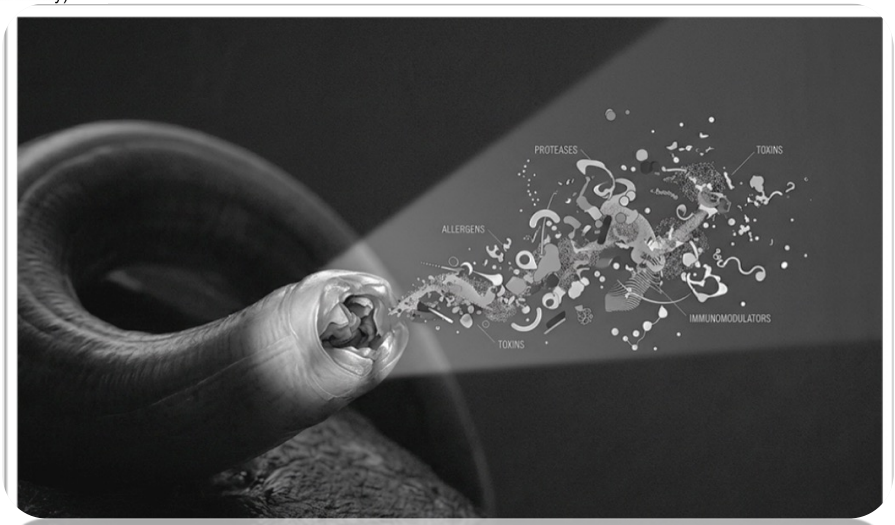
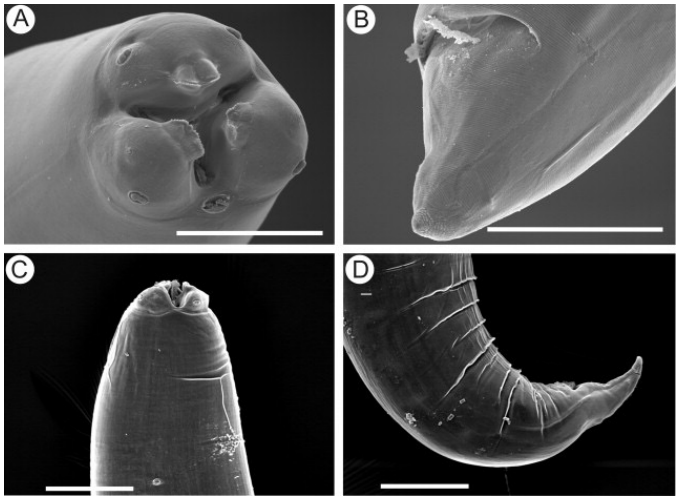
European Union Reference Laboratory for Parasites (EURL-P; food safety)

DEPARTMENT INFECTIOUS DISEASES

EURL PUBLIC HEALTH
FOOD-, WATER- AND VECTOR-BORNE HELMINTHS AND PROTOZOA





From foodborne parasite to intestinal hazard: *Anisakis* spp. extracellular vesicles impact on human colonic organoids

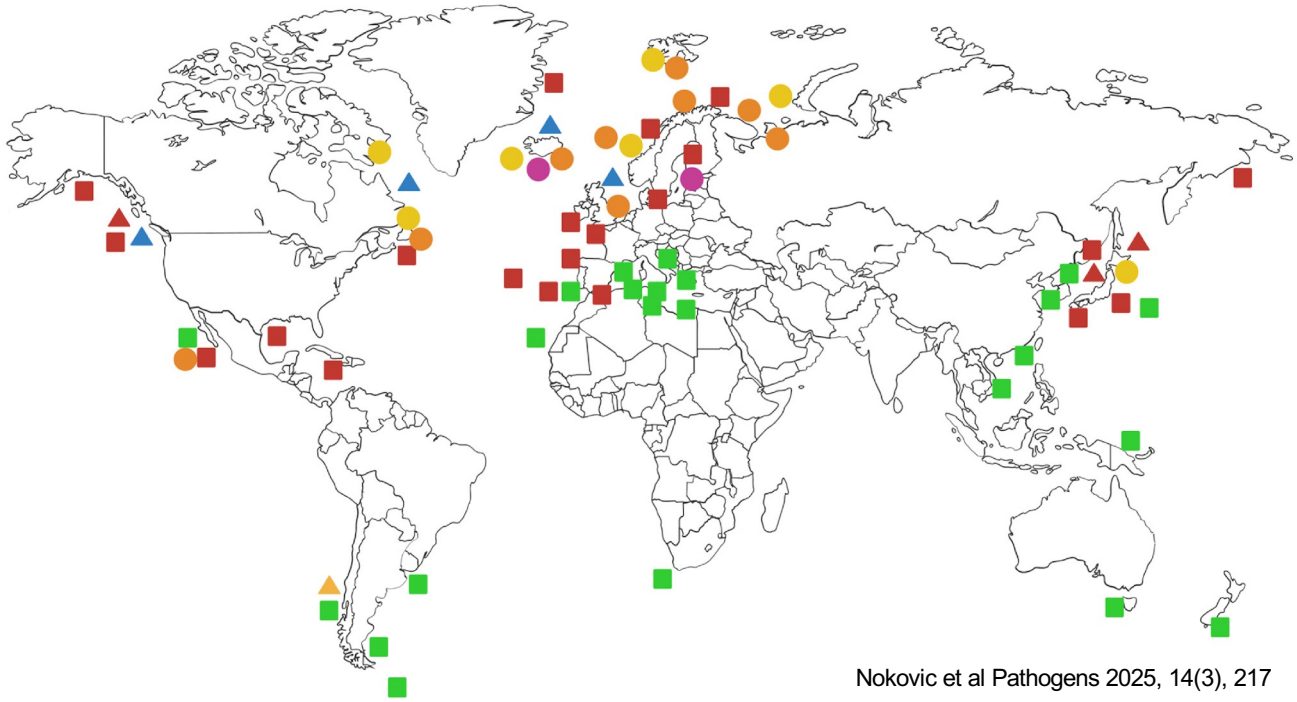
XXI Workshop of the National Reference Laboratories for PARASITES
28-29 October 2026

