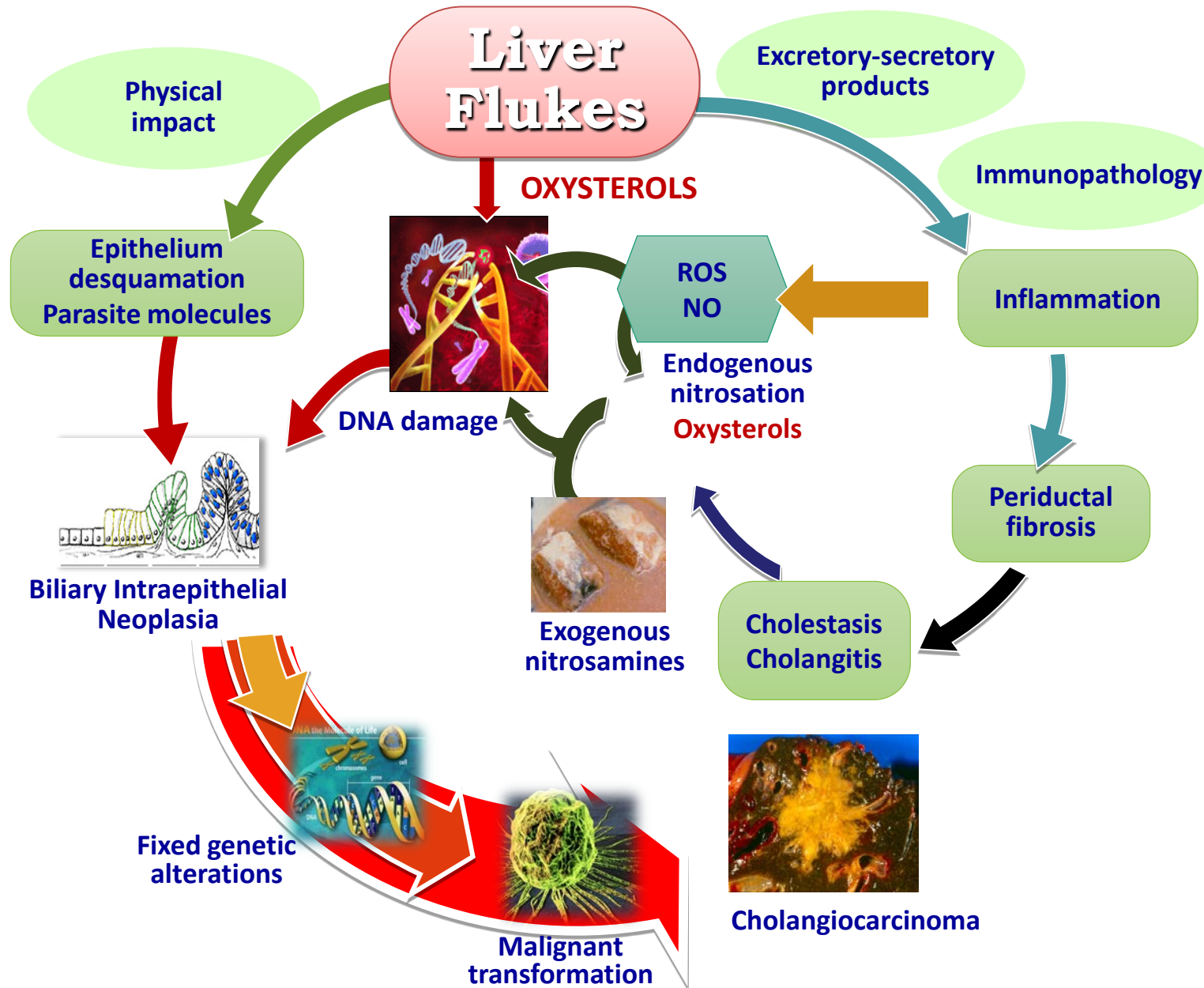
Metabolite (m/z)	(from <i>O. felineus</i>)	(from <i>O. felineus</i> and after metabolization)
259, 325		317, 539, 588, 667

Urine

Metabolite (m/z)	(from <i>O. felineus</i> and after metabolization)
125, 166, 188, 231, 292, 322, 387, 467, 523	



Potential mechanisms of liver fluke induced carcinogenesis



Modified
Sripa & Pairojkul (2008)

- **Search of potential molecular targets for new anthelmintics**
- **Combinatorial treatment of liver fluke infection**

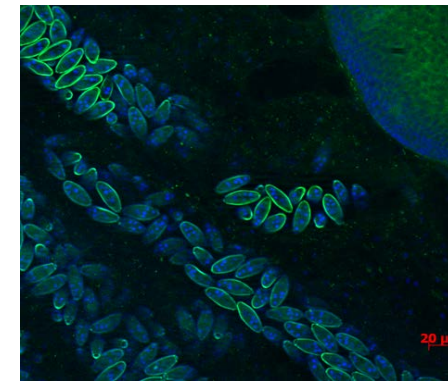
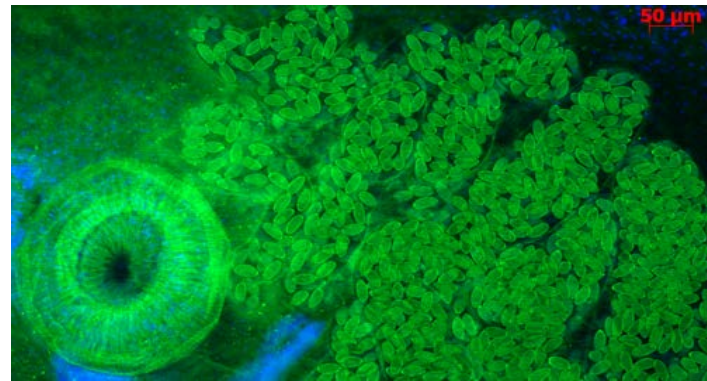
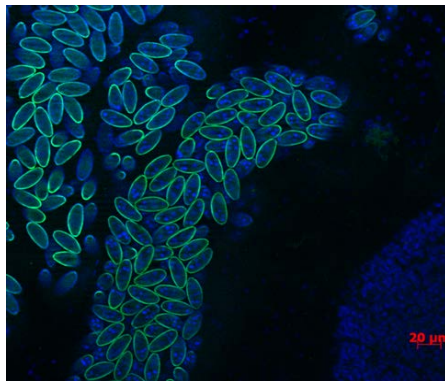
Praziquantel: *in vivo* study

PZQ	Dose, mg/kg	Animals	Worms per animal, \pm SD	Worms mortality, %
-	0	22	34 \pm 12	0
+	75	17	10 \pm 4	70%
+	400	7	6 \pm 4	81%

