PREPARE4 VBD

Horizon 2020
European Union Funding
for Research & Innovation

A Cross-Disciplinary Alliance to Identify, PREdict and prePARe for Emerging VectorBorne Diseases - PREPARE4VBD Project

Copromicroscopic techniques and photographic illustrations of eggs for the diagnosis of *Fasciola* spp. and Paramphistomidae

2nd Edition

UNIVERSITÀ
DEGLI STUDI DI NAPOLI
FEDERICO II

Dipartimento
Medicina Veterinaria
Produzioni Animali

Centro Regionale Monitoraggio Parassitosi
CREMOPAR

Fasciola hepatica and *Calicophoron daubneyi* eggs

Egg of *Fasciola hepatica* (liver fluke):
Oval, thin-shelled, with operculum, 130-145 x 70-90 µm, golden-yellow, contains the fertilised egg cell and yolk cells.

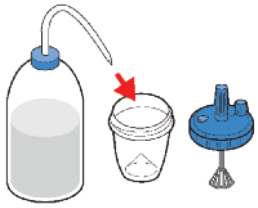
Egg of *Calicophoron daubneyi* (rumen fluke):
Oval, thin-walled, with operculum, 140-180 x 76-95 µm, light grey, completely filled with granular material (zygote and yolk cells).

Copromicroscopic techniques

Mini-FLOTAC Technique

MINI-FLOTAC
Technique

STEP 1



Add 45 ml of FS-Zinc sulphate 1,350

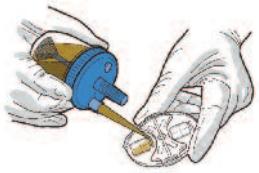
STEP 2

Fill the conical collector with 5 g of faeces

STEP 3

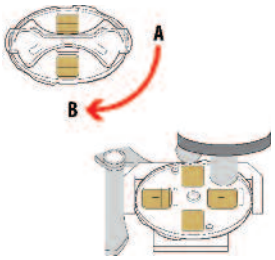
Homogenize the sample

STEP 4

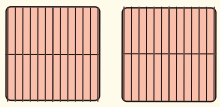


Fill the Mini-FLOTAC

STEP 5



Wait for 10 minutes, translate and examine under the microscope



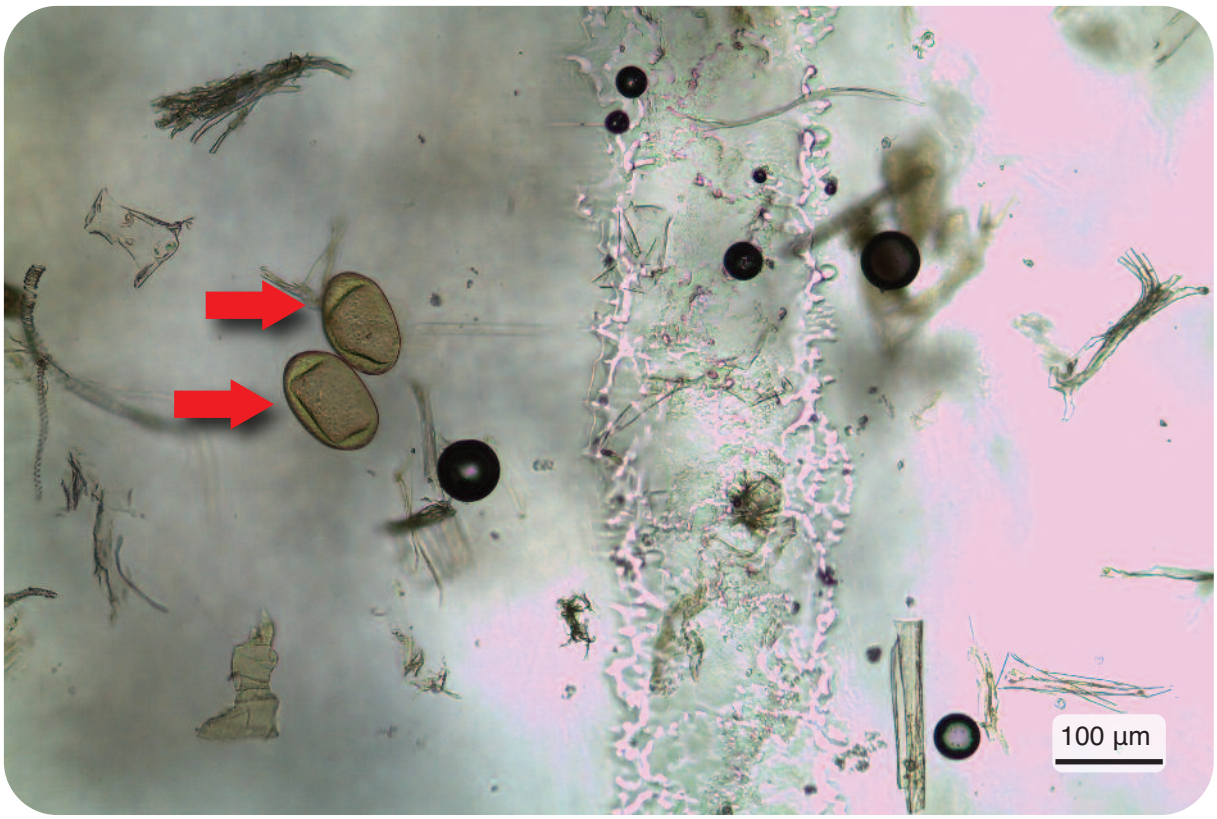
Analytic sensitivity and multiplication factor = **5 Eggs per gram (EPG) of faeces**

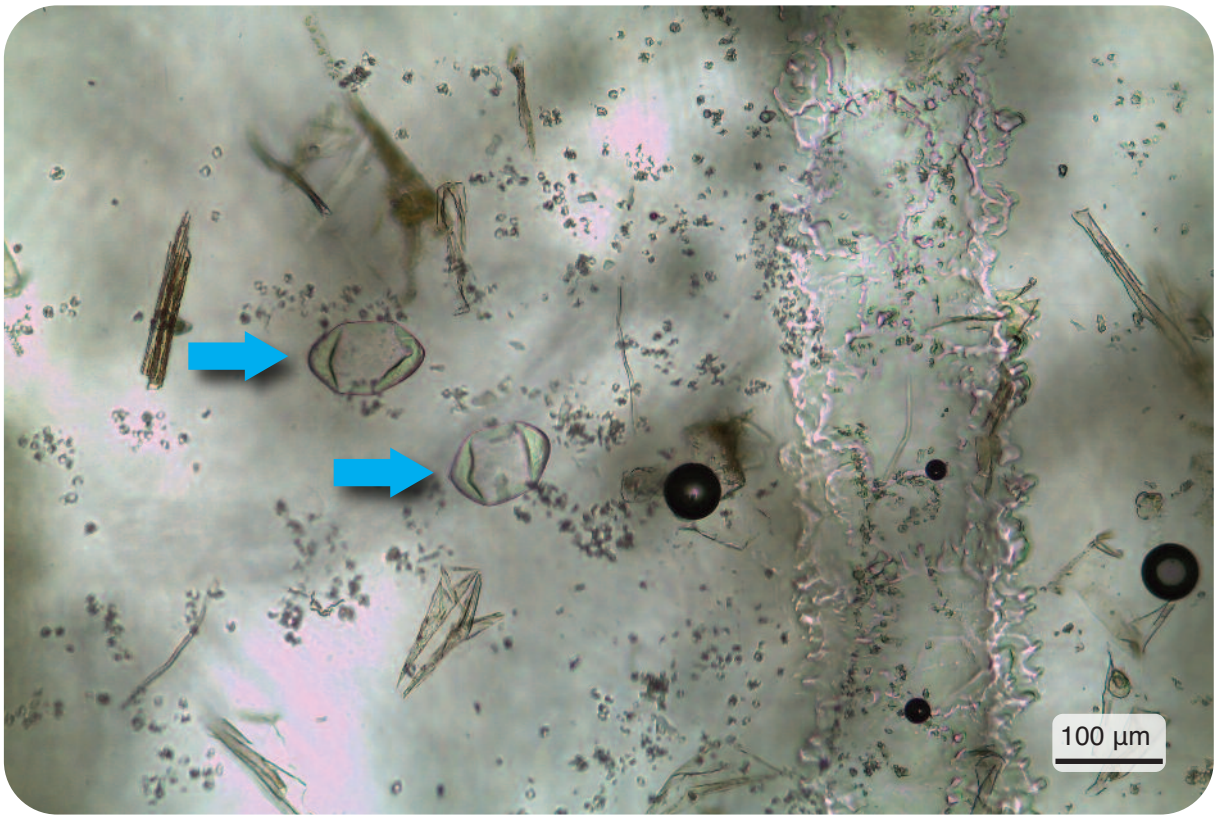
Eggshells (eggs slightly deformed due to flotation solution) of *Fasciola hepatica* (red arrow) and *Calicophoron daubneyi* (blue arrow) under Mini-FLOTAC (100× magnification): *F. hepatica* eggshells are golden-yellow, whereas *C. daubneyi* eggshells are light grey in colour.