Dr. Ilaria Bellini

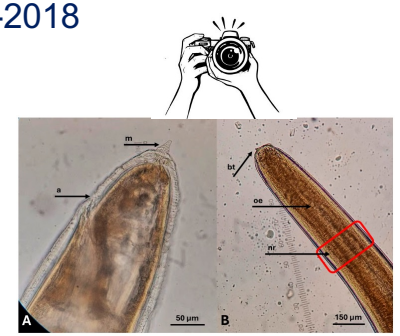
Email: ilaria.bellini@iss.it

Anisakis spp.: *a marine nematode with a long* journey

WORLD DISTRIBUTION OF ZOONOTIC SPECIES IN THE PERIOD BETWEEN 1986-2018



Nokovic et al Pathogens 2025, 14(3), 217

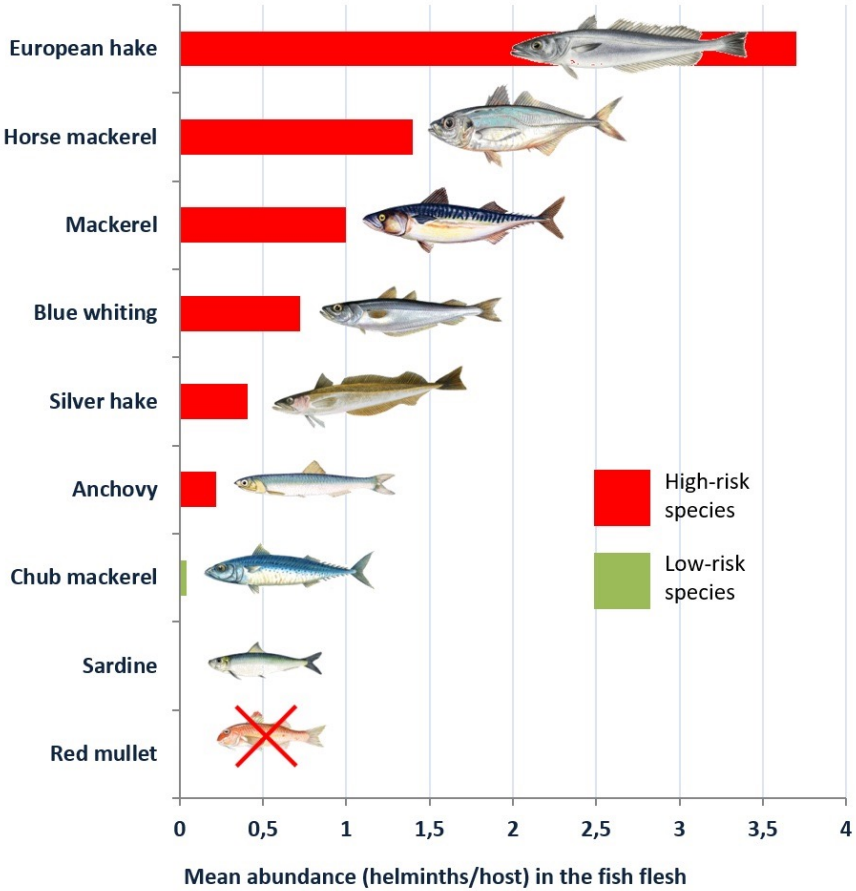


Anisakis pegreffii *Anisakis simplex* s. s. *Phocanema decipiens* s.s. *Phocanema azarasi*
Phocanema cattani *Contracaecum osculatum* s. s. *Contracaecum osculatum* A
Contracaecum osculatum B



Most of the human infections are due to two main species:
Anisakis simplex and Anisakis pegreffii.

Risk of transmission

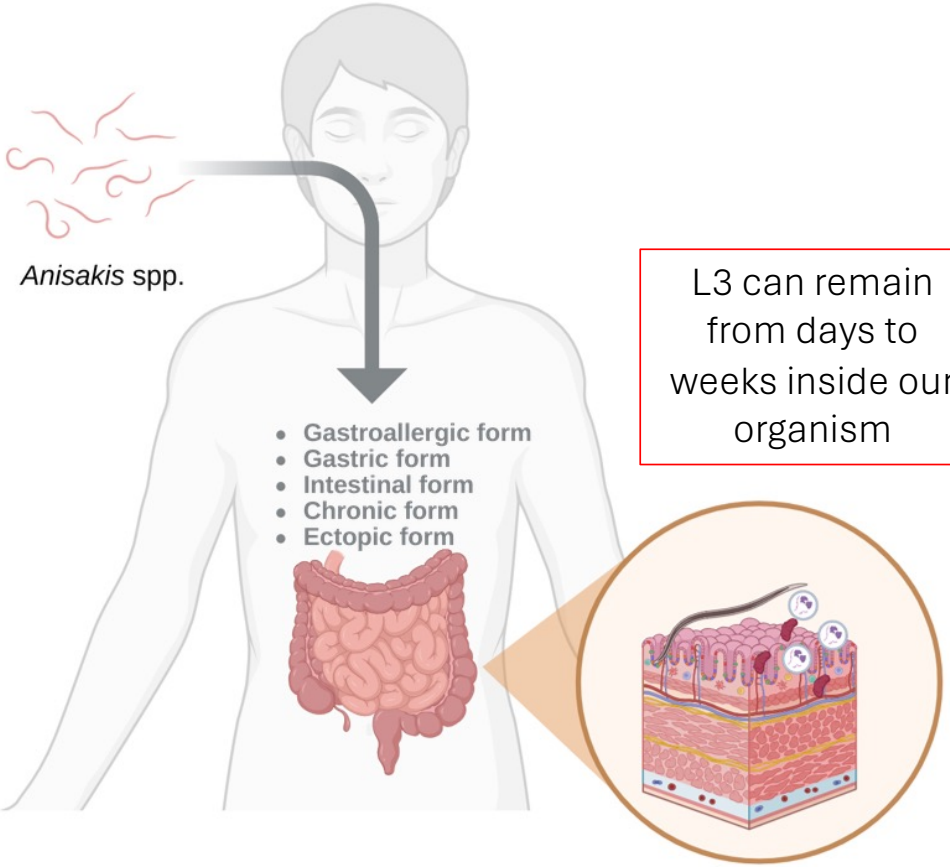


Prevalence (%) and mean abundance of *Anisakis* type I larvae in fishes according to the geographic origin