DAPI staining of *O. felineus* eggs

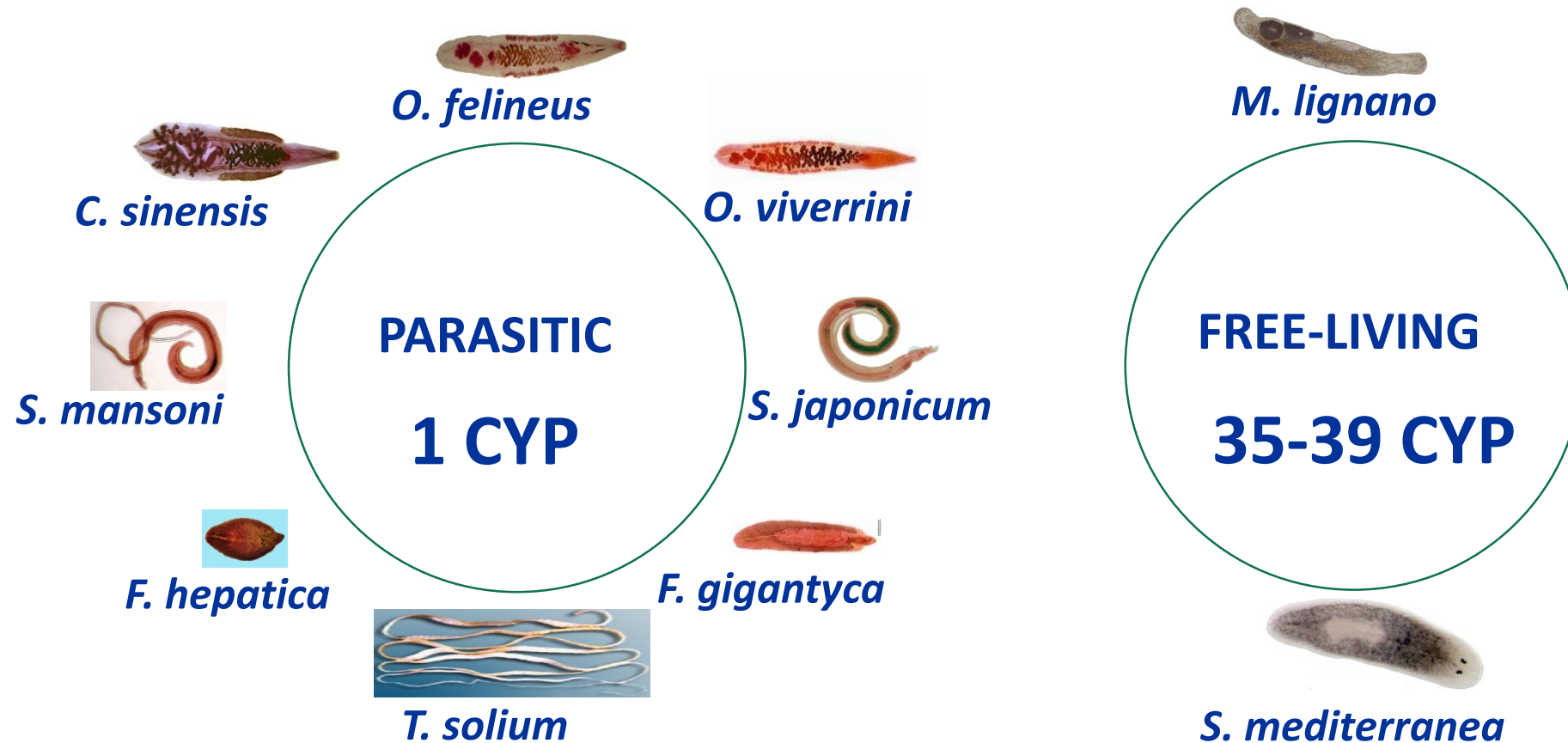
Control

Praziquantel, 21 days after treatment



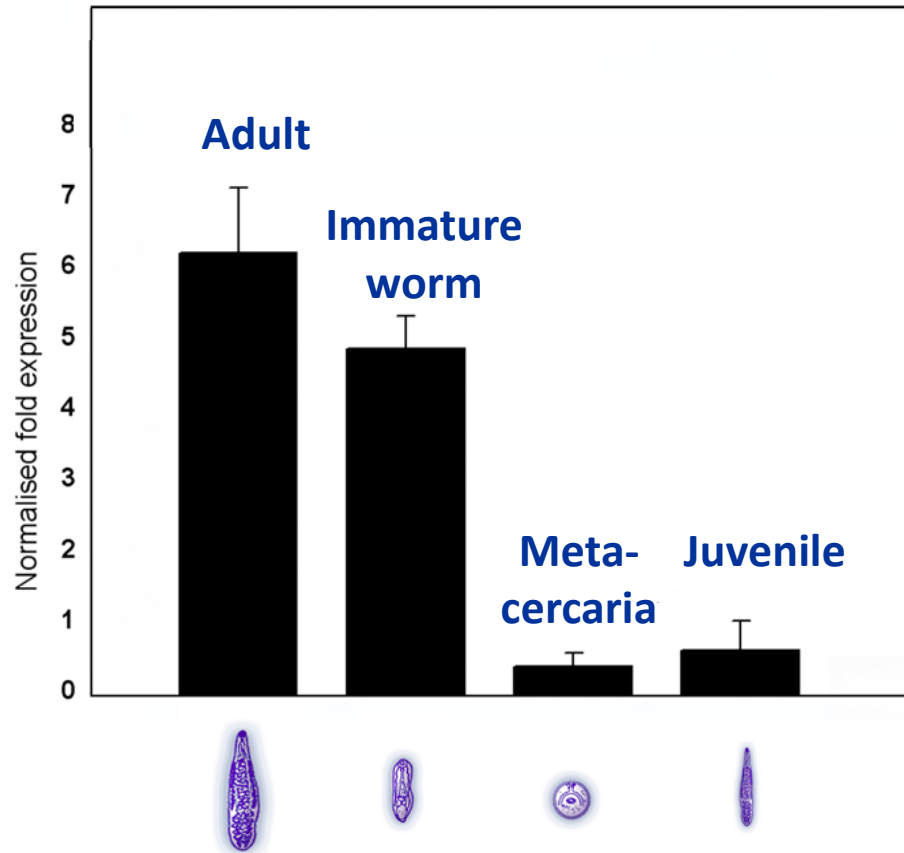
After treatment with praziquantel, 20 - 30% of the worms have demonstrated the normal body structure, motility, and state of eggs

In silico: CYP450 in Platyhelminthes



There is only one cytochrome P450 in parasitic flatworms. In addition, there are no any flavin-containing monooxygenases in nucleotide databases of *O. felineus* and other parasitic flatworms