Mini-FLOTAC Technique
(100x magnification)

F. hepatica eggshells

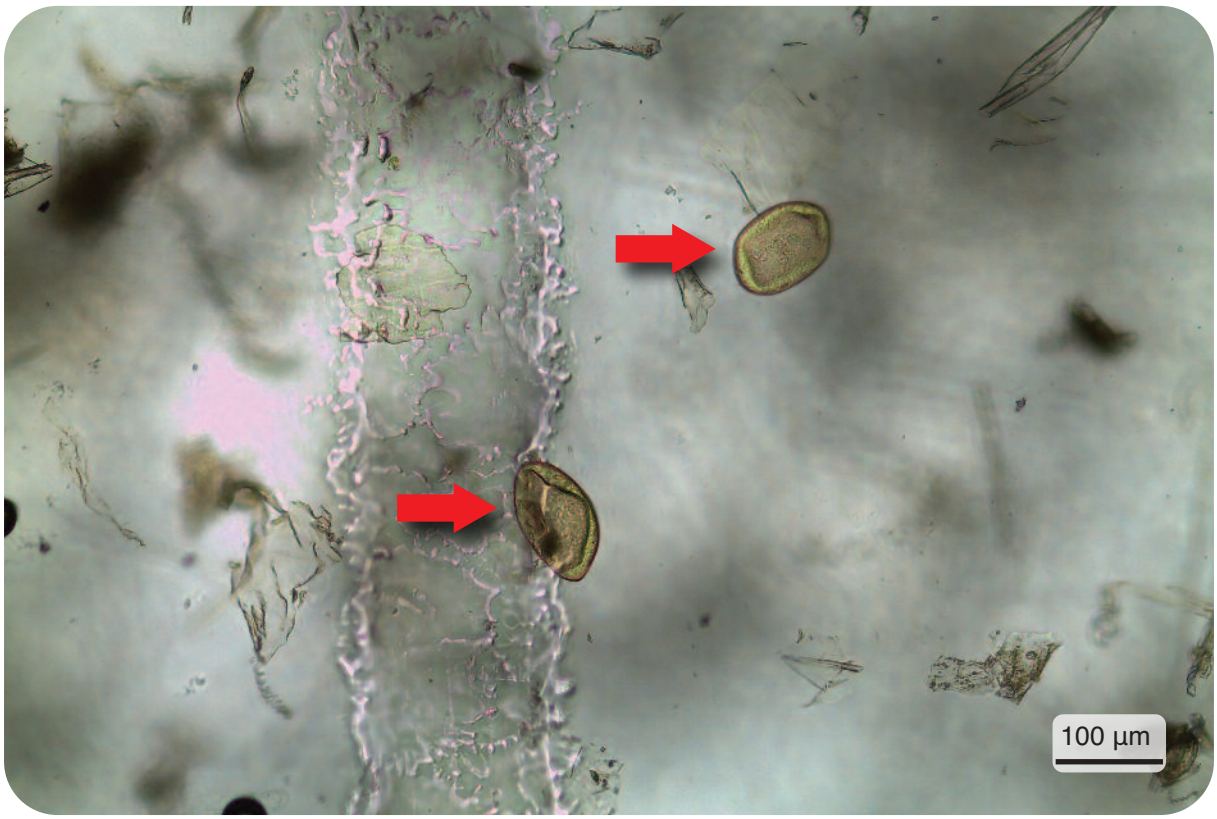
C. daubneyi eggshells

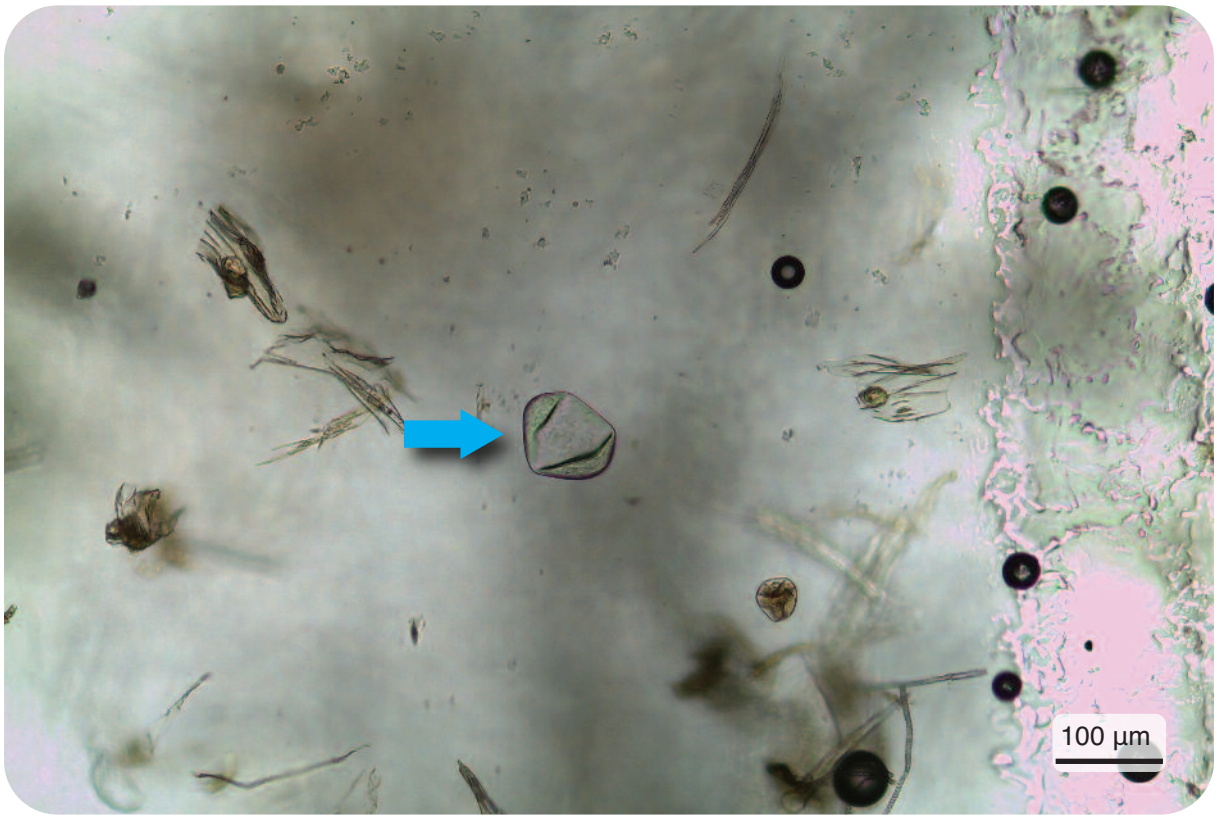




Mini-FLOTAC Technique
(100x magnification)