Fish Species	Atlantic Ocean		Mediterranean Sea	
	Prevalence (%)	Mean Abundance	Prevalence (%)	Mean Abundance
Blue whiting (*Micromesistius poutasou*)	77.51	4.83 (3.08–6.58)	19.13	2.45 (0.00–6.25)
Hake (*Merluccius merluccius*)	84.00	20.34 (5.40–35.28)	6.00	0.05 (0.00–0.10)
Silver hake (*Merlucccius bilinearis*)	59.48	1.42 (1.06–1.78)	-	-
Red mullet (*Mulllus barbatus*)	-	-	3.23	0.05 (0.00–0.09)
Mackerel (*Scomber scombrus*)	76.43	7.29 (4.16–10.41)	30.77	3.20 (1.57–4.83)
Chub mackerel (*Scomber japonicum*)	19.18	1.84 (0.00–5.12)	14.29	0.17 (0.20–0.33)
Horse mackerel (*Trachurus trachurus*)	100.00	52.50 (35.26–69.74)	32.00	0.94 (0.43–1.45)
Sardine (*Sardina pilchardus*)	11.43	0.34 (0.08–0.61)	2.13	0.05 (0.00–0.12)
Anchovy (*Engraulis encrasicolus*)	18.87	0.43 (0.21–0.66)	9.87	0.11 (0.05–0.17)
Total	52.90	4.56	11.92	0.79

Debenedetti et al «Prevalence and Risk of Anisakid Larvae in Fresh Fish Frequently Consumed in Spain: An Overview« Fishes 2019, 4(1), 13

When *Anisakis* meets the human accidental host .....



L3 can remain from days to weeks inside our organism



Broad spectrum of symptoms:

Acute	Chronic
• Diarrhea	• Ulcerous lesions
• Abdominal pain	• Eosinophilia
• Urticaria	• Granulomas
• Anaphylaxis	• Inflammation

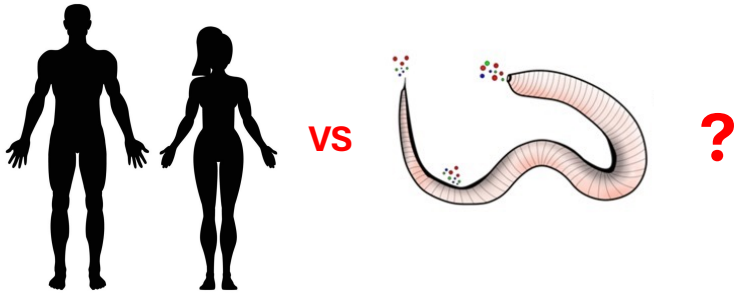
....along with asymptomatic cases:



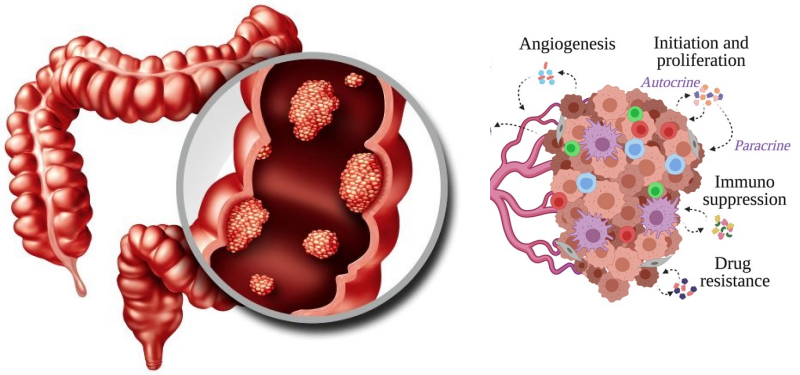
PROBLEMS IN DIAGNOSIS AND EPIDEMIOLOGICAL DATA!

Why are we interested in Anisakiasis?

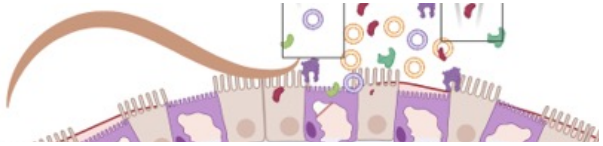
Little is known about *Anisakis*-human interactions and consequences



Potential risk factor for gastric-colon cancer
Potential association with IBD



Anisakis spp. Bystander or instigator of a tumorigenic microenvironment ?

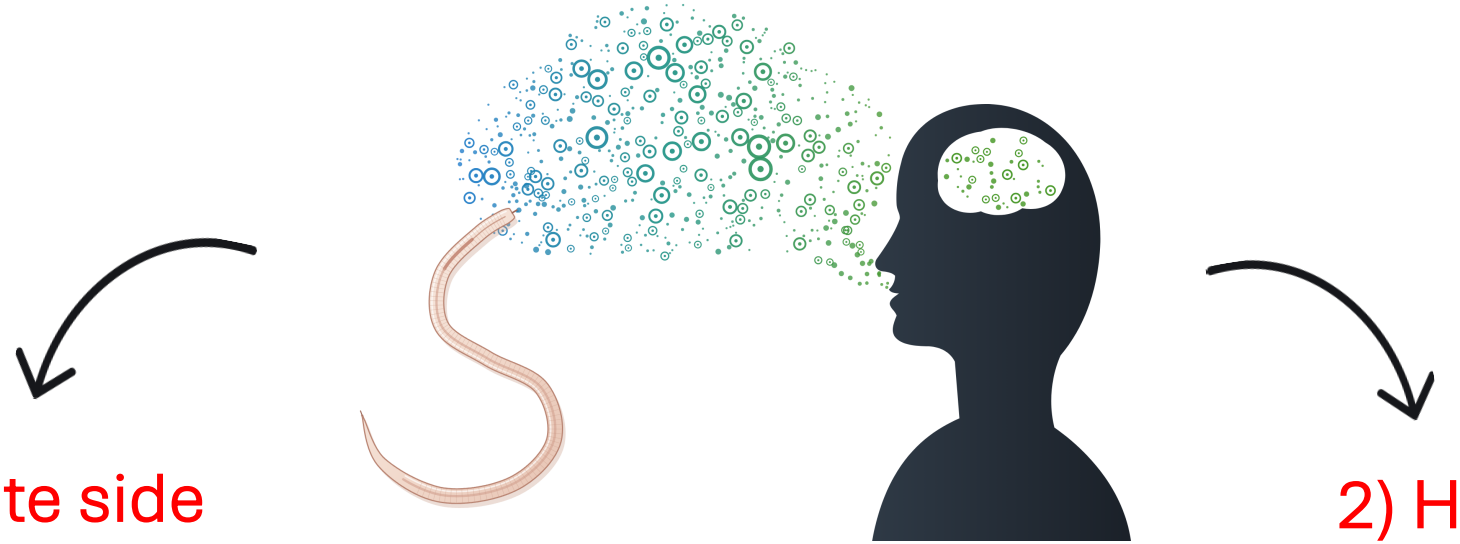


Need to investigate the molecular dialogue occurring at the host-parasite interface