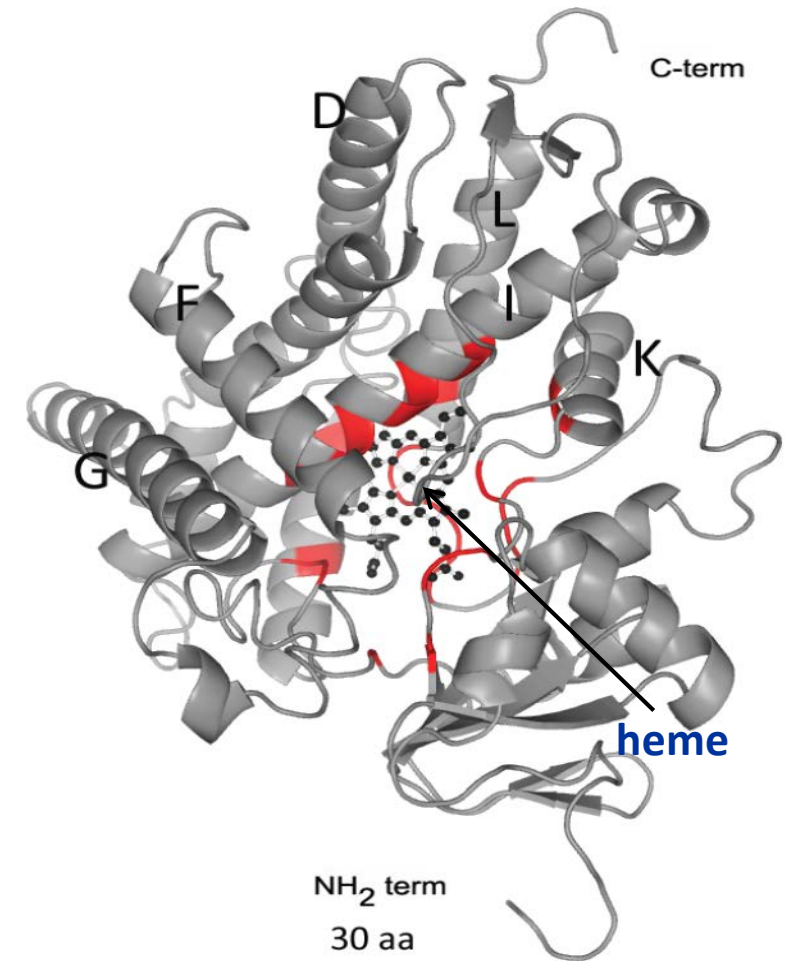
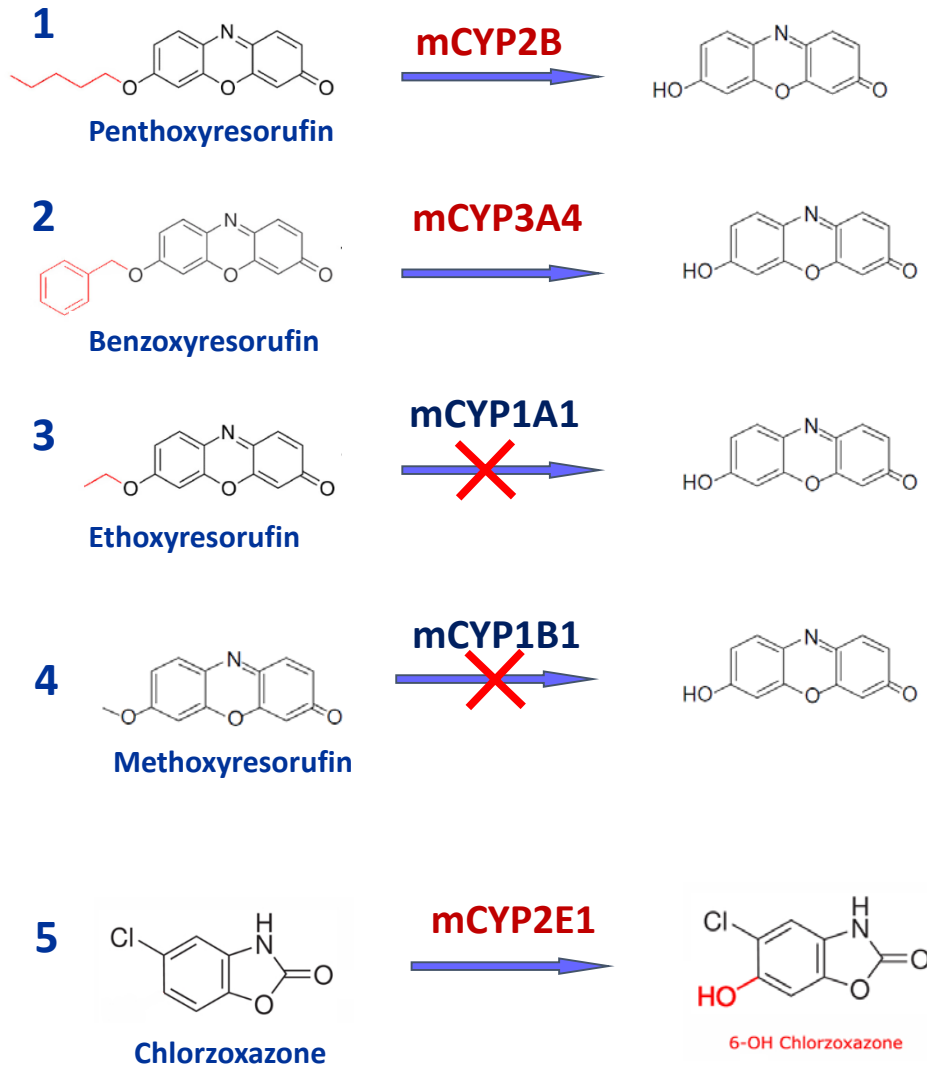
O. felinus CYP450 mRNA levels at different life stages



- CYP is differentially expressed throughout the *O. felinus* life cycle
- The level of CYP mRNA expression in adults is significantly higher than in other life stages
- The level of CYP gene expression in adult worm is comparable to the expression of such housekeeping genes as paramyosin, alfa tubulin and ubiquitin-like protein

