ZOONOTIC TREMATODES IN MARINE AND FRESHWATER FISH FROM FRANCE:

UPDATES AND NEW DATA ON DISTRIBUTION AND TOOLS

MÉLANIE GAY & MAUREEN DUFLOT

Cryptocotyle: life cycle

Duflot et al., 2021a

Metacercariae encysts (skin, muscle)

Free swimming cercariae from periwinkle looks for the second intermediate host

Evolution within 4 successive larval stages

Miracidia → Sporocyst → Rediae → Cercariae

Embryonated eggs pass into bird feces

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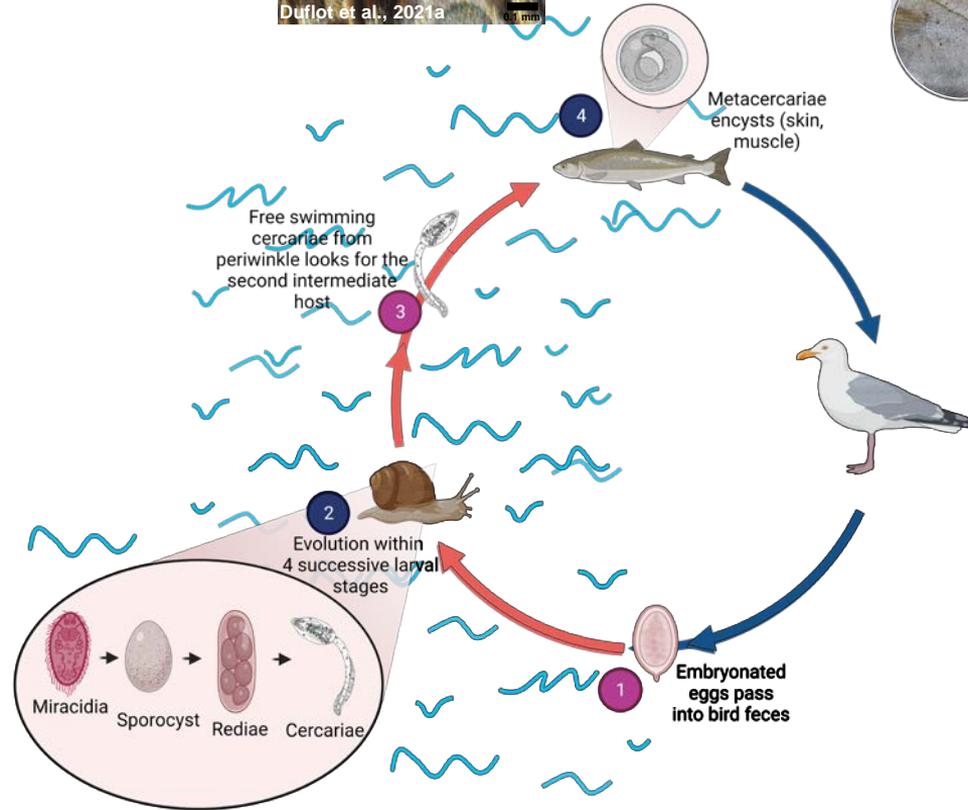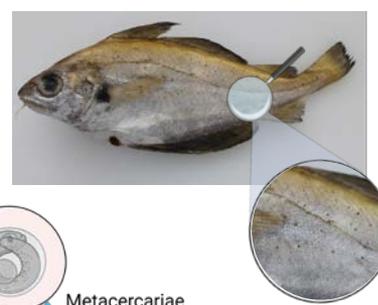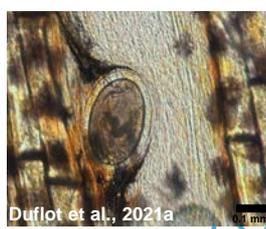
Cryptocotyle: life cycle
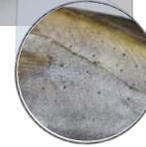
Phylogenetic position among Opisthorchioidea superfamily including **18 zoonotic genus**

- Impact on human health
  →unknown
- Few publications

First investigation of **black spot disease** on **commercial fish** from **the English Channel and the North Sea**

Duflot et al., 2021a

Metacercariae encysts (skin, muscle)

Free swimming cercariae from periwinkle looks for the second intermediate host

Metacercariae excysts in the bird intestine + adult stage evolves

Evolution within 4 successive larval stages

Miracidia Sporocyst Rediae Cercariae

Embryonated eggs pass into bird feces

duodenum
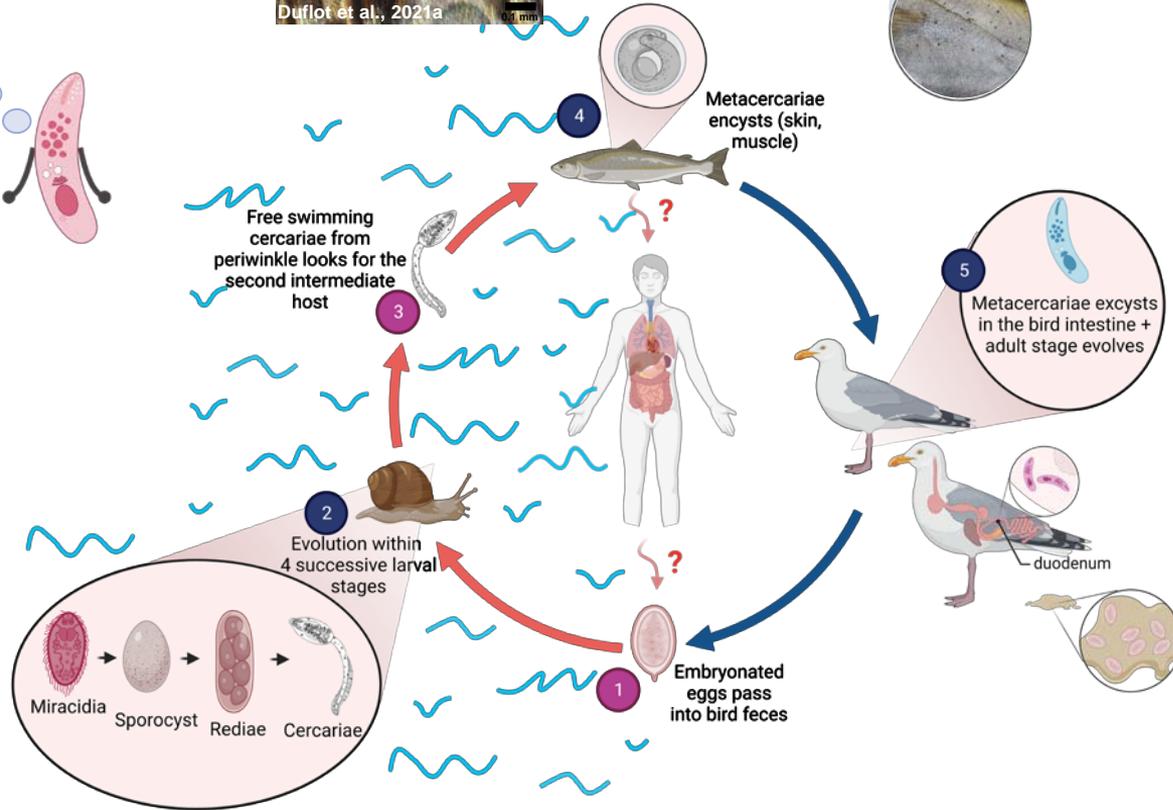
Cryptocotyle: Material and methods: detection and identification tools

According to Buchmann, 2007

Isolation of parasites

Morphological identification by microscopy
- Colorization and assembly on slide
- Observation of the morphology and measurements