Anisakis extracellular vesicles elicit immunomodulatory and potentially tumorigenic outcomes on human intestinal organoids

Ilaria Bellini [1], Daniela Scribano [1], Cecilia Ambrosi [2 3], Claudia Chiovoloni [1], Silvia Rondón [1], Annamaria Pronio [4], Anna Teresa Palamara [1 5], Agostina Pietrantoni [6], Anna Kashkanova [7], Vahid Sandoghdar [7], Stefano D'Amelio [# 1], Serena Cavallero [# 8 9]

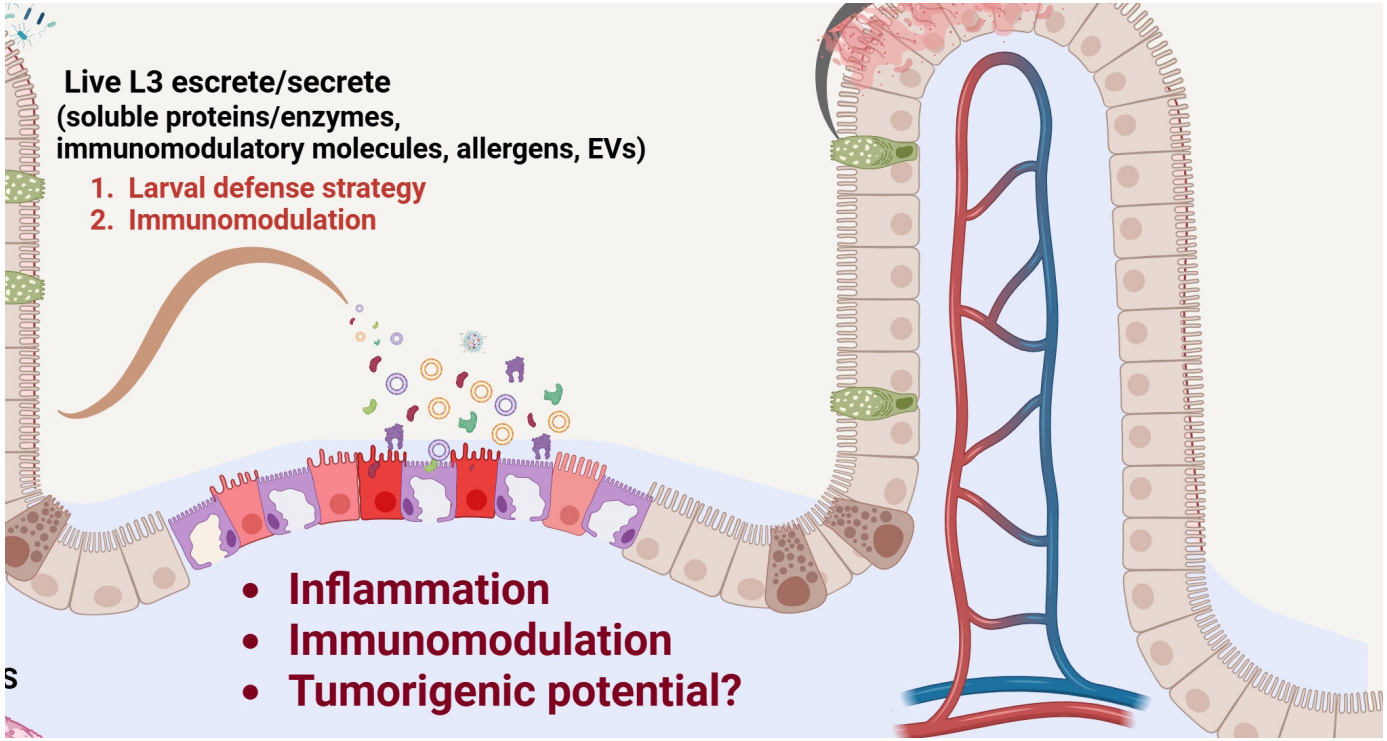
Aim: TO EXPLORE THE MOLECULAR DIALOGUE



1) Parasite side

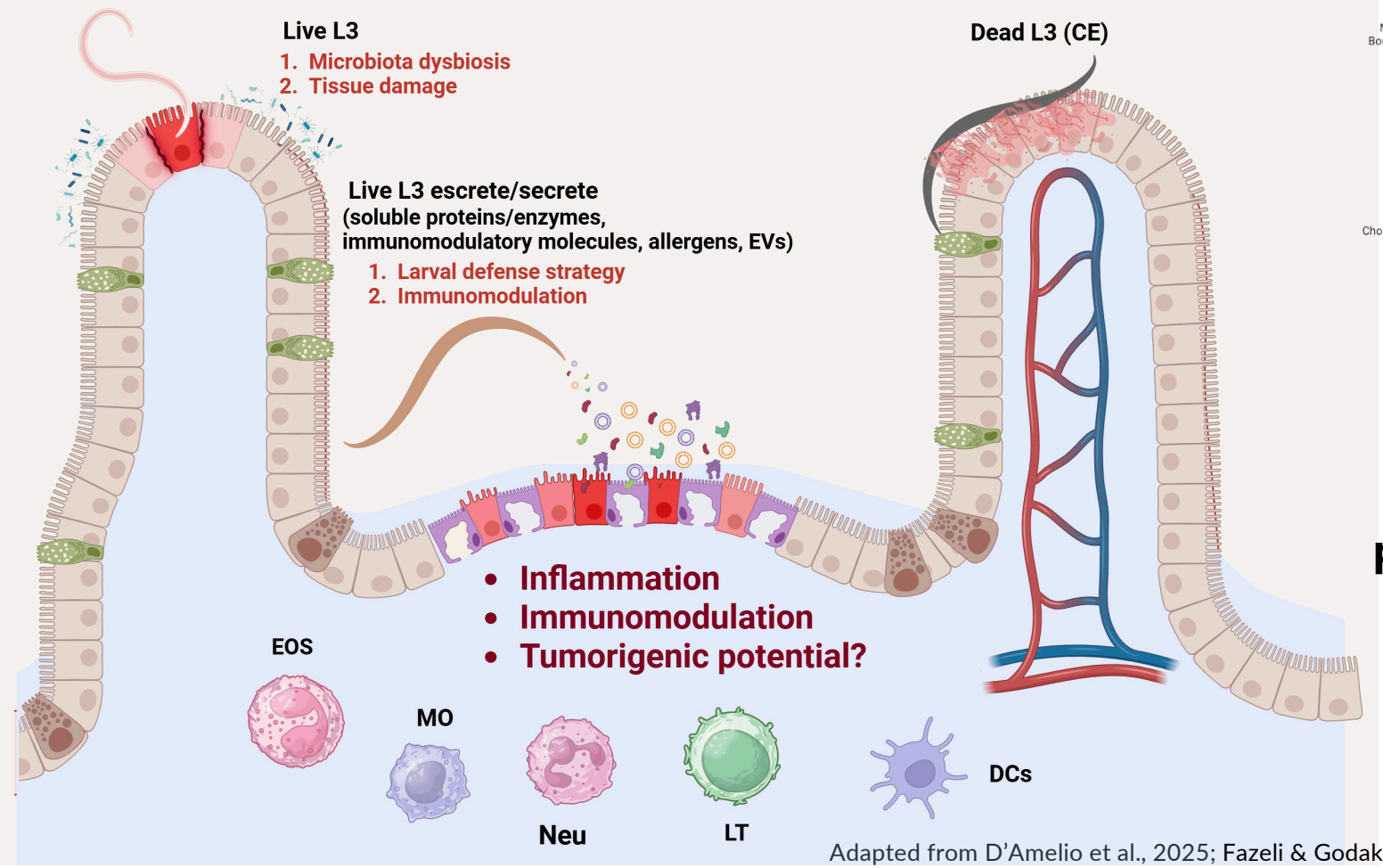
2) Host side

1) Parasite side:



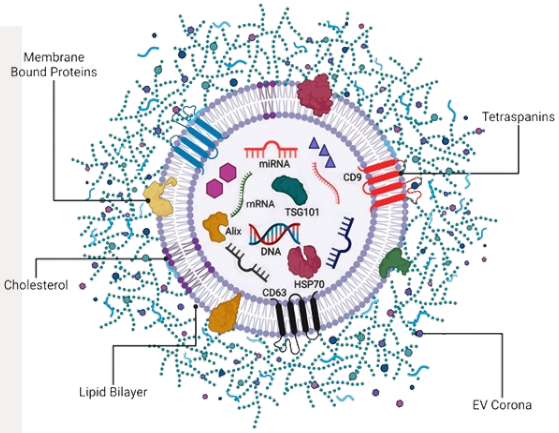
Adapted from D'Amelio et al., 2025

Recently emerged messenger of pathogenicity



Adapted from D'Amelio et al., 2025; Fazeli & Godakumara 2024

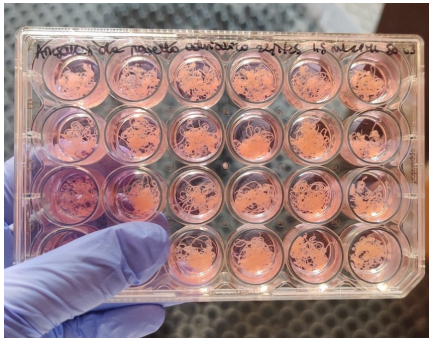
Extracellular vesicles (EVs)



30 nm – 10 µm

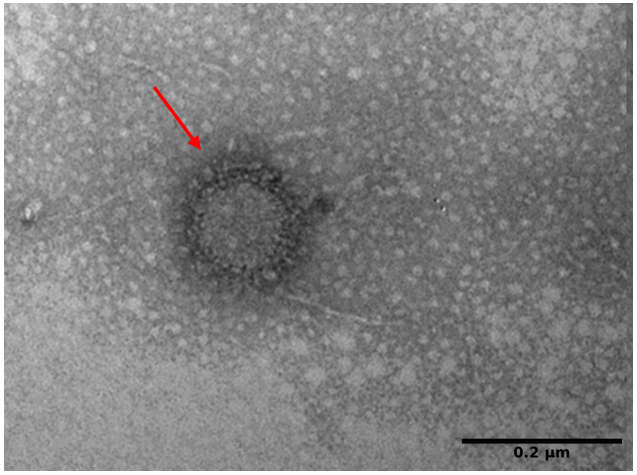
EVs are **key messengers in parasite–host** cross-talk, like package "sent" by the parasite and "read" by the host that are able to shape their cellular responses

Anisakis extracellular vesicles

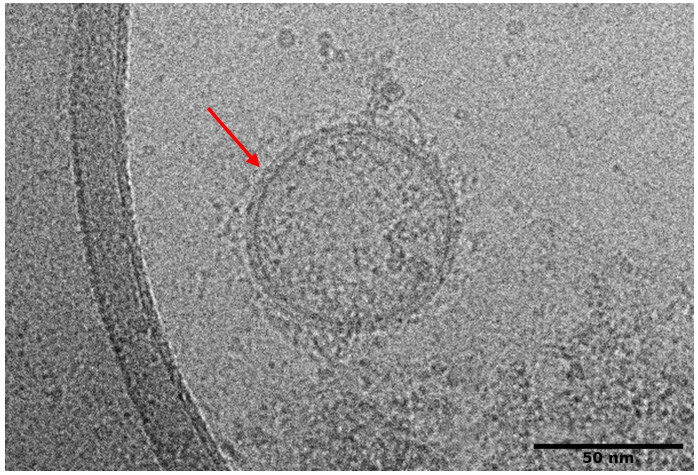


Anisakis spp. L3 cultures at 37°C 5% CO2

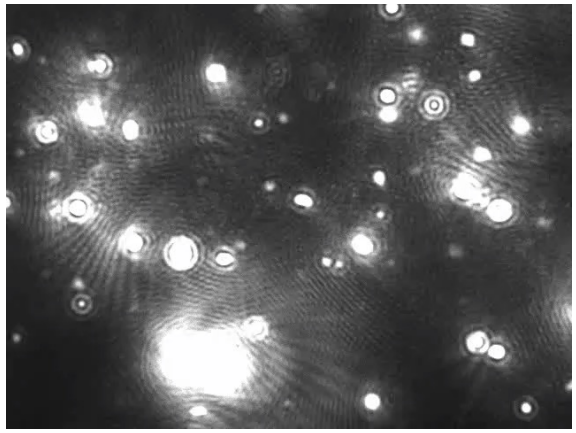
Transmission Electron Microscopy



CRYO- Transmission Microscopy

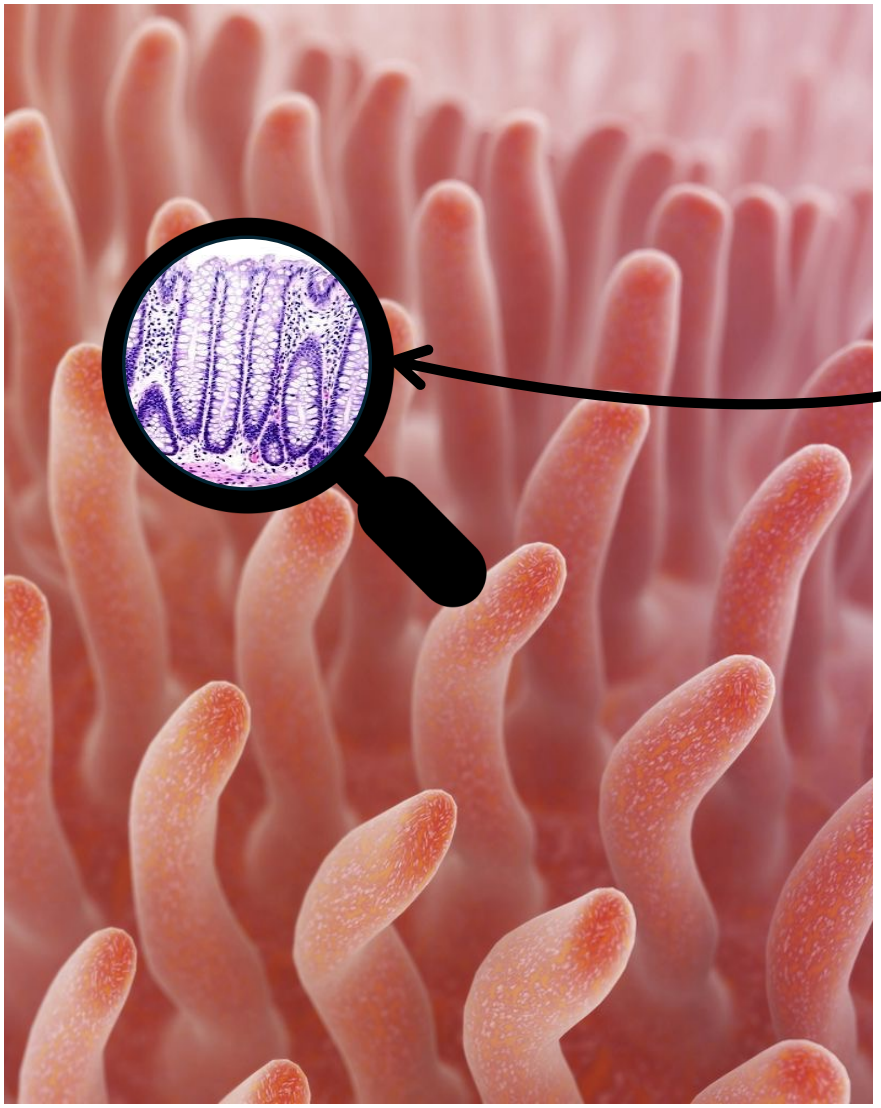


Personal images, unpublished

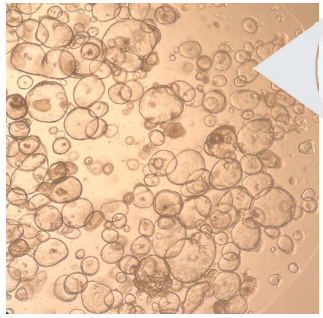


Nanoparticle tracking analyses NTA

2) Human side: Intestinal Organoids from colon biopses (HIOs)

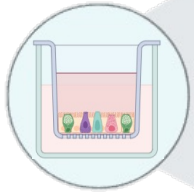


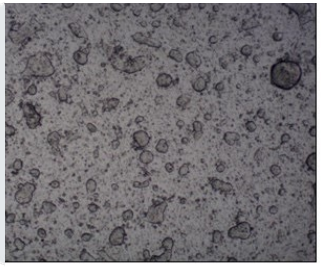


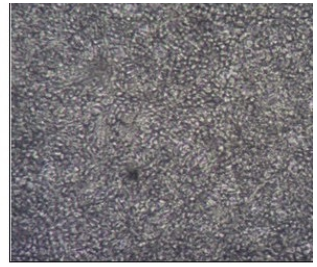


HIOs
(**3D**)

HIOs plated on
transwell (**2D**)





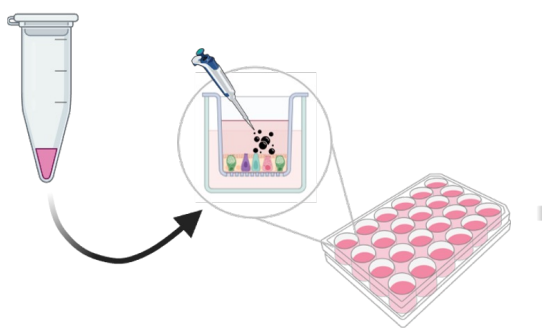


Model advantages

- ✓ **Self renewal** and organization
- ✓ Retain **organ specificity** and **genome stability**
- ✓ Different **cell populations** (enterocytes, Goblet cells)

Anisakis EVs vs Human Intestinal Organoids

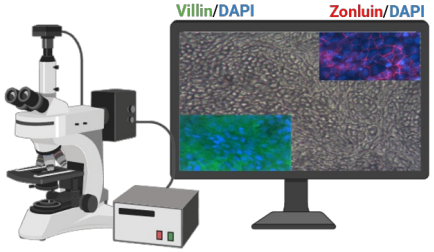
Anisakis
EV sample



3 biological replicates

1) Evaluate host barrier integrity

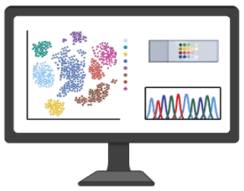
Immunostaining for junctional and differentiation markers



+

2) Evaluate host gene expression alteration

Comparative transcriptomic analysis



+

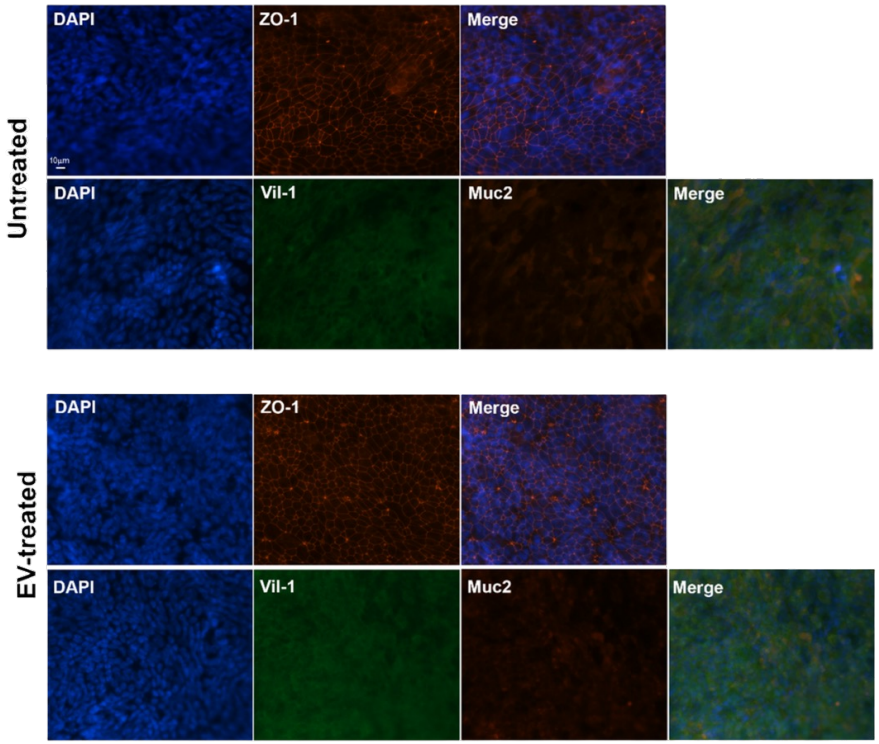
3) Evaluate host response

Multiplex assay for soluble factors

Standard-sensitivity protocol	High-sensitivity protocol
IL-8 (CXCL8)	IL-6
IL-10	IL-1β
ST2 (IL-33)	IL-23
CD40	IL-17A
CEACAM-5	IL-15
CEACAM-1	IL-33
CXCL5 (ENA-78)	GM-CSF
	IL-2
	IL-13
	IL-22



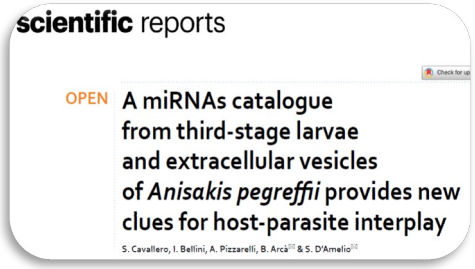
1) The intestinal epithelial barrier was preserved



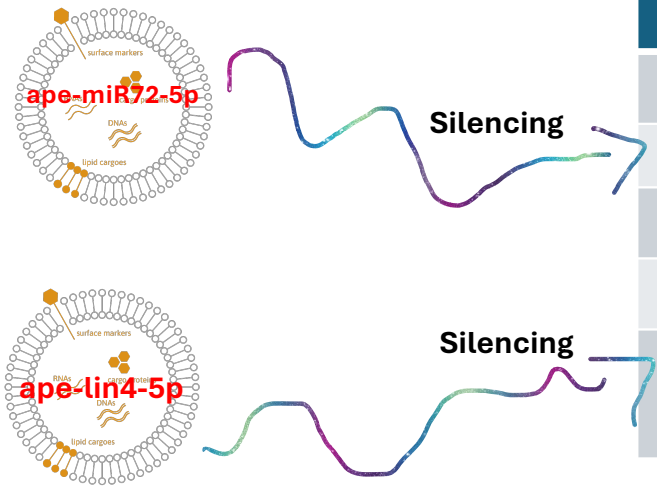
Immunostaining analyses for **ZO-1**, **Vill-1** and **Muc2** markers showed **no differences** between treated and control samples

2) Comparative transcriptomic analysis

7 significantly differentially expressed genes: 2 UPREGULATED AND 5 DOWNREGULATED



DEG (UP)	Types of cancer
NUPR1	breast, Lung cancer tissues and cell lines, Colorectal Cancer, Human gastric cancer tissues
HIST1H2BD	Cholangiocarcinoma, esophageal carcinoma, glioblastoma multiforme (GBM), head and neck squamous cell carcinoma



Putative gene targets of two miRNAs enriched in *Anisakis* EVs

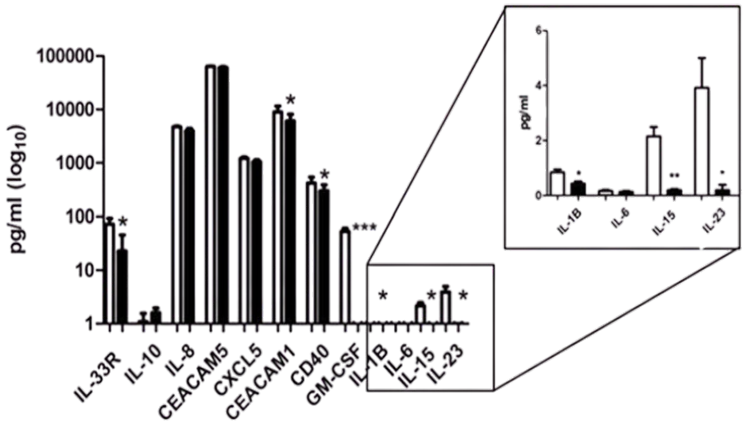
DEG (DOWN)	Types of cancer
LEFTY1	Endometrial, Merkel cell carcinoma, Breast
TACC1	breast, Lung, Uterus, Ovary
MYBL2	human breast cancer, hepatocellular carcinoma (HCC)
MKI67	Renal cellcarcinoma, Lung, Bladder Breast, Cervical, Upper urinary tract, Lymphoma
EPHB2	Prostate, Gastric, Colorectal, Bladder Cholangiocarcinoma, Lung

Bellini et al., 2024

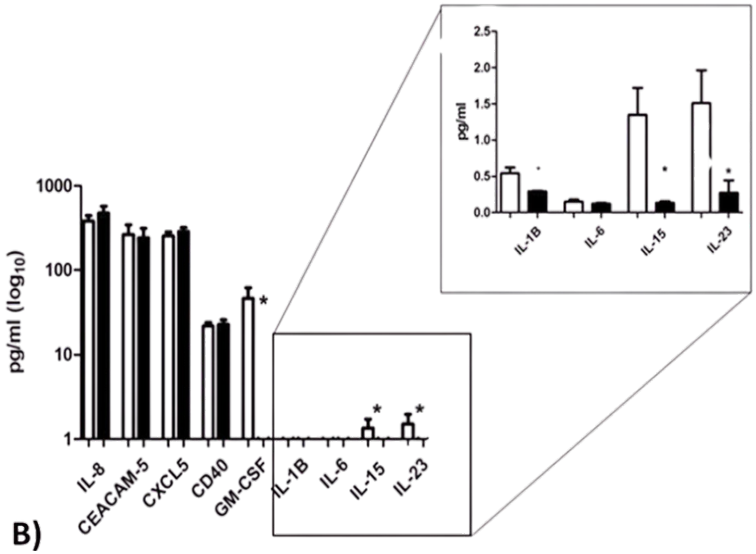


All the seven genes are involved in pathways related to **chronic inflammation and cancer**

3) Multiplex ELISA assay



*IL-33R, CD40, CEACAM-1, IL-1β, GM-CSF, IL-15, and IL-23



*IL-1β, GM-CSF, IL-15, and IL-23

IL-33: involved in helminth expulsion
IL-15: LT proliferation
Il-23: activates Th17 helper T cells and stimulates the production of interleukin-17 (IL-17), promoting inflammatory responses

Immunosuppression

Downregulation of cytokines involved in host immune strategy against helminths

Bellini et al., 2024

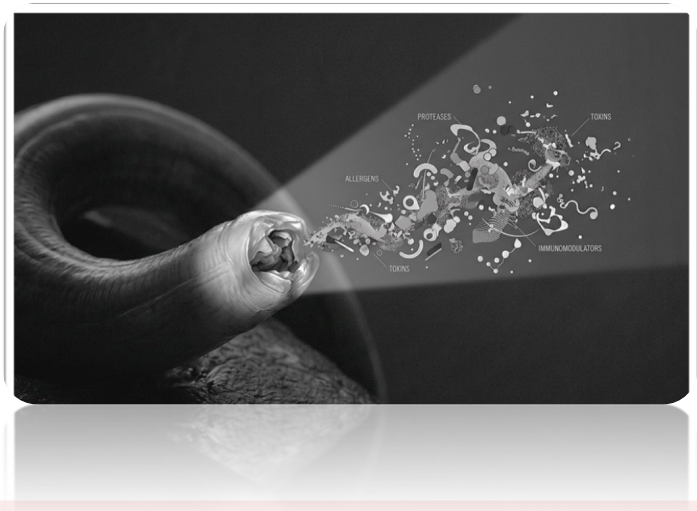
Anisakis extracellular vesicles VS human accidental host

Anisakis products (Evs) can alter the expression of genes involved in inflammation and **Downregulate** key factors involved in host defence **strategy against helminths**

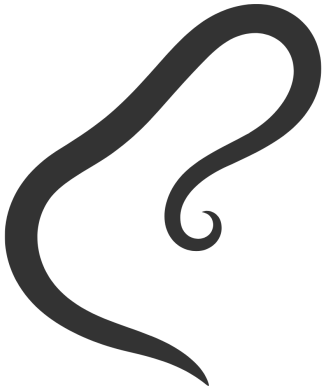
Mechanical movement



Excreted/secreted products



Somatic antigens released after death



Anisakis is **not** only a matter of **larvae** and **allergy**: what it releases, even after larval death, may also have effects on our organism.

Conclusions and future perspectives:



Control of fish products
prevents the ingestion of live larvae

Correct freezing and cooking
inactivates larvae

Larger efforts are needed on three sides:

The human side

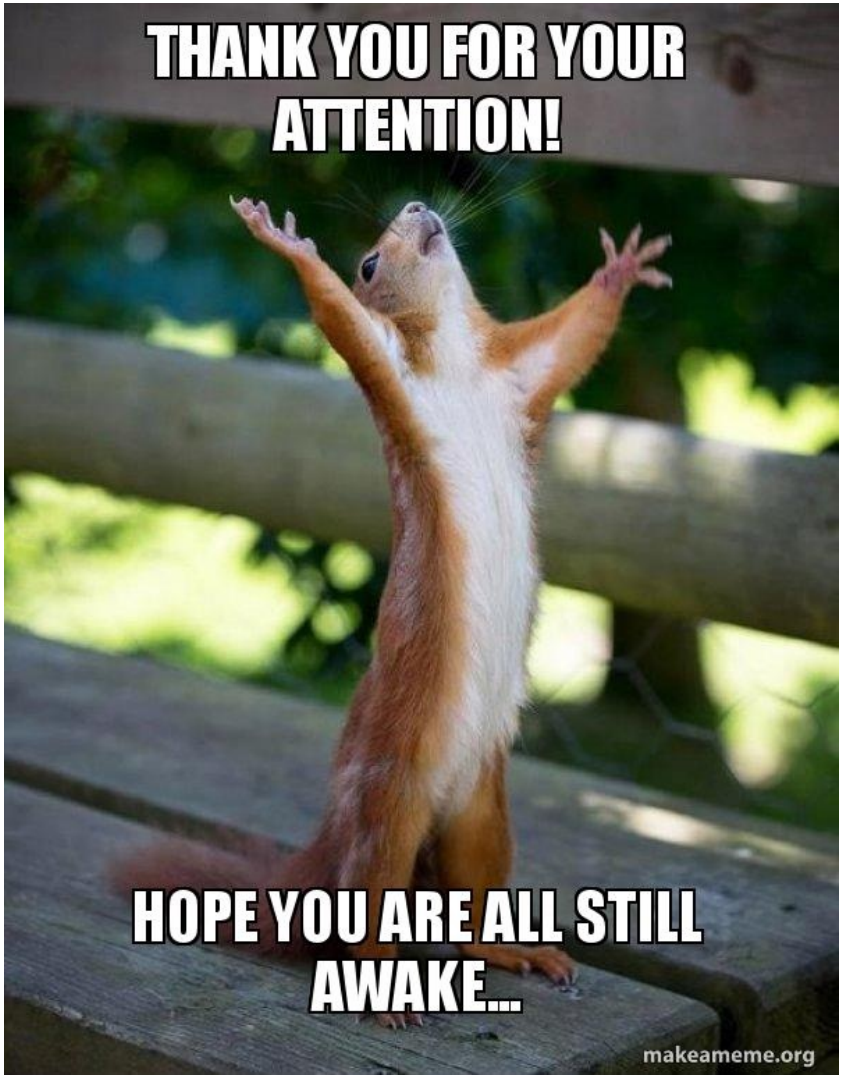
Allergy and tumorigenic potential;
Role of individual genetic background
Monitoring of potential sequeale
Improvement of diagnostic tools

The parasite side

More data on the parasite's molecular "messages" (omics, standardization of protocols..); more evidence from *in vitro and in vivo* models

The natural host side

Continuous monitoring of *Anisakis* in fish and in the marine environment



Thanks to:

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