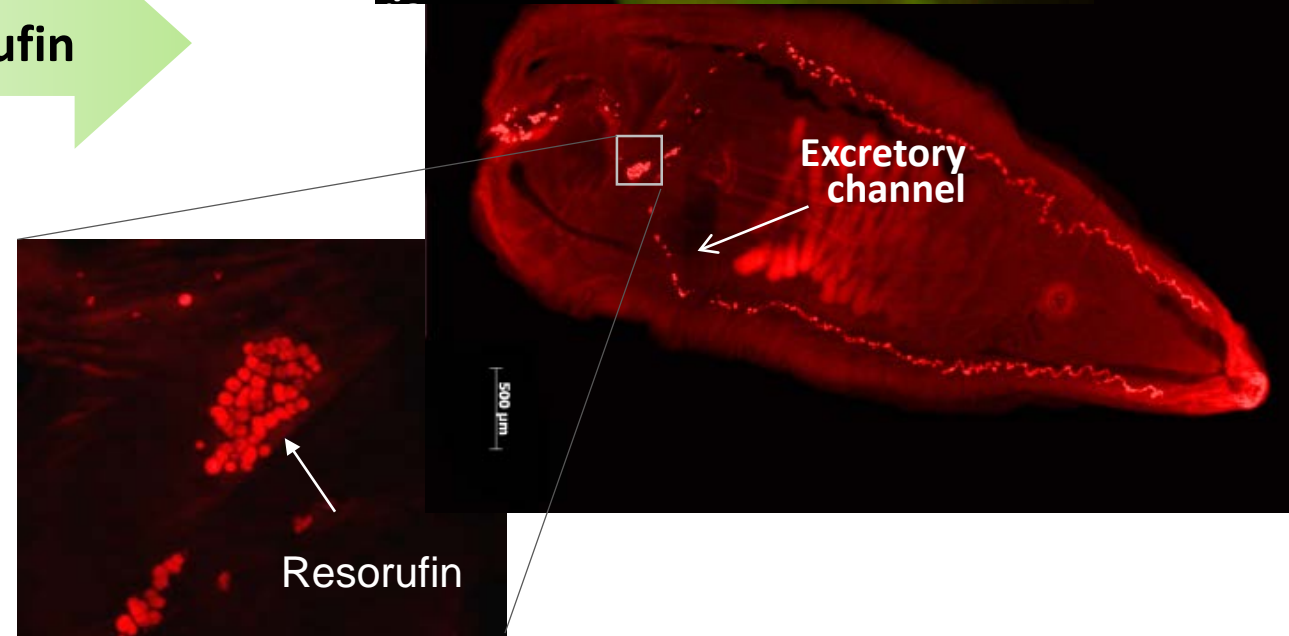
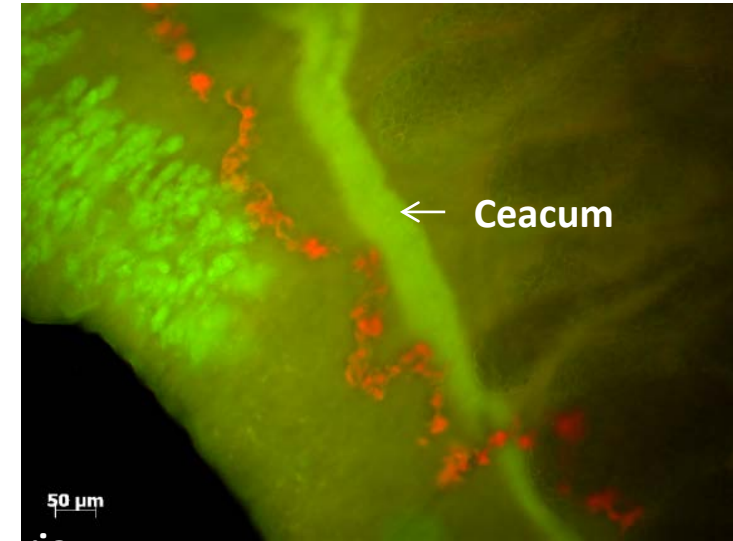
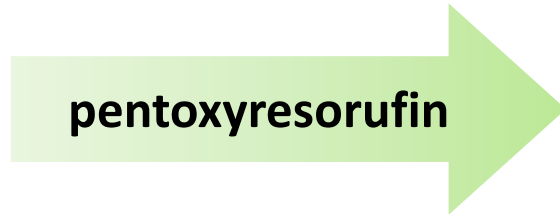
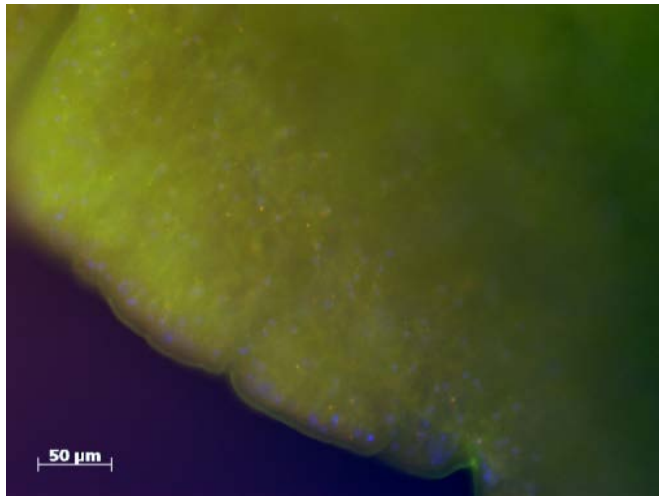
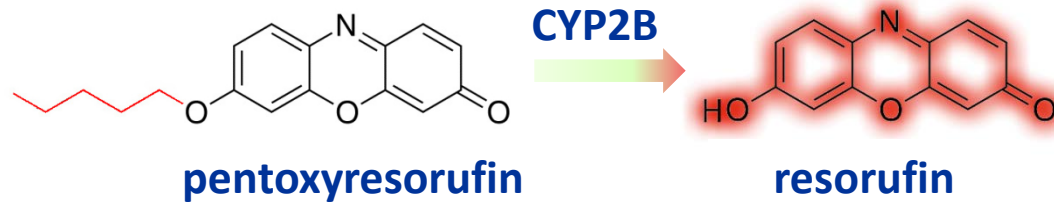
Internal RT-PCR controls: paramyosin, alfa tubulin, ubiquitin-like protein, mitochondrial ribosomal protein L14