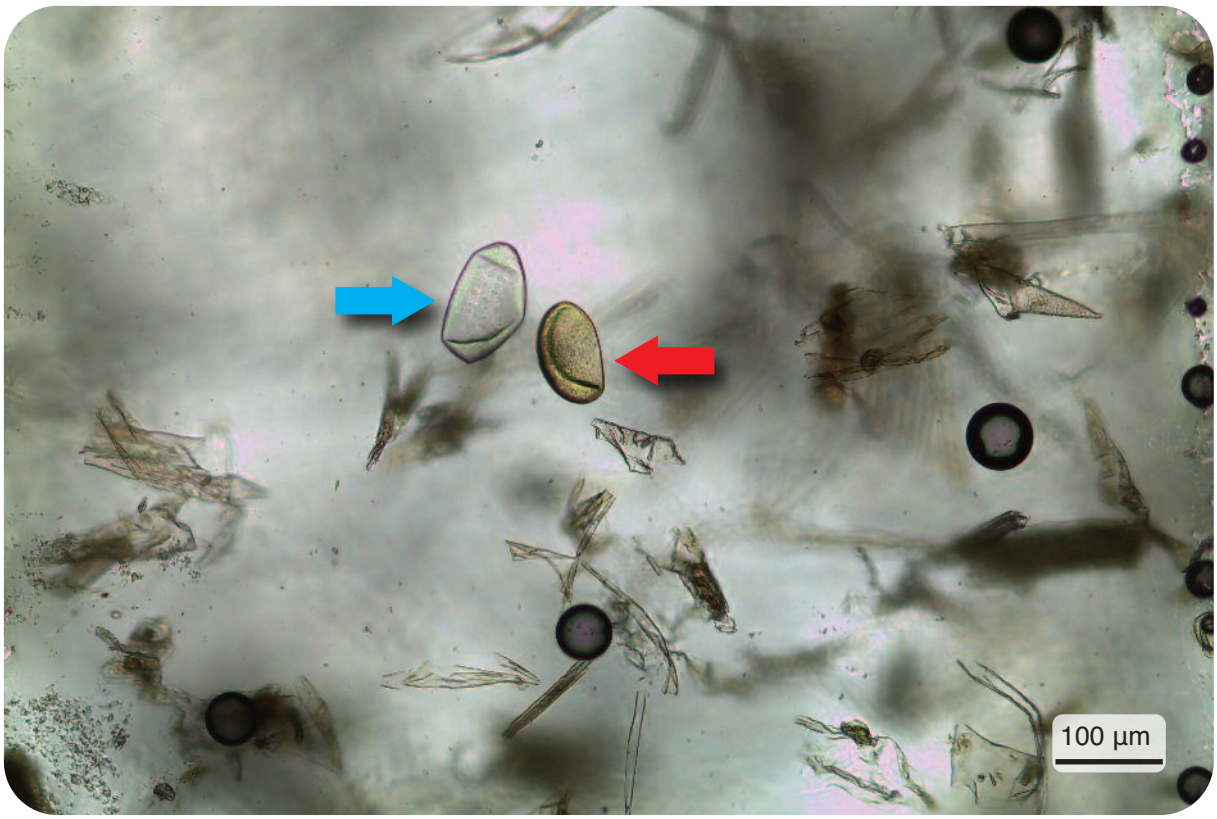
F. hepatica eggshells *C. daubneyi* eggshells

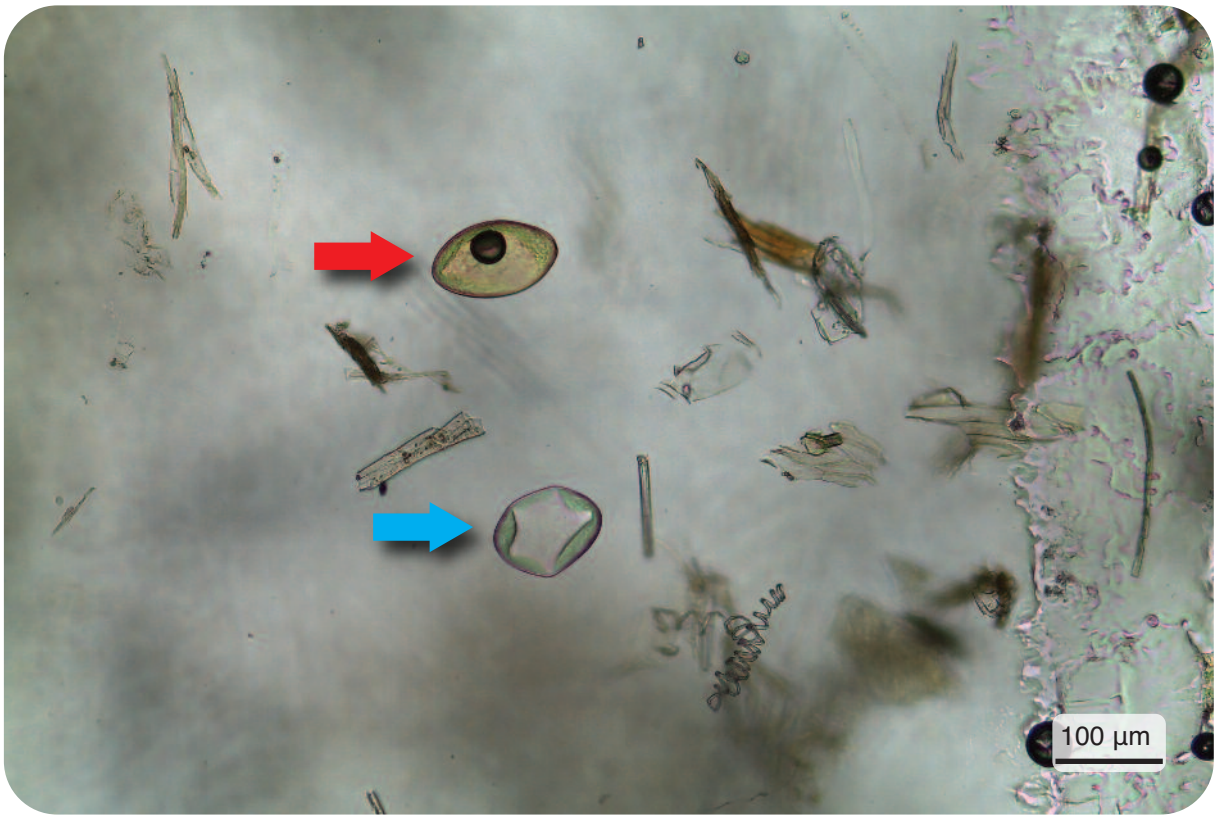




Mini-FLOTAC Technique
(100x magnification)