Molecular identification by PCR
- Enzymatic lysis
- PCR
- Electrophoresis
- Sanger sequencing
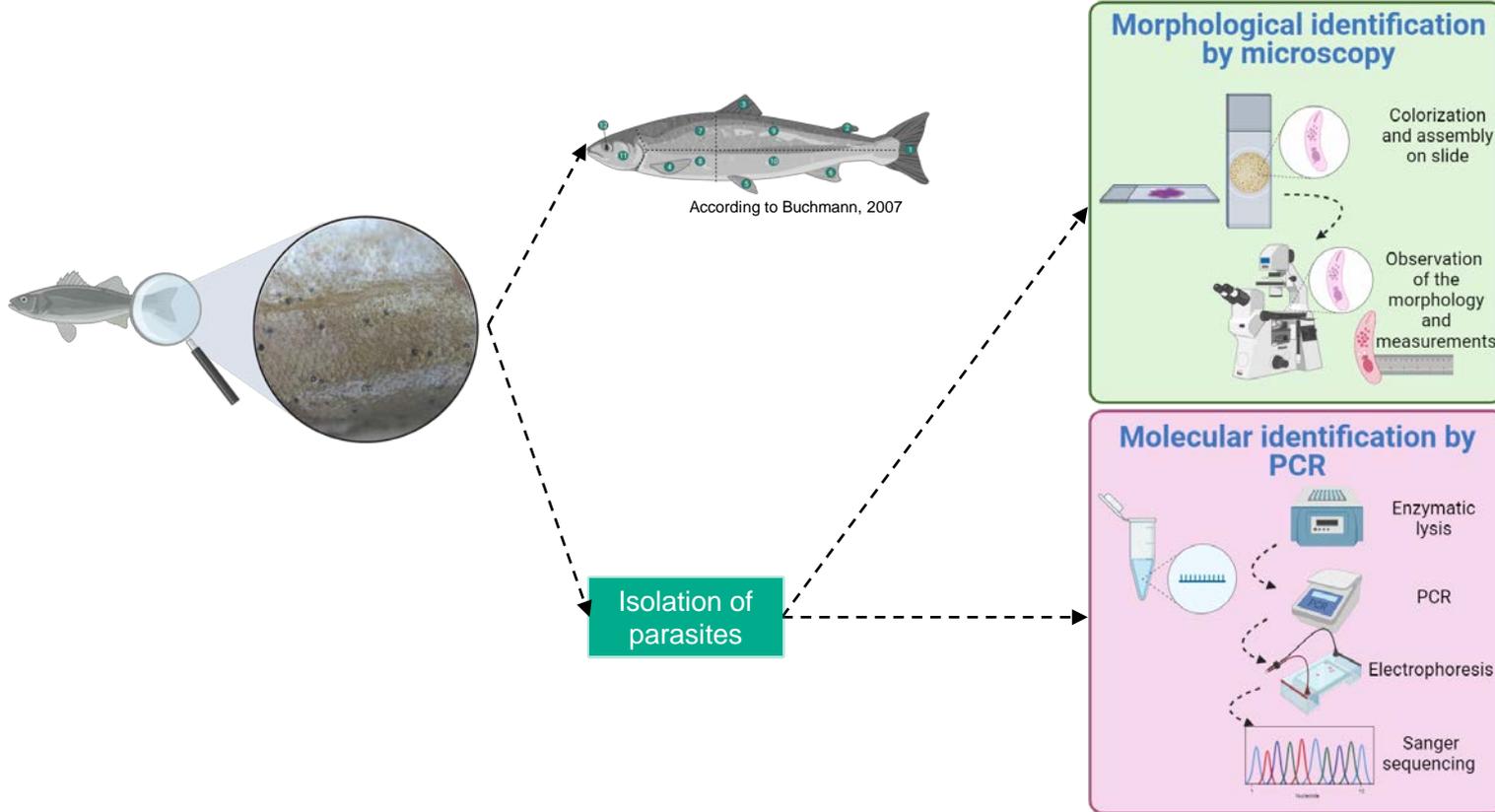
Cryptocotyle: Materials and methods - Sampling
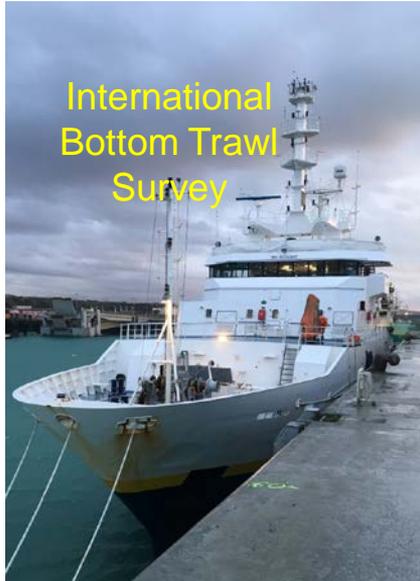
International Bottom Trawl Survey

Central North Sea CNS

Western North Sea (WNS)

Eastern North Sea (ENS)

Southern Bight of the North Sea (SBNS)

Eastern English Channel (EEC)
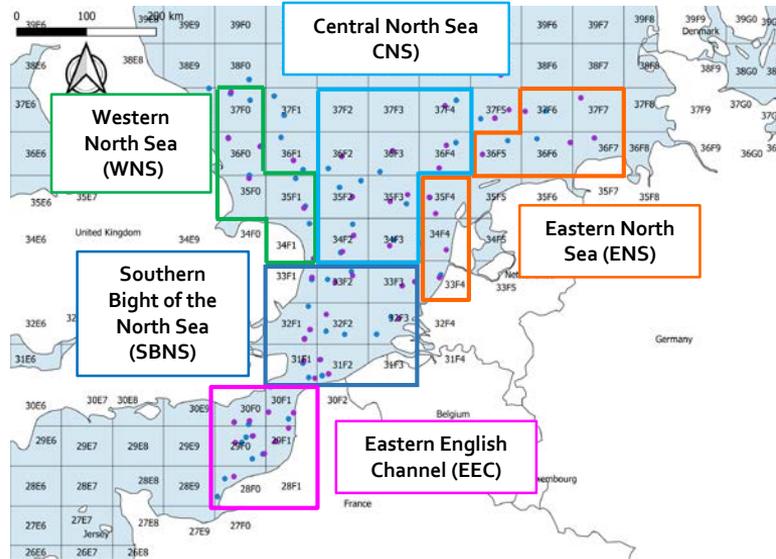
1 trawl per subarea = 40 individuals by fish species

= 1586 fish

Common name	Scientific name	Photo
Herring	*Clupea harengus*	
Sprat	*Sprattus sprattus*	
Whiting	*Merlangius merlangus*	
Pout	*Trisopterus luscus*	
Dab	*Limanda limanda*	
Flounder	*Platichthys flesus*	
Plaice	*Pleuronectes platessa*	

Cryptocotyle Results – Prevalence of black spots infection

Legend: Whiting, Sprat, Herring, Pout, Dab, Flounder, Plaice (2019); Whiting, Pout (2020)
* No individual

Map regions: Western North Sea, Central North Sea, Eastern English Channel, Eastern North Sea, Southern Bight of the North Sea
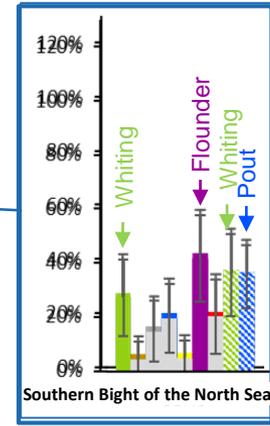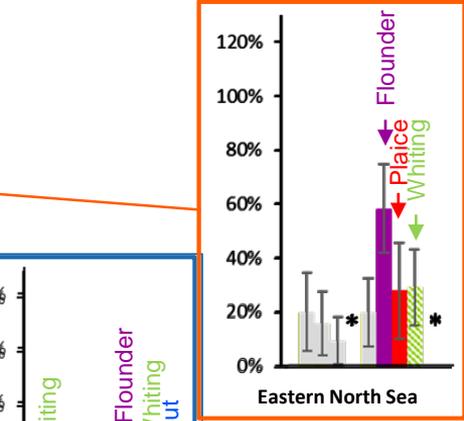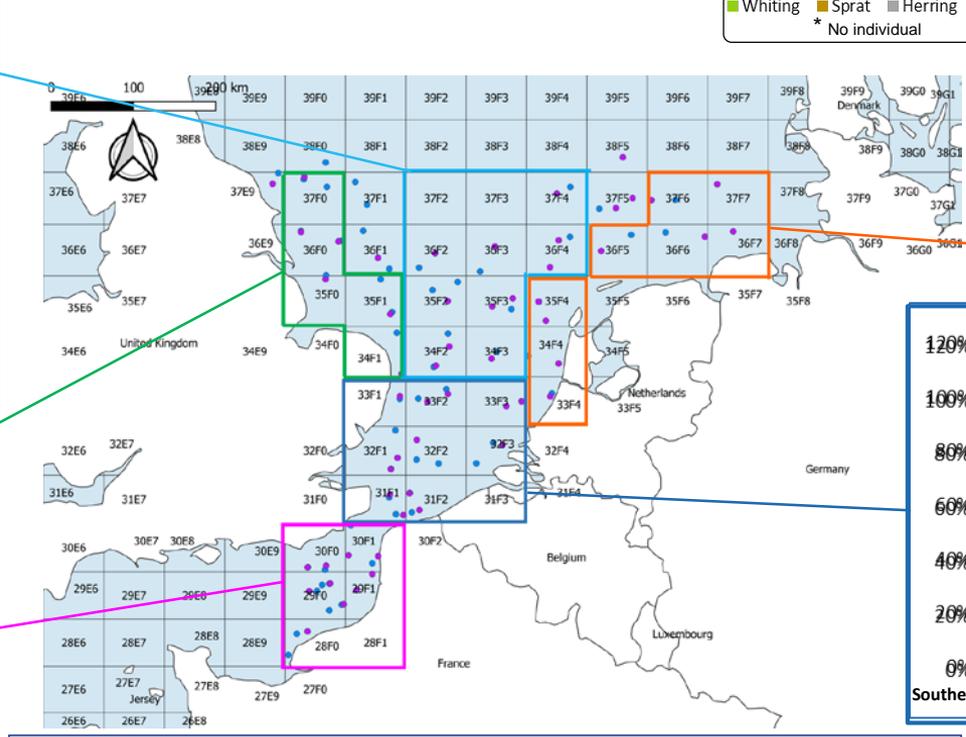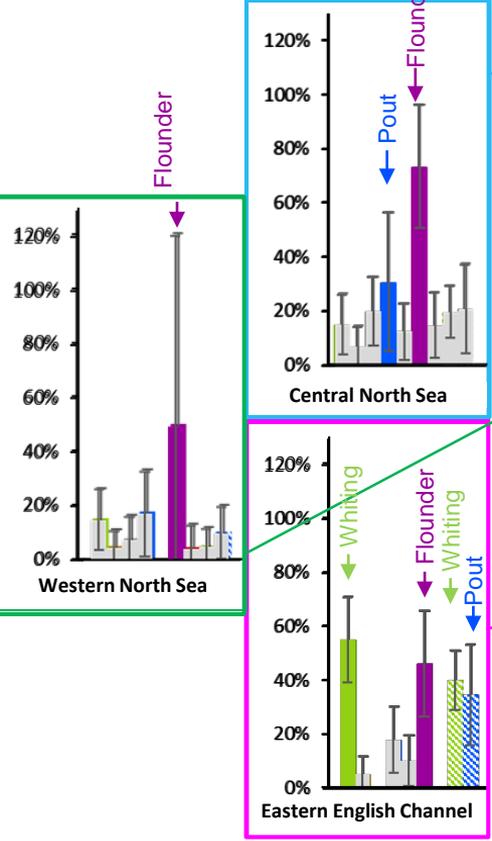
Infected fish = fish with at least one black spot

7 selected species were infected

Globally, the most infected species = **pout, whiting and flounder**

Cryptocotyle: Conclusions & outlooks

✓ Presence of metacercariae responsible for black spot diseases in 7 commercial fish species from the Eastern English Channel and the North Sea

✓ **Identification** (both morphological and molecular)

- Large majority of ***Cryptocotyle lingua***

- Few ***C. concava***

- Some undetermined **Bucephalidae**

⇒ Broad range of hosts for *C. lingua*

❖ Further samplings to better define the distribution of each *Cryptocotyle* species and to understand/determine drivers of their distribution

❖ New samplings on other hosts of *Cryptocotyle* life cycle for a better understanding of the circulation of these parasites in a marine ecosystem.

❖ Experimental *in vivo* studies to determine the zoonotic potential of *Cryptocotyle*

Clinostomum complanatum: taxonomy and life cycle

- **Class: Trematode**
- **Subclass: Digenea**
- **Order: Diplostomida**
- **Superfamily: Schistosomatoidea**
- **Family: Clinostomidae**

Adults live in Ardeidae throat

Eggs are shed through saliva or feces

Eggs hatch in water

Free miracidium penetrates in snail

Metacercariae pass to bird when fish host is eaten

Asexual reproduction

Cercariae actively penetrate fish

Swimming free cercariae emerge from snail

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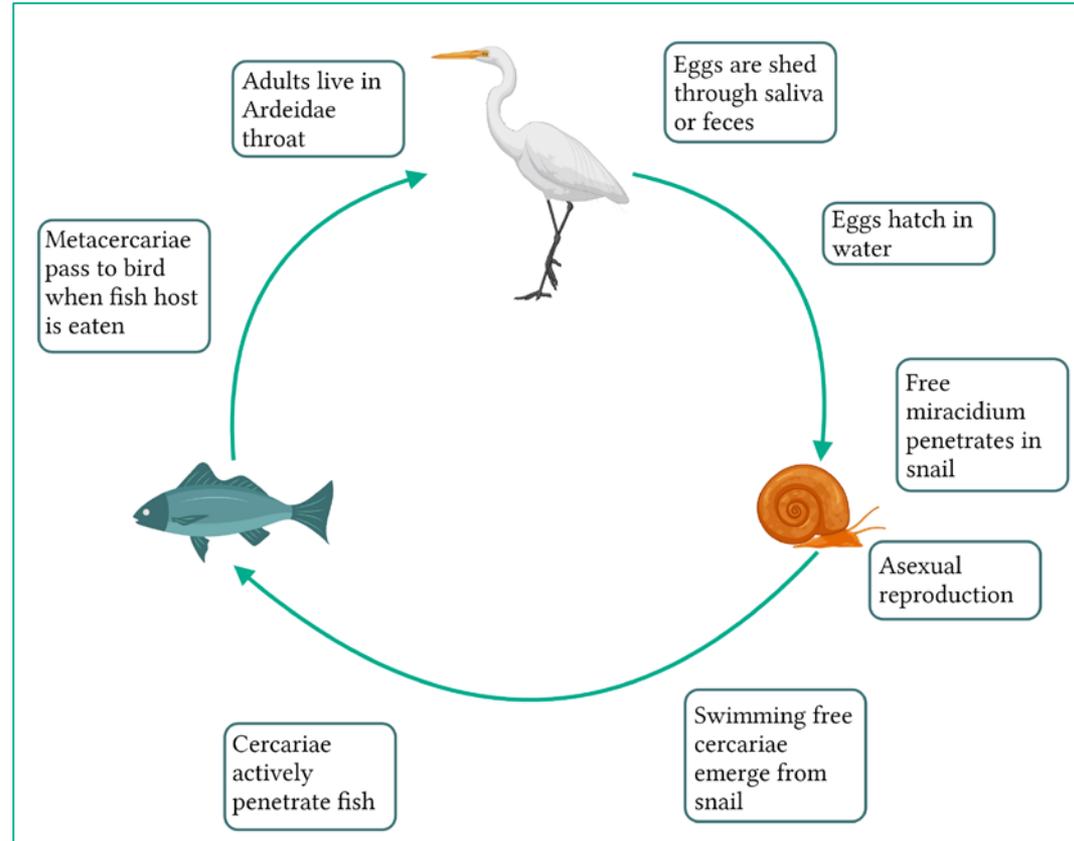
Clinostomum complanatum: Geographical distribution and Hosts

- **Europe :**
  - Central-Eastern Europe along the Danube basin
  - North East of Italy
  - Turkey
  - Israel
- **America (North and South)**
- **Africa**
- **Asia**

- **Definitive host:**
  - Piscivorous birds, mainly Ardeidae (heron, egret, bittern)
  - Rarely reptiles or mammals
- **1st intermediate host: aquatic snail (Lymnaeidae)**
- **2nd intermediate host: fish (wild and aquacultured)**
  - Cyprinidae (barbels, chub, carp)
  - Percidae (perch, pike-perch)
  - Cobitidae (loach)
  - Centrarchidae (rainbow perch, largemouth black bass)

- **Human cases** followed consumption of **raw or undercooked/processed fish** (carp, perch)
- Human cases described in **Japan, Israel, Korea, India and Thailand**
- Pathologies due to the establishment of the worm in the pharynx or larynx of humans
- Remedy = elimination of parasite through endoscopy

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Clinostomum complanatum in France ?

- **First observation in December 2019 in the river Doubs (Jura, North East France): from recreative angler, white cysts on perch**

    => **morphological identification =** *Clinostomum complanatum*

- **Since then, several observations:**

11/2021
Perch
Wild recreational fishing
River and pond

12/2020
Perch
Wild professional fishing
Pond

05/2021
Rudd
Aquaculture
Pond

12/2019 & 12/2020
Perch
Wild recreational fishing
River

09/2021
Perch

River

06/2021
Perch

River

# *Clinostomum complanatum*: some French data

- **1 batch of 33 perch**

  - ✓ Prevalence 97%

  - ✓ Abundance 0 to 33 parasites

  - ✓ No correlation with length, weight, age, sex (but limited data)

  - ✓ Heterogeneous distribution
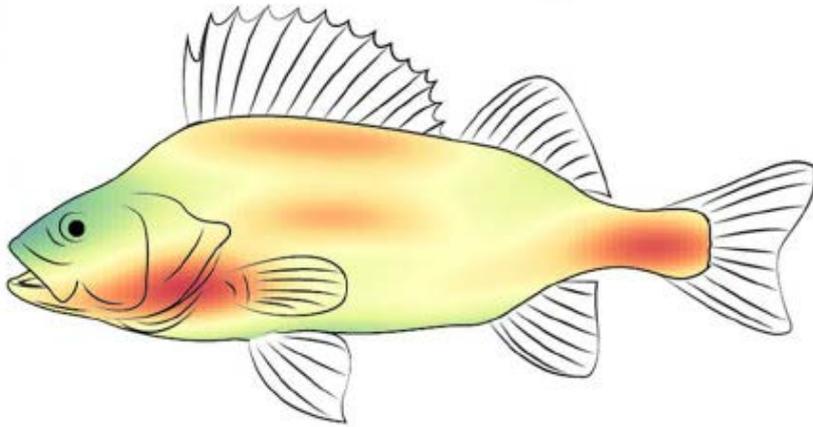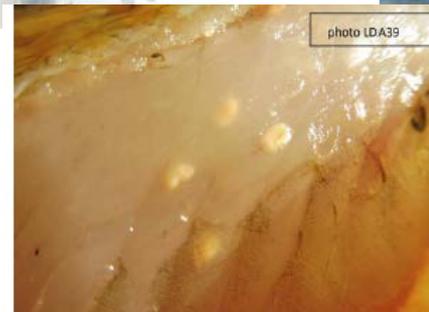
Clinostomum complanatum: some French data

Molecular identification: Sanger sequencing of partial rRNA 28S and COI gene
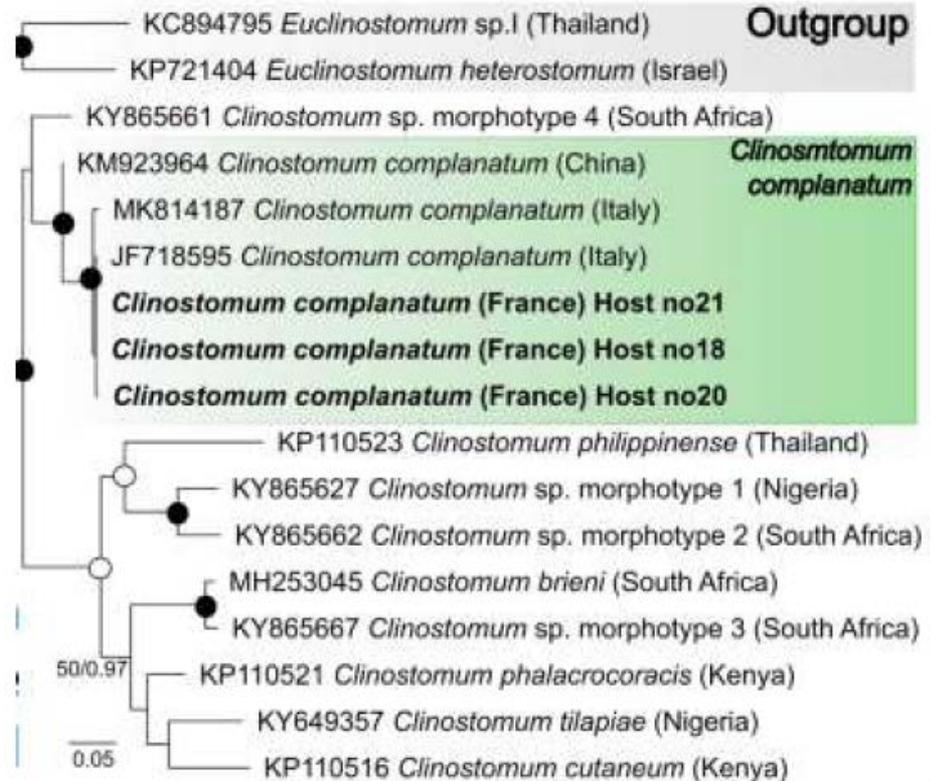
Maximum likelihood
phylograms based on COI
mtDNA gene sequences

Clinostomum complanatum: situation in France and Europe

Parasites present on fish of high economic value and with tradition to be consumed raw

Unknown host spectra, infestation levels and distributions, scarce data

No reliable data on treatments effective to kill these parasites

Taxonomic uncertainties

Need of reliable epidemiological data

Need of technological data

Need of population genetic data

French working group (health and food authority, recreational and professional fishermen, fish farmers, scientists)

Clinostomum complanatum: conclusions

✓ **Applied research needs: Efficiency of processes to kill these parasites (smoking, salting, cooking and freezing)**

✓ **Fundamental research needs:**

  ✓ What is the distribution of the parasite? Wild vs aquaculture? Which species? Which environment? Other stages?

  ✓ Which origin of this emergence? Fish stocks? Migratory birds?

  ✓ Which species? Clarification of taxonomy

"*ClinExplor*": first epidemiological study of *C. complanatum* distribution in European perch in France

Thanks for your attention

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