Spectrum of activity of *O. felineus* CYP

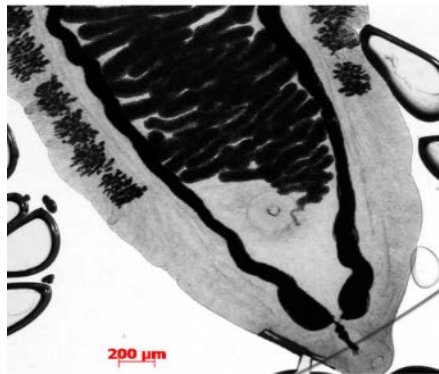
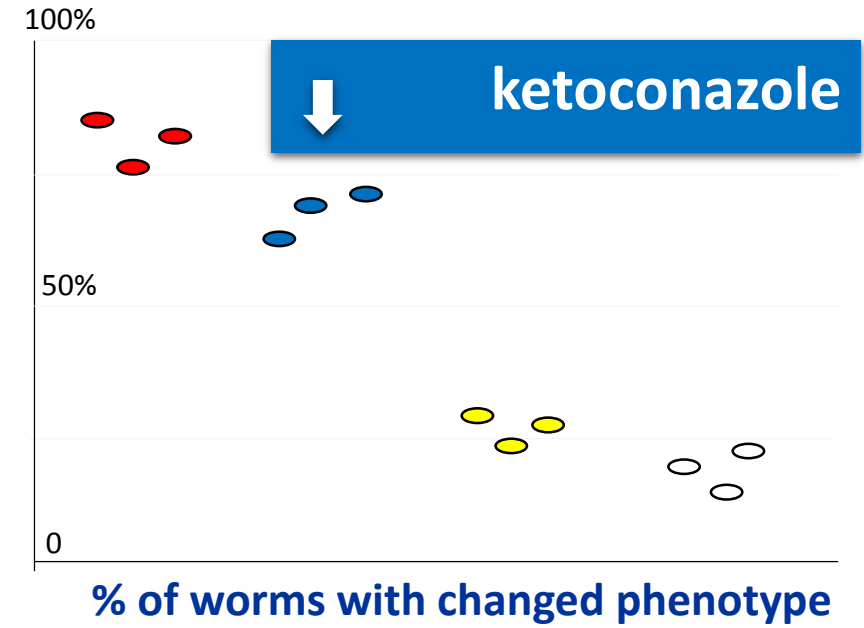
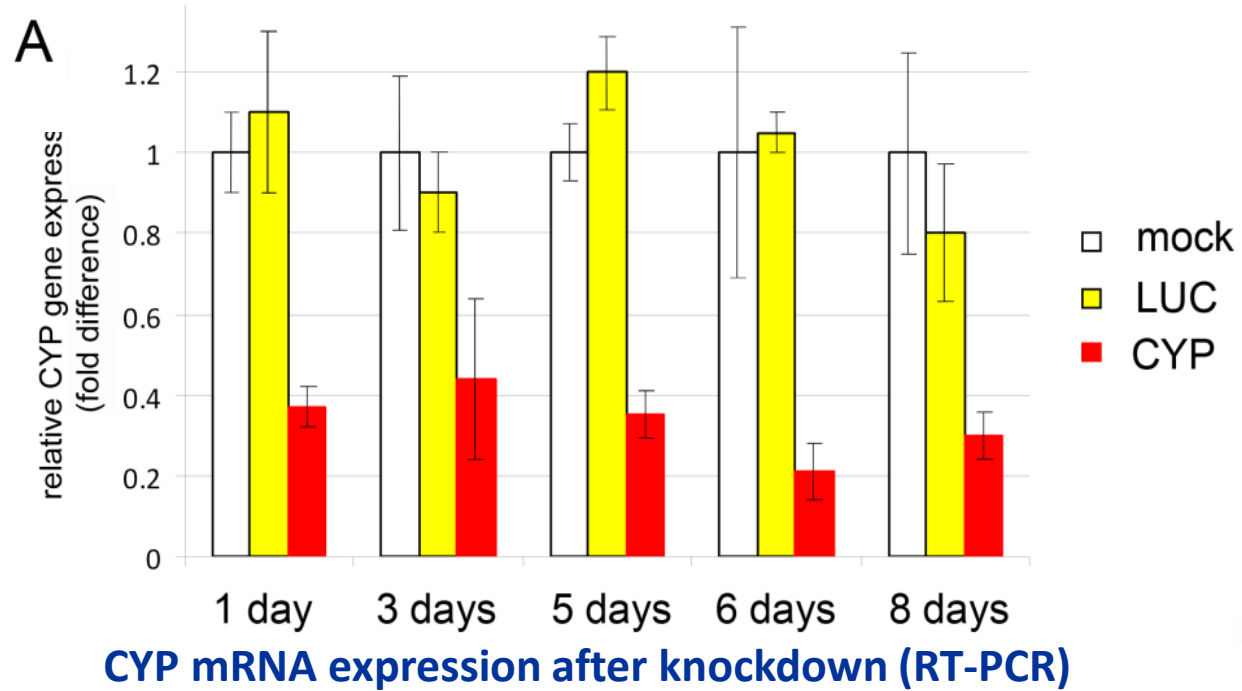


3D modeling of *O. felineus* CYP450

O. felineus CYP activity in the fluke tissue

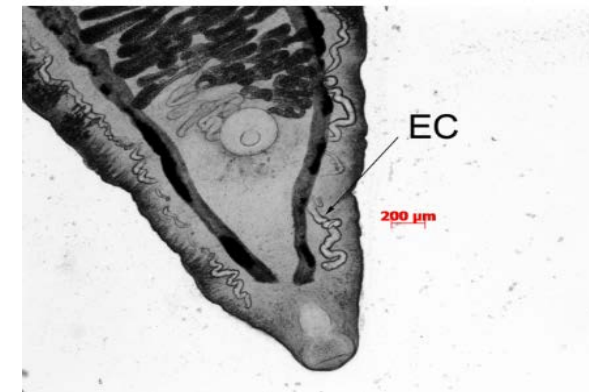


CYP gene knockdown and ketoconazole effects on *O. felinus* adults



Control

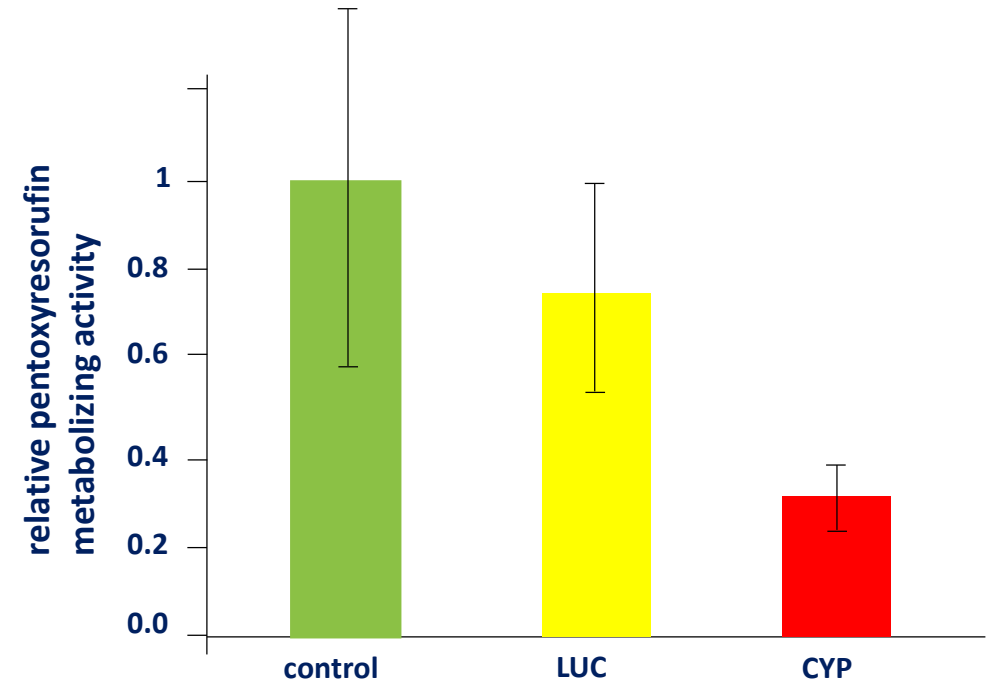
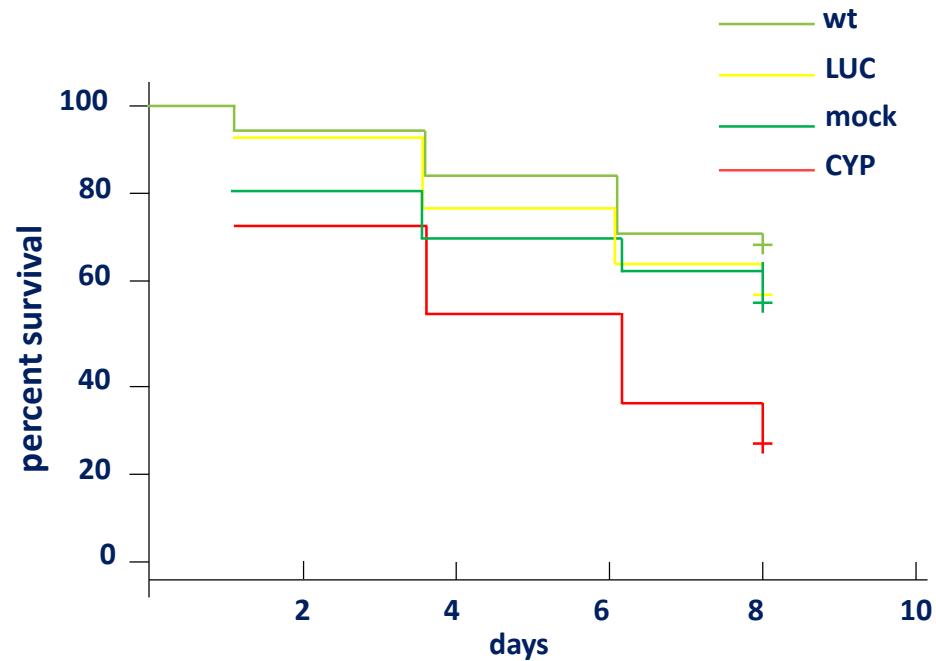
CYP gene knockdown or ketoconazole