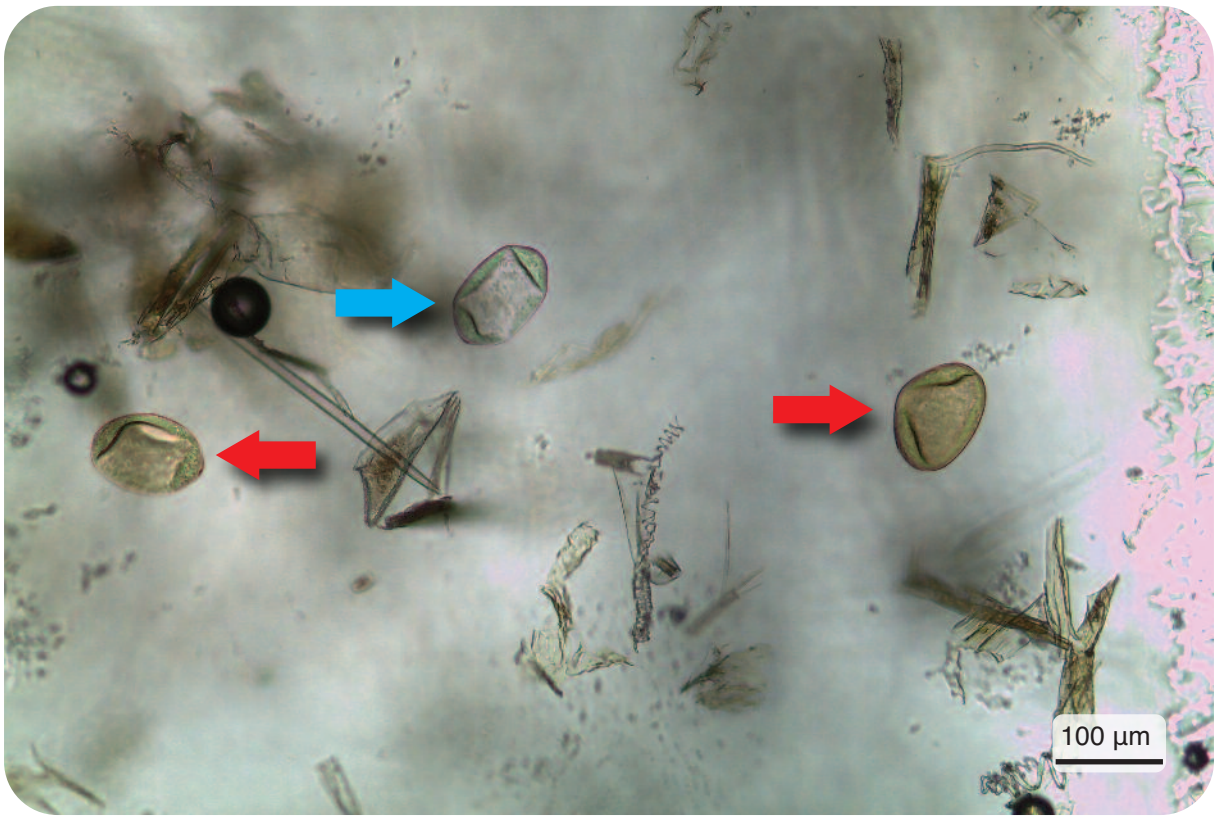
F. hepatica eggshells *C. daubneyi* eggshells

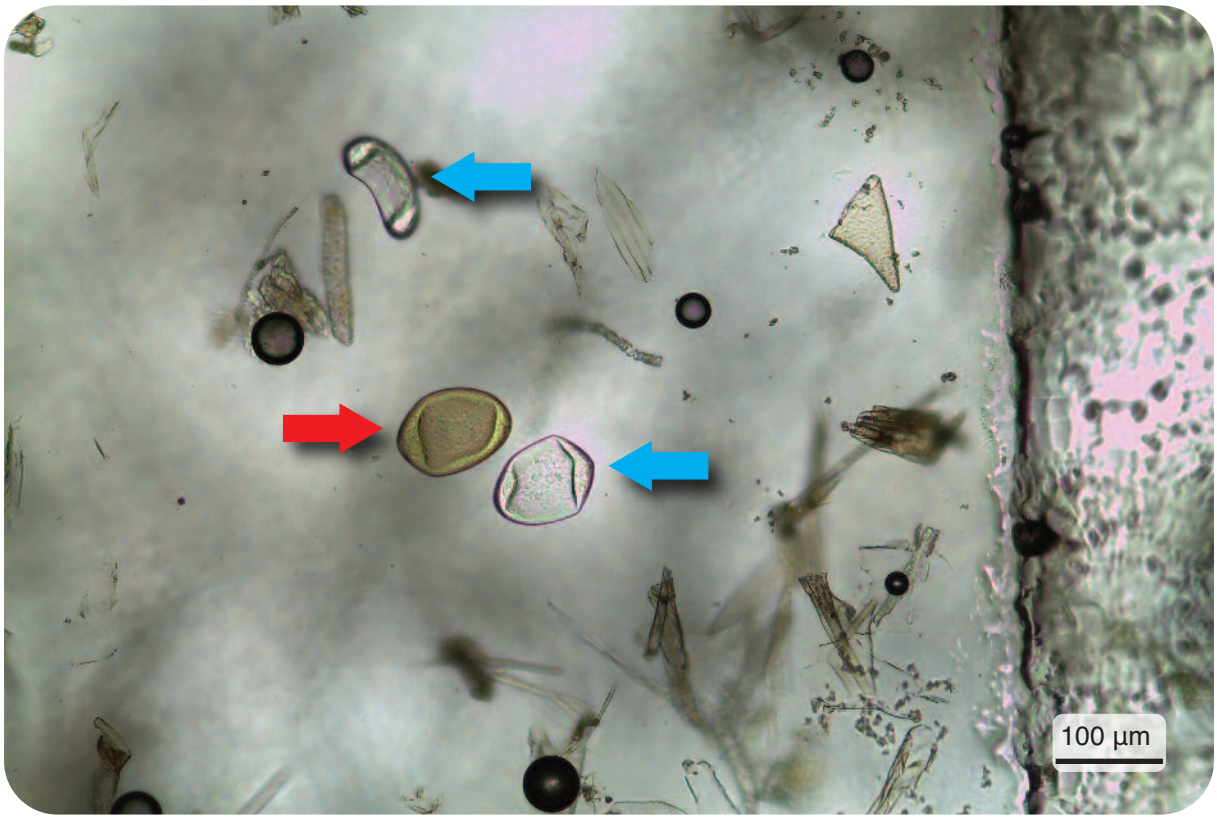




Mini-FLOTAC Technique
(100x magnification)

F. hepatica eggshells *C. daubneyi* eggshells





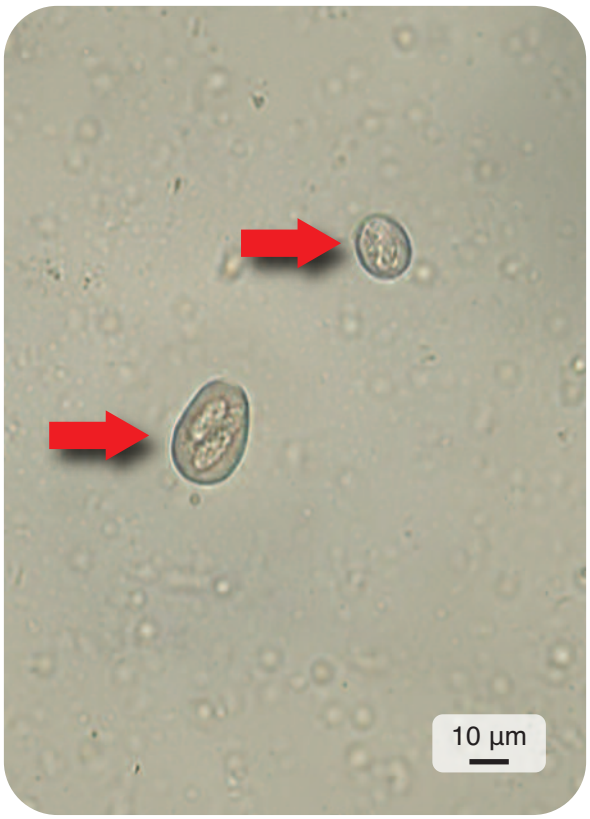
APPENDIX

Mini-FLOTAC Technique

Other parasitic elements (eggs, oocysts, cysts), artifacts and pseudo-parasites

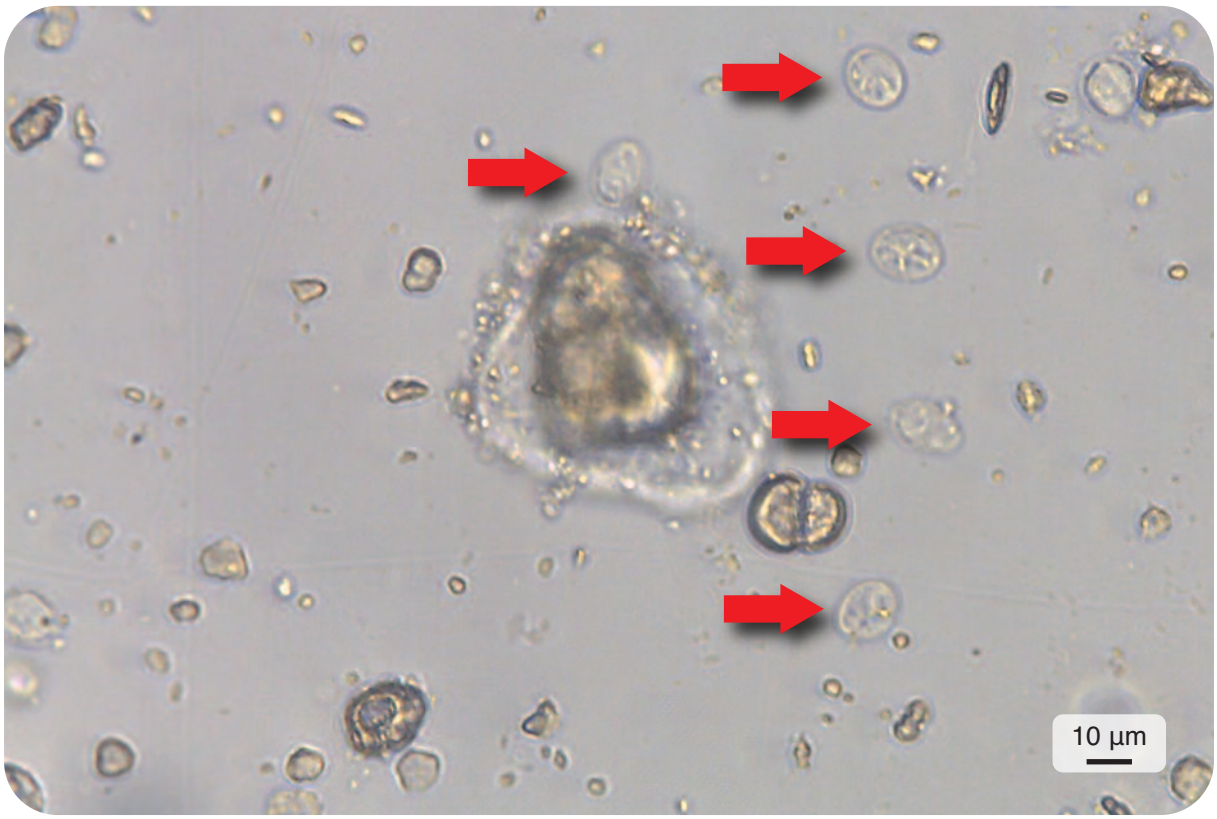
***Eimeria* spp.**
Oocysts of *Eimeria* spp. are ovoid or subspherical and colourless (20-34 x 12-23 µm). The species can be differentiated after sporulation.

Eimeria spp. oocysts



***Giardia* spp.**
Cysts of *Giardia* spp. are ovoid, colourless and contain four nuclei (8-12 x 7-10 µm).

Giardia spp. cysts

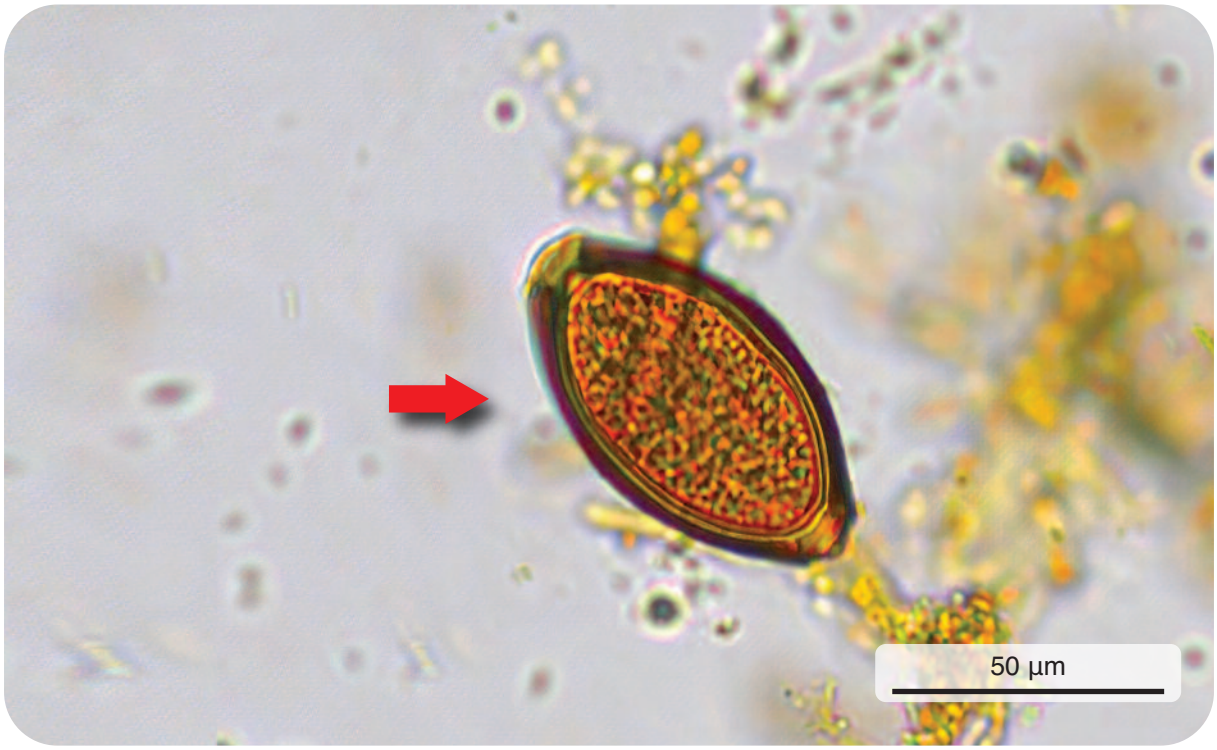


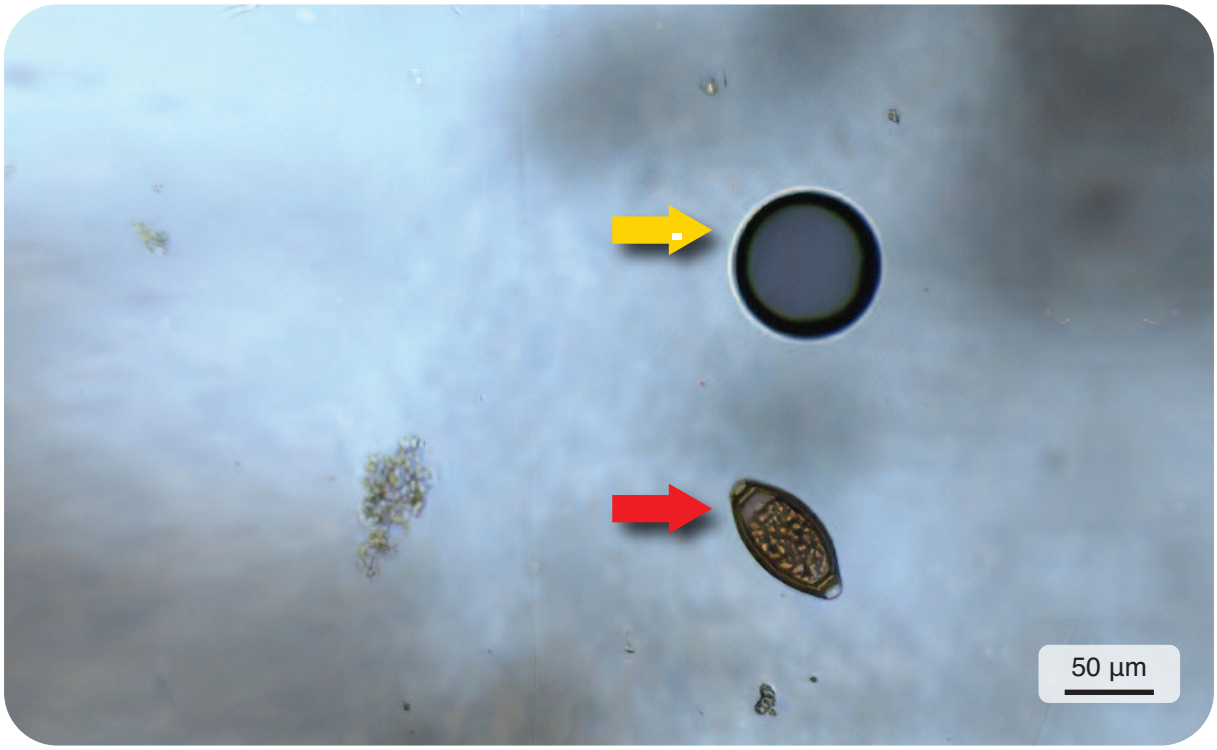
Trichuridae

***Trichuris* spp.**

Eggs of *Trichuris* spp. are medium-sized (70-80 x 30-42 µm), thick walled, lemon-shaped, curved side walls, with two transparent polar plugs, dark brown, unsegmented and granular contents.

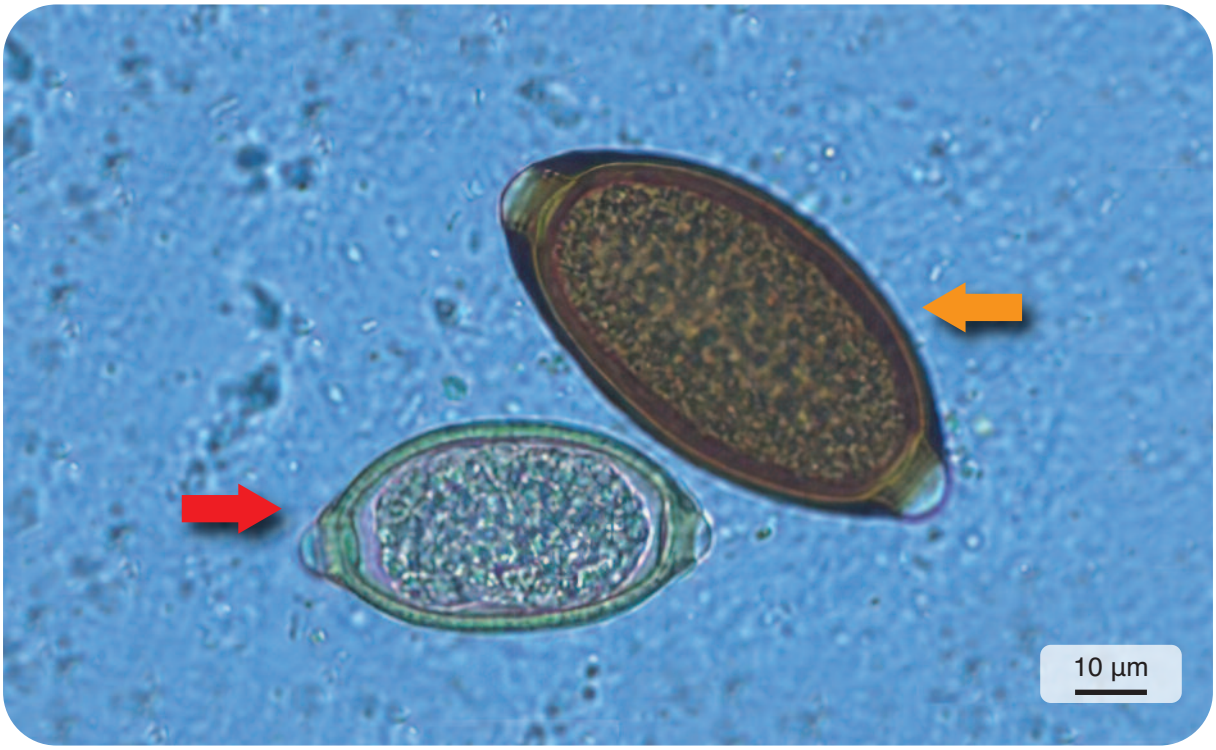
Trichuris eggs Air bubbles





***Capillaria* spp.**
Eggs of *Capillaria* spp. are barrelshaped (similar to *Trichuris*), 45–50 × 22–25 µm, colourless and have thick shells that are slightly striated with bipolar plugs.

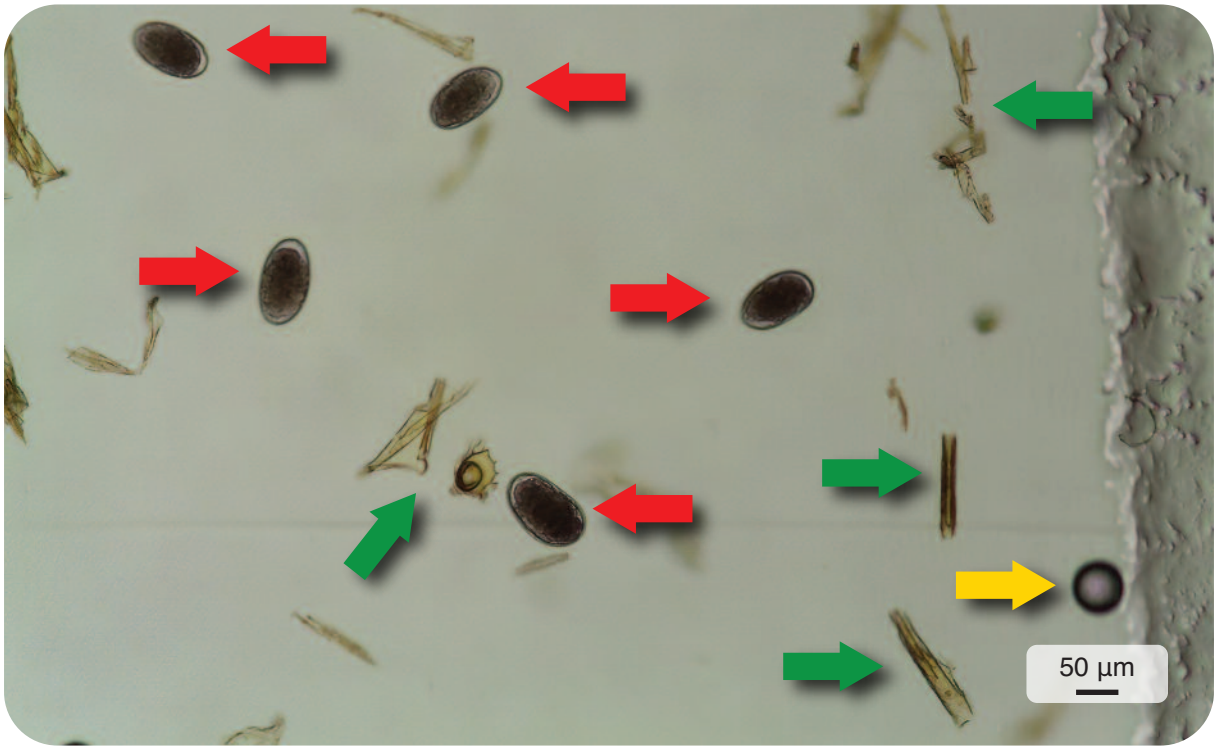
Capillaria eggs *Trichuris* eggs



Gastrointestinal nematodes (GINs)

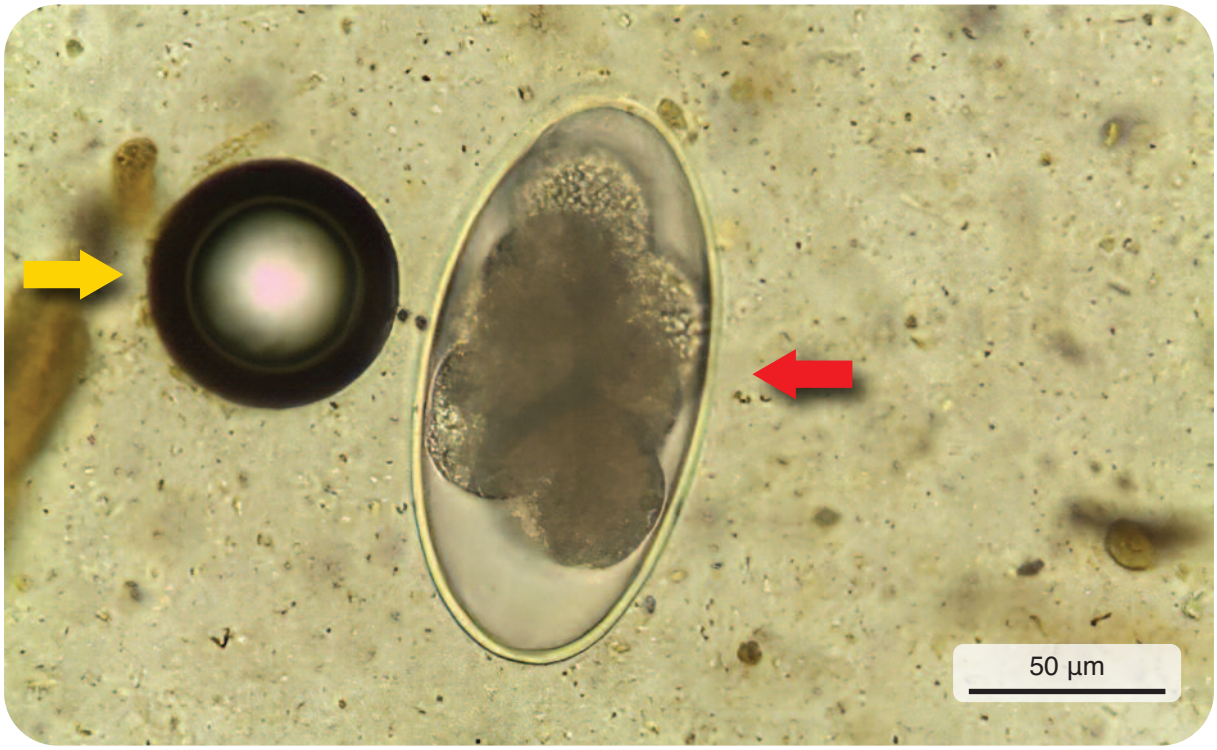
Eggs of GINs are medium-sized (range 70-98 x 30-50 µm), more than 16 blastomeres, thin-walled, oval with equal poles or one more tapered pole and colourless. Eggs are very similar, differentiation is possible after coproculture by identification of third-stage larvae (L3).

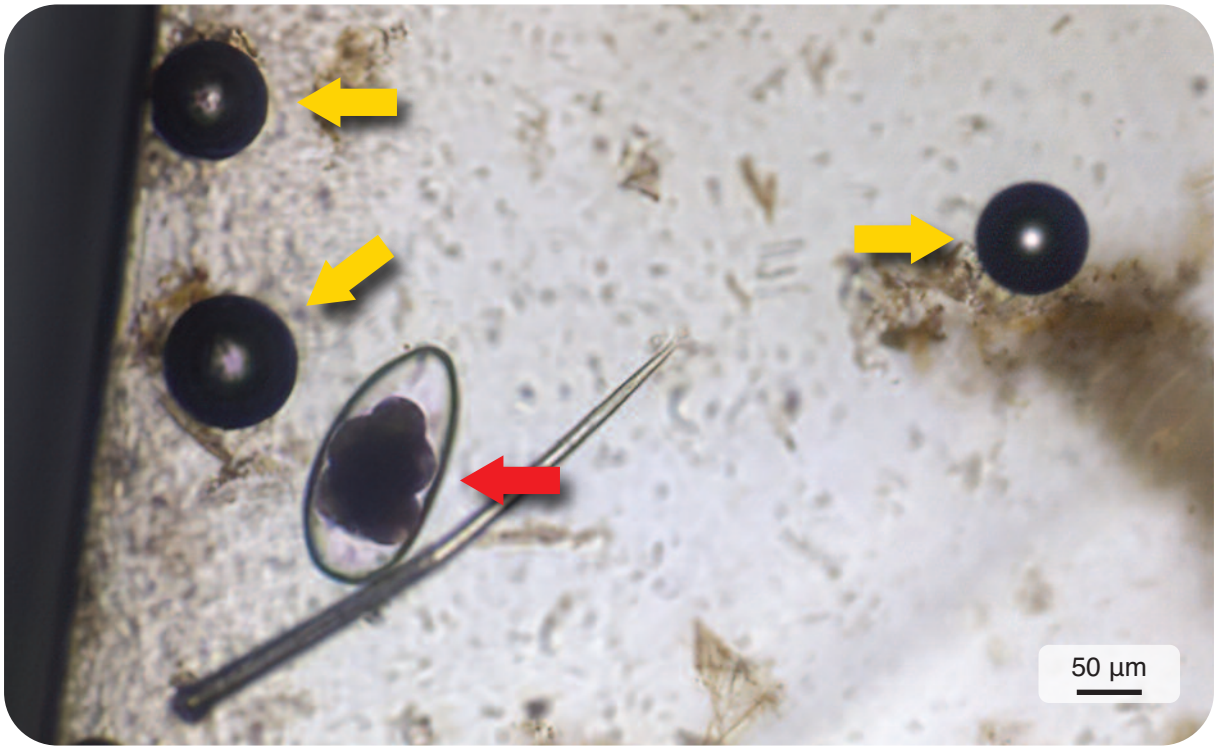
GIN eggs Plant matters Air bubbles



***Nematodirus* spp.**
Eggs of *Nematodirus* spp. are very large (range 150-200 x 75-100 µm), curved side walls of medium thickness, contains less than 16 blastomeres, poles more tapered.

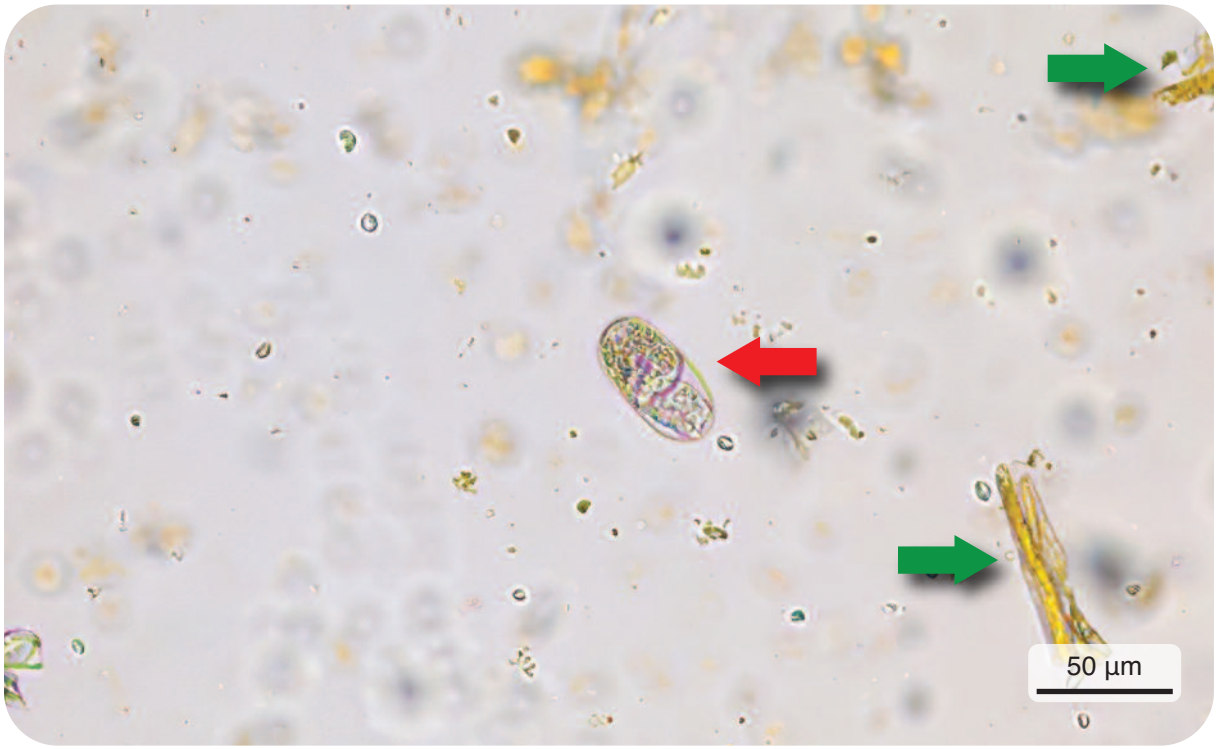
Nematodirus eggs Air bubbles





***Strongyloides* spp.**
Eggs of *Strongyloides* are small (40-55 x 30-40 µm) thin-walled, wide ellipsoid with flattened poles, colurless, with embryo.

Strongyloides eggs Plant matters

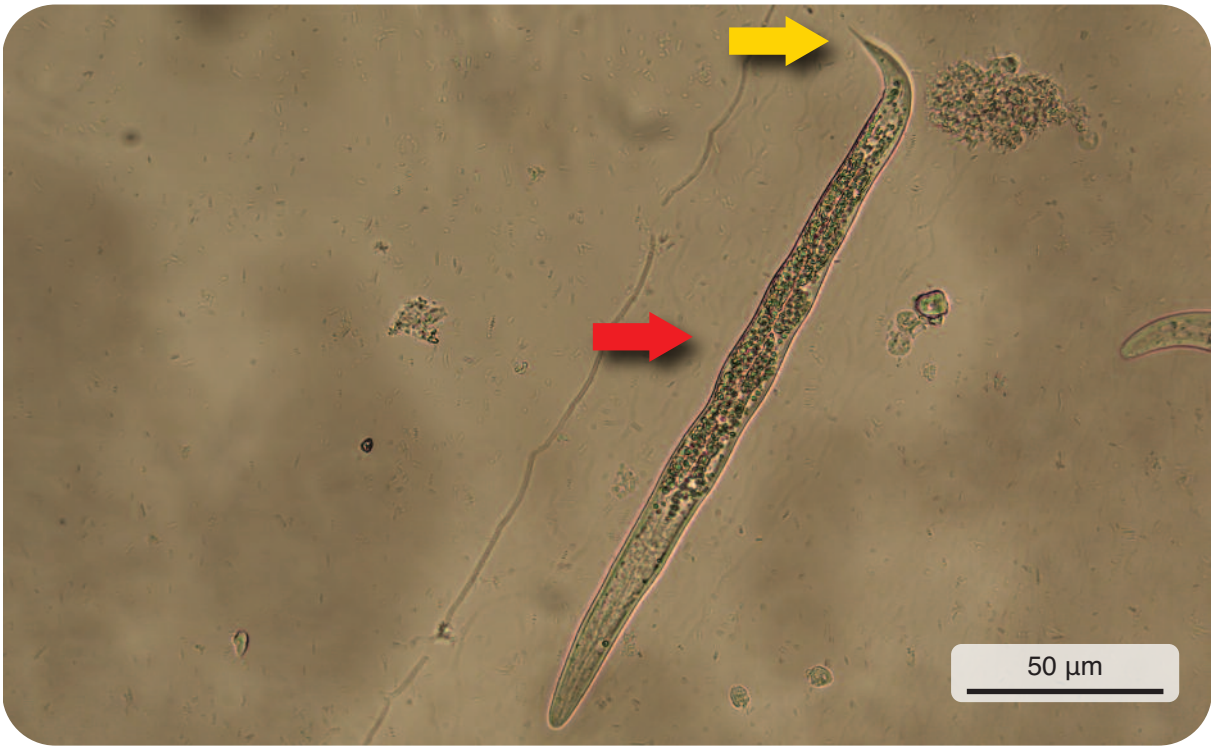


Dictyocaulus viviparