

# *Opisthorchis felineus* AND *Metorchis bilis*: DEVELOPMENT OF ITS-2 BASED MULTIPLEX PCR ASSAY FOR THEIR IDENTIFICATION AND DISCRIMINATION

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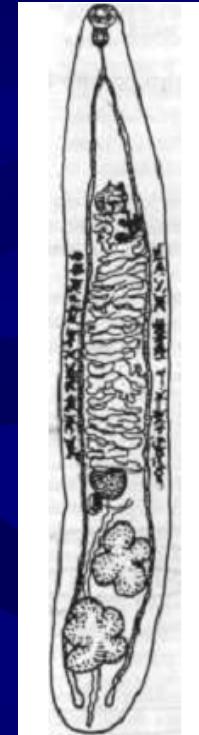
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# Several opisthorchiidiasis causing agents in Northern Eurasia

The situation with opisthorchiasis assessment and studies is complicated at the Northern Eurasia by its overlapping with another opisthorchiidases:

- metorchiasis caused by *Metorchis bilis*,
- pseudamphistomiasis caused by *Pseudamphistomum truncatum*,
- clonorchiasis caused by *Clonorchis sinensis* as well.

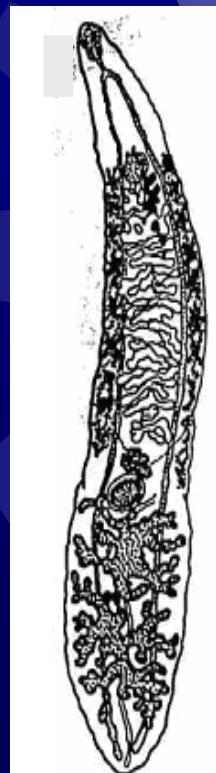
*Opisthorchis felineus*



*Metorchis bilis(albidus),*



*Clonorchis sinensis*

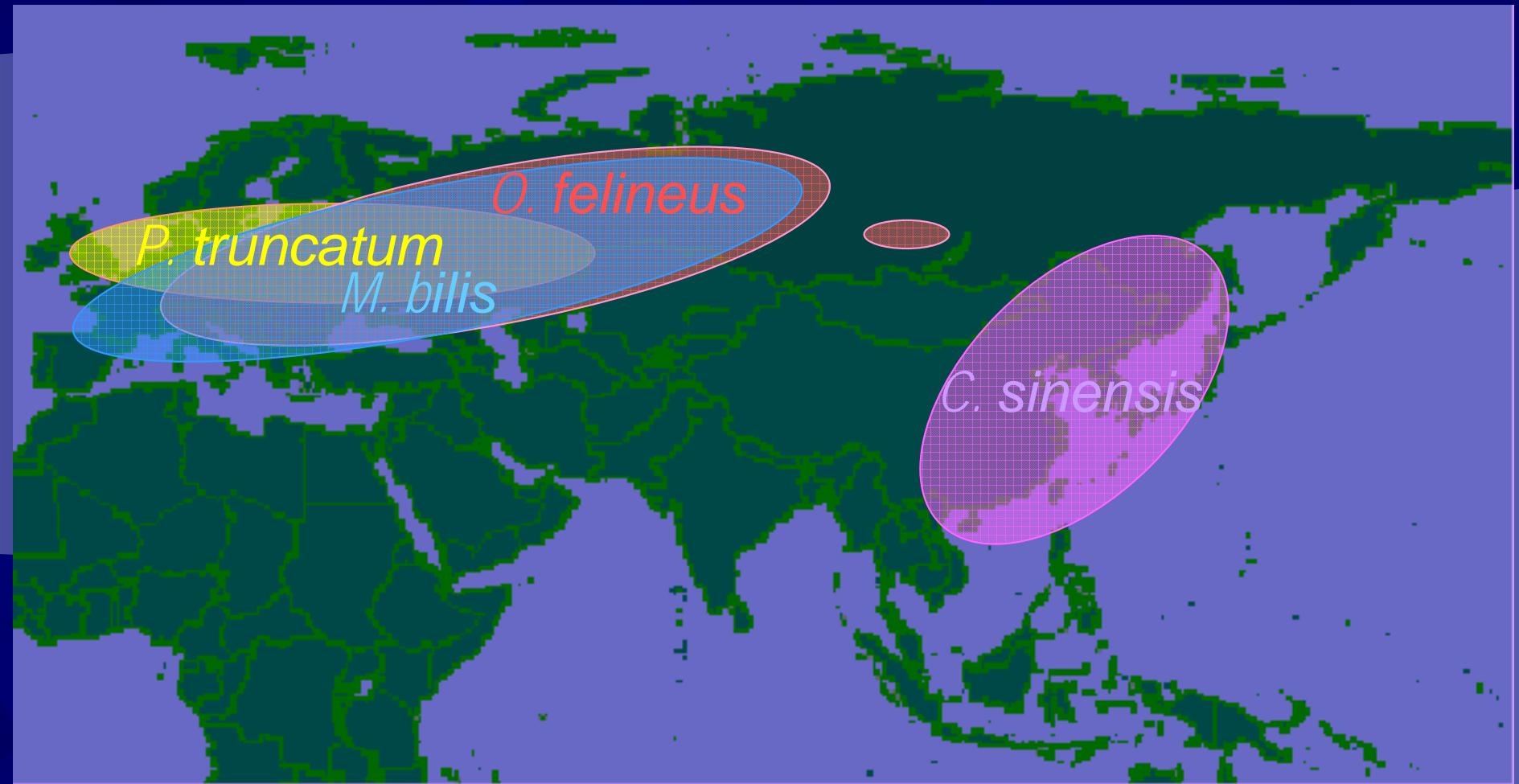


*Pseudamphistomum truncatum*



*M. bilis* and *P. truncatum* affect mainly gall bladder in animals. But there are evidences that they are capable to parasitize in humans too.

# Scheme of areals of opisthorchiidiasis causing agents in Northern Eurasia



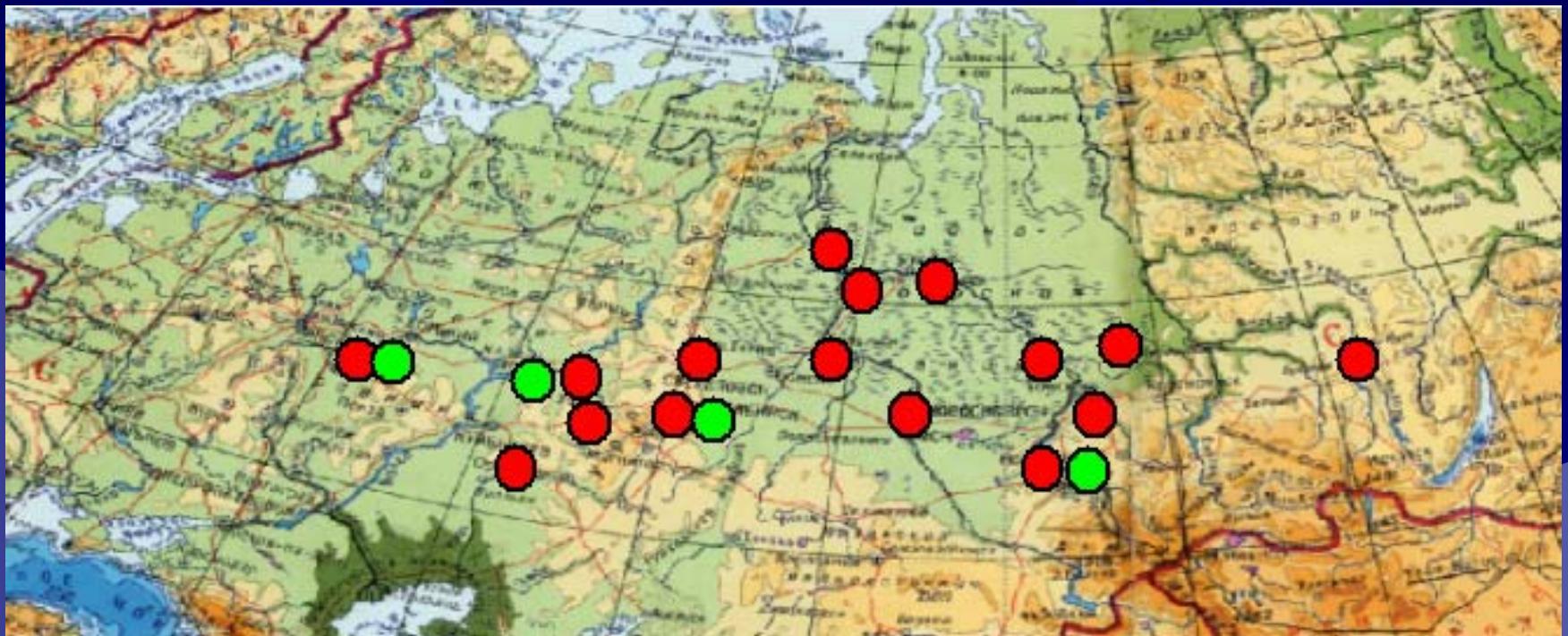
Overlapping scheme of *O.felineus*, *Metorchis bilis*,  
*Pseudamphistomum truncatum*, and *Clonorchis sinensis* areals.

# Objectives



The main current objective of our work is to develop a multiplex PCR assay for identification and discrimination of *Opisthorchis felineus* and *Metorchis bilis*.

# Studies on genetic variation in *Opisthorchis felineus* and *Metorchis bilis*



Red dots – collection points of *O. felineus* samples,  
Green dots - collection points of *M. bilis* samples.

Sampling localities in: Irkutsk obl., Tomsk obl., Novosibirsk obl.,  
Tumen' obl., Khanty-Mansy Autonom.Okrug, Cheljabinsk obl.,  
Bashkortostan, Orenburg obl., Voronezh obl.

Genetic markers used:

- mitochondrial COX1, COX3, ND1 genes;
- nuclear ITS1, ITS2, 9<sup>th</sup> intron of paramyosin gene.

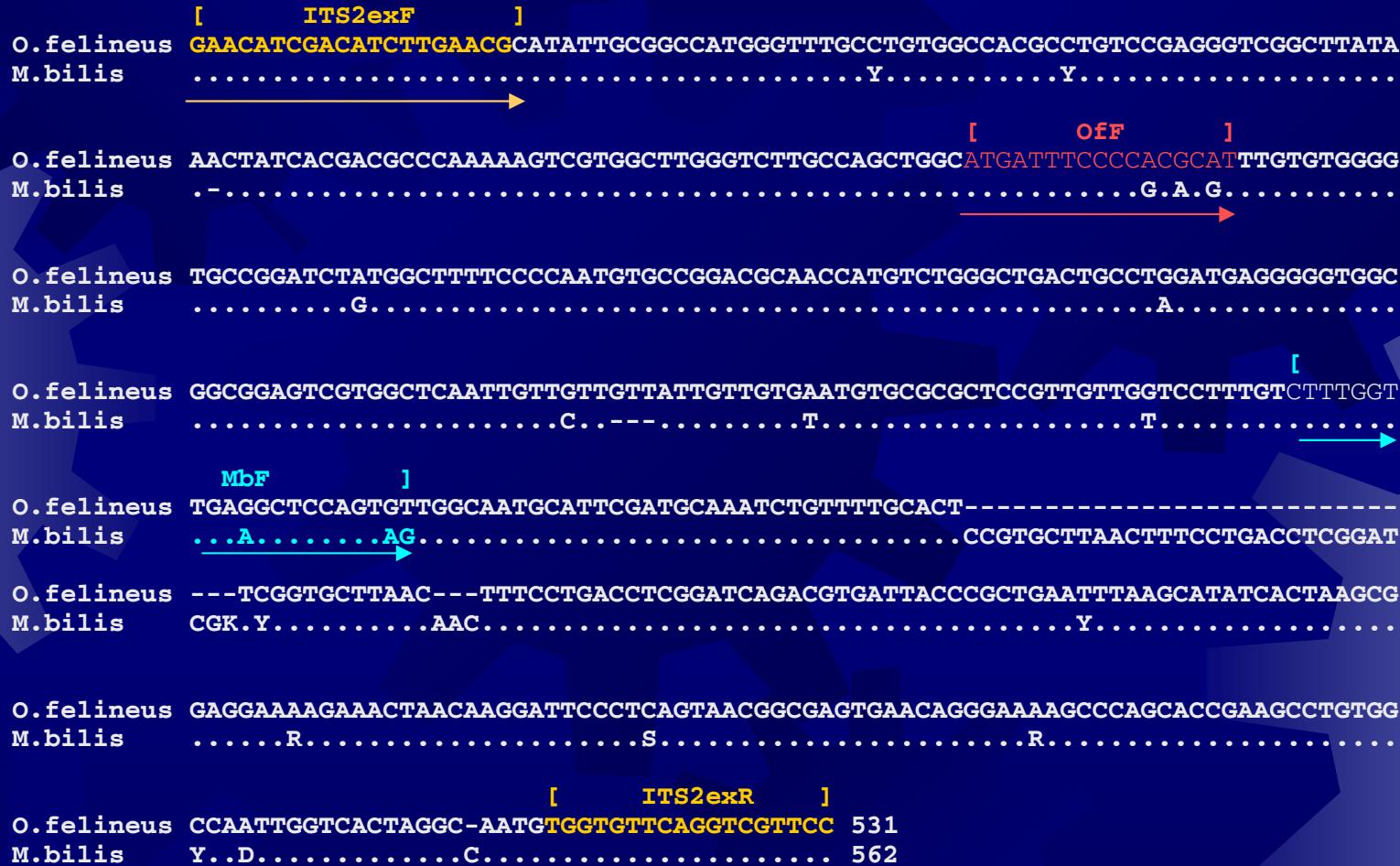
# ITS2 marker variation as base for PCR assay development

The common respective ITS2 marker genotypes are present in all populations studied with rare occurrence of point-specific genotypes, mainly in heterozygotic occurrence.

SPECIES	LOCALITY	ITS2 GENOTYPE
<i>O. felineus</i>	Novosibirsk obl. Tomsk obl. Khanty-Mansy Autonom.Okrug Tumen' obl. Irkutsk obl. Cheljabinsk obl. Bashkortostan Orenburg obl. Voronezh obl.	1, 2, 3 1, 2 1, 2, 5 1 1 1 1, 2 1 1
<i>M. bilis</i>	Novosibirsk obl. Cheljabinsk obl. Bashkortostan Voronezh obl.	1 1 1 1

So the data on nucleotide sequences of ITS2 marker were used for development of PCR diagnostics for the two opisthorchiids species.

# Development of ITS2 based PCR assay for *O. felineus* and *M. bilis*



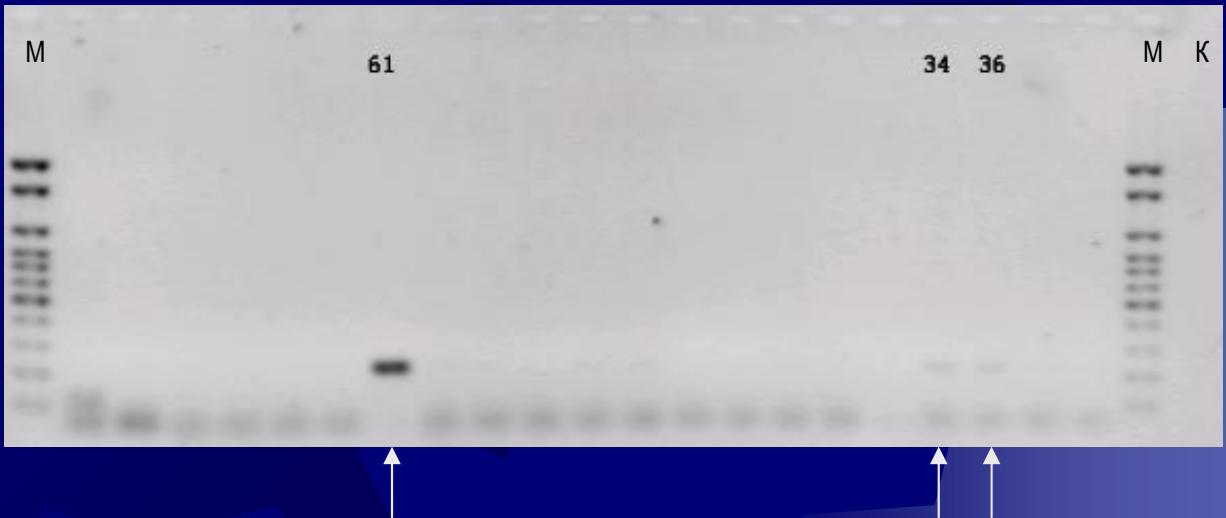
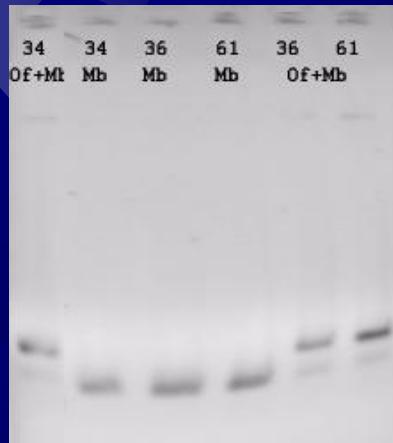
The pair of universal primers (ITS2exF and ITS2exR) were designed to first amplify the ITS2 DNA. Then the species specific primers for multiplex PCR (MbF and OfF) were designed to produce in pair with common reverse primer ITS2exR amplicons of different size: 300bp - for *O. felineus* and 500bp – for *M. bilis*.

# Evaluation of ITS2 based PCR diagnostics for *O. felineus* and *M. bilis*

## Products of multiplex PCR with the species specific primers

Clinical samples (stool samples from Tomsk) being coproovoscopically positive for opisthorchiasis were analysed with using the primer specific for *M. bilis*:

Primers used:



The clinical samples showing the signal for *M. bilis* were reanalysed with using both species specific primers to proof the mix-invasion.

So ~10% of the clinical samples showed *M. bilis* signal aside with *O. felineus* one.

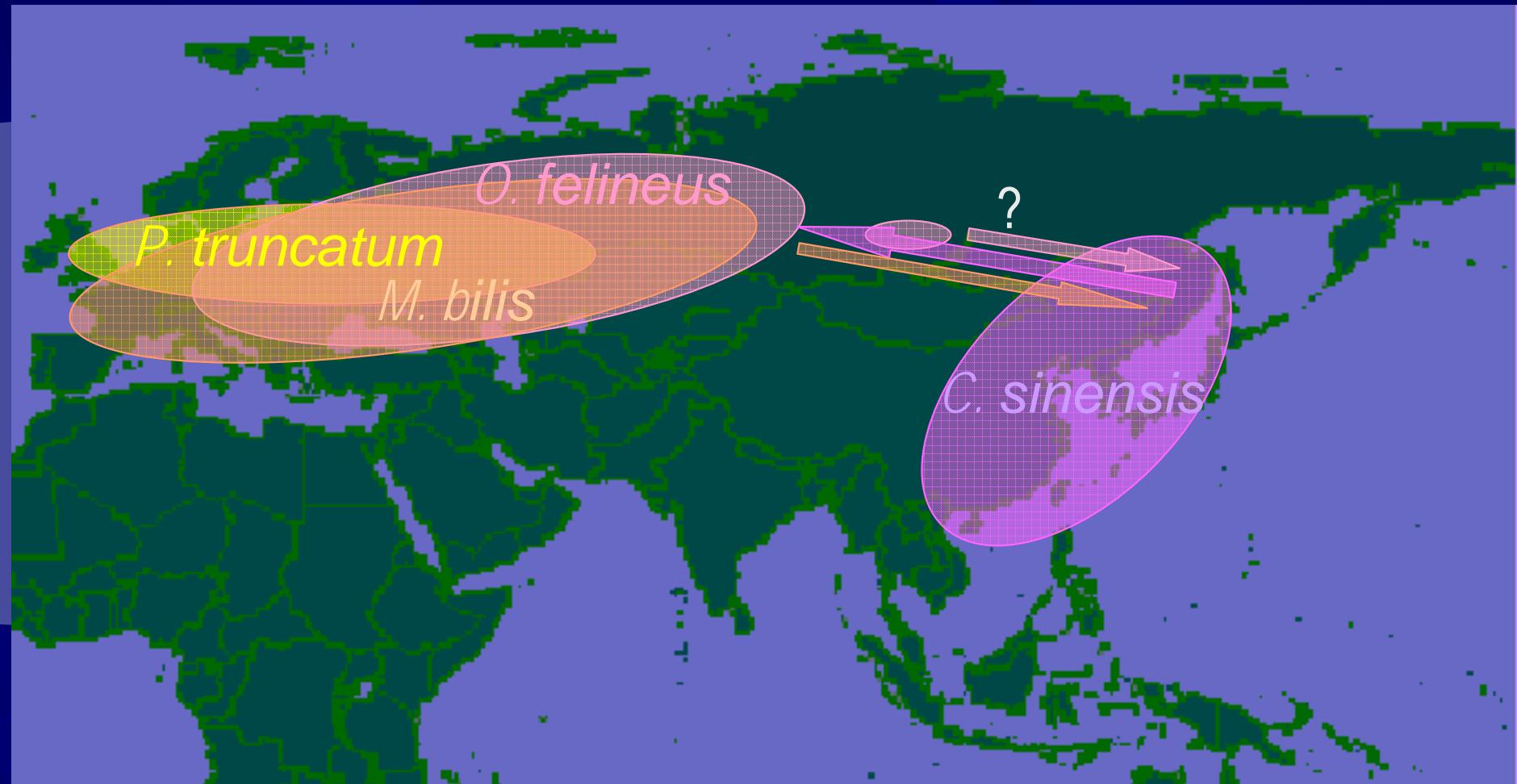
## Conclusions

The multiplex PCR with species specific primers allows us to identify even in enough difficult clinical samples cases of mix-invasion by two opisthorchiidiasis causing agents - *O. felineus* and *M. bilis*.

The PCR assay is ready to be applied to more representative sample set to estimate sensitivity and specificity values.

Direction for further development – to extend the assay for identification and discrimination others opisthorchiidiasis causing agents.

# Possible mobility of areals of opisthorchiidiasis causing agents in Russia



*O. felineus/M.bilis* and *C. sinensis* areals can be more dynamic  
due to either human activity or climate changes.



**Thank you for attention.**