Experiment

Deformation of the excretory channels (EC)

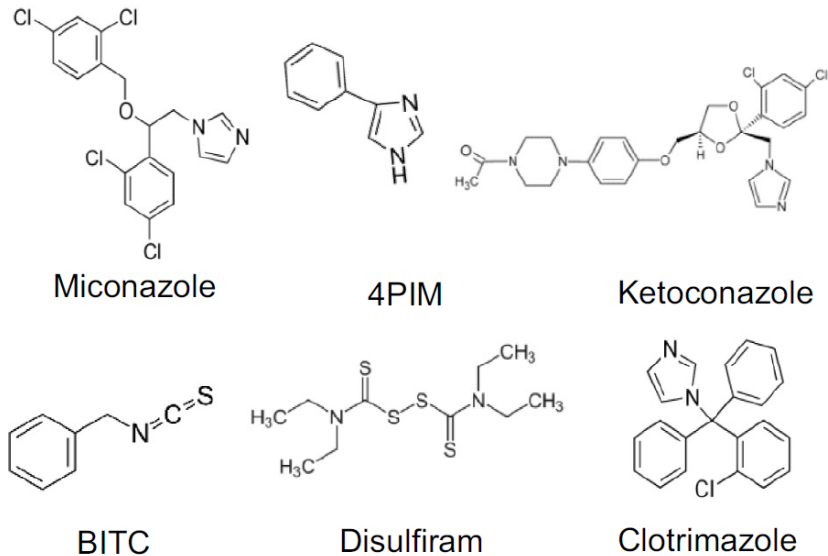
Survival curves and pentoxyresorufin metabolizing activity in worm tissues under the influence of RNA interference



Decrease of CYP gene level expression and CYP activity led to change of the phenotype and increase in worms death

Anthelmintic activity of cytochrome P450 inhibitors: in-vitro effect on the liver fluke *Opisthorchis felineus*

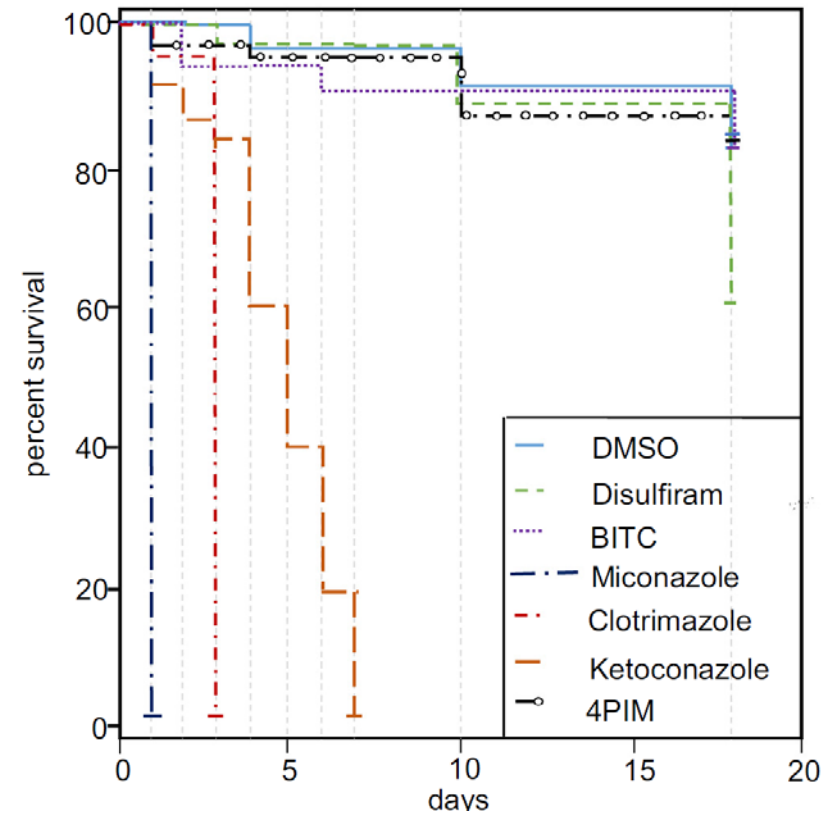
Structures of the cytochrome P450 inhibitors



	Adults IC ₅₀ (μM)	NEM IC ₅₀ (μM)
Praziquantel	0.47	0.98
Miconazole	20.05	0.79
Clotrimazole	18.03	1.25
Ketoconazole	13.77	16
BITC	27.2	16
4PIM	>>100	>>100

4PIM, 4-phenyl imidazole; BITC, benzyl isothiocyanate

Survival of newly excysted metacercariae



Kaplan–Meier survival curves. Inhibitors: 10 μM BITC, 10 μM disulfiram, 10 μM miconazole, 10 μM clotrimazole, 10 μM 4PIM, 40 μM ketoconazole or DMSO.

Disodium salt of glycyrrhizic acid

A novel supramolecular delivery system for Praziquantel

Structural formulas of PZQ and glycyrrhizic acid

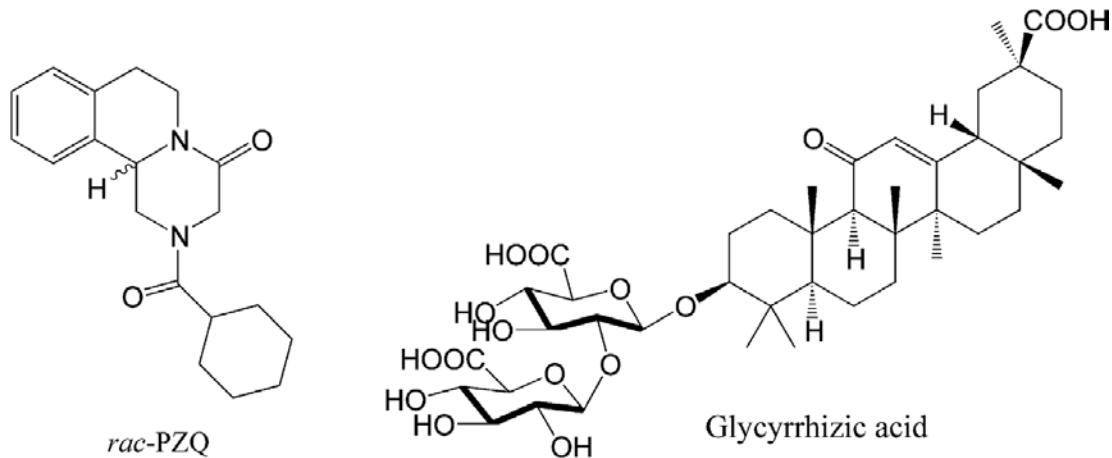
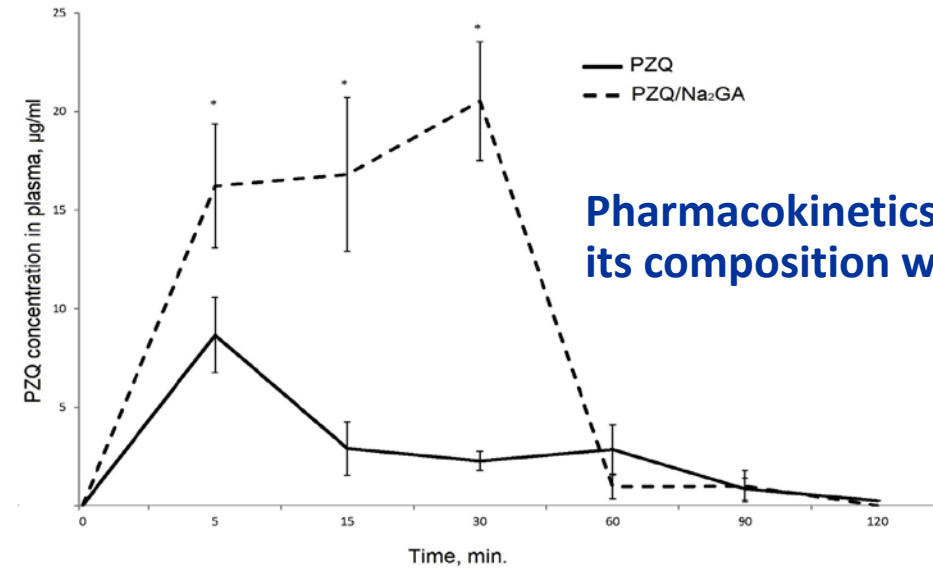
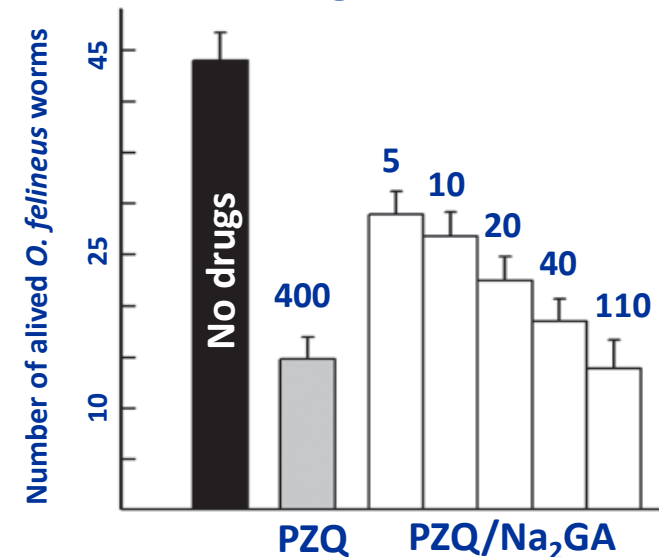


Table 1. Solubility of PZQ samples and compositions

Sample composition, weight ratio*	PZQ solubility in water, g/L, 37°C	Increase in solubility, <i>n</i> times
Original PZQ	0.234	—
PZQ/Na ₂ GA 1/5	0.557	2.38
PZQ/Na ₂ GA 1/10	0.687	2.94
PZQ/Na ₂ GA 1/20	0.817	3.49



In vivo testing of PZQ and PZQ/Na₂GA 1/10



Up to 10-fold increase in the anthelmintic activity of PZQ in the composition as compared to PZQ alone

Acknowledgements



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University of Porto



José Manuel Correia da Costa, Nuno Vale - University of Porto, Portugal

Banchob Sripa, Thewarach Laha - Khon Kaen University, Khon Kaen, Thailand



TOPIC
TOMSK OPISTHORCHIASIS
CONSORTIUM

Tomsk Opisthorchiasis Consortium

Thank you for your attention!

A study of tribendimidine effects *in vitro* and *in vivo* on the liver fluke *Opisthorchis felineus*

In vitro

Efficiency of tribendimidine (TBN)

IC50 = 0.23 μ M for newly excysted metacercariae

IC50 = 0.19 μ M for adult

Efficiency of praziquantel (PZQ)

IC50 = 0.98 μ M for newly excysted metacercariae

IC50 = 0.47 μ M for adult worms

In vivo

Chronic infection

TBN at 400 mg/kg - 77.2% worm burden reduction

PZQ at 400 mg/kg - 76% worm burden reduction

CONCLUSION: The differences between worm burden reduction values after PZQ and TBN treatment were not significant, thus TBN was as effective as PZQ against *O. felineus* liver flukes. Given the broad-spectrum activity of TBN and efficacy against *O. felineus*, this drug may be a promising candidate for the treatment of opisthorchiasis felinea and other liver fluke infections.



Institute of Cytology and Genetics (ICG) Siberian Branch of the Russian Academy of Sciences



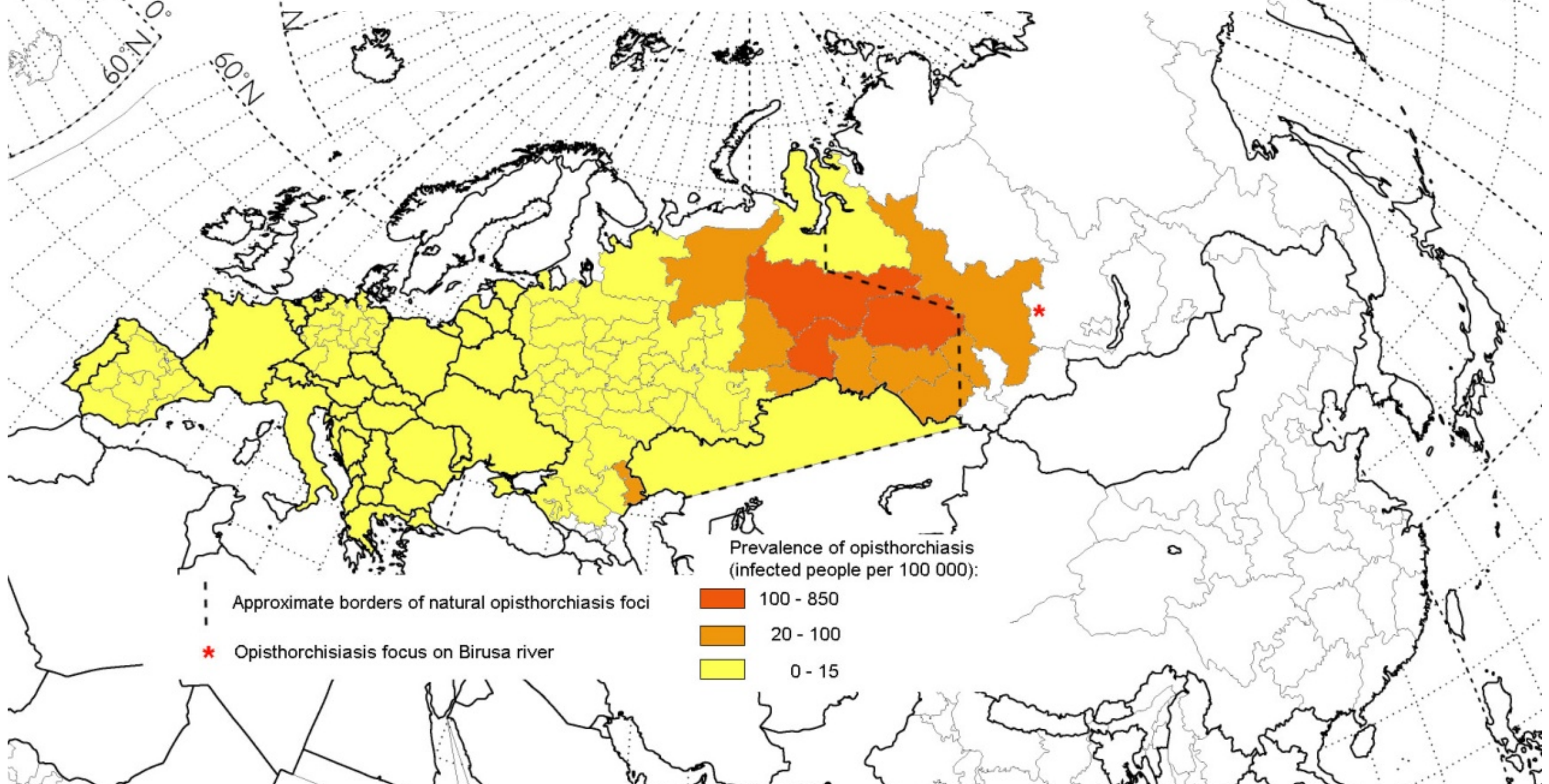
Permanent staff: 1400

Post-graduate students: 96

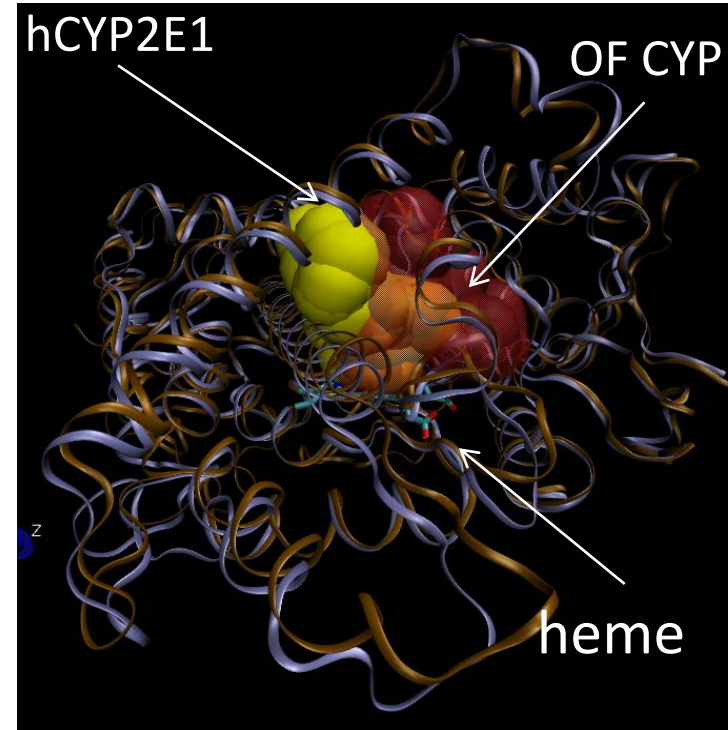
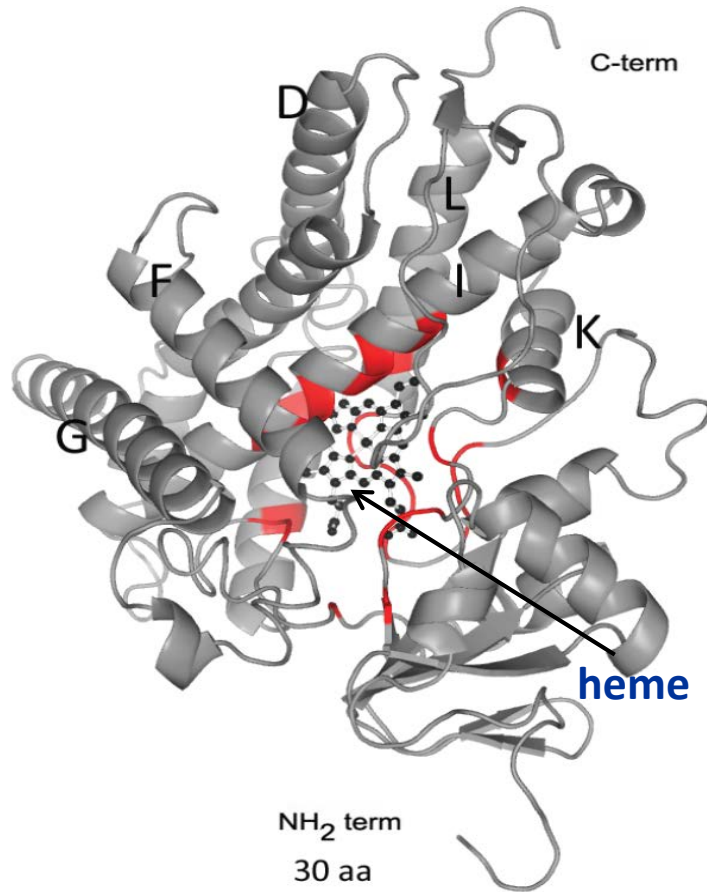
Graduate students: 160

<http://www.bionet.nsc.ru>

Geographical range of *Opisthorchis felineus* and the prevalence of opisthorchiasis



3D modeling of *O. felinus* CYP450



➡ The predicted secondary structure showed high level of similarity with the CYP2 proteins of mammals, especially with human CYP2E1, which was the reason for selecting the structure of 2E1 as a reference for the 3D modeling (Phyre2 multi-template modeling, 6 templates).

Praziquantel is metabolized in the liver with involvement of 2B1 and 3A4 isoforms of cytochrome P450 (CYP2B1 and CYP3A4)

Whether liver fluke *O. felineus* has functionally active CYP(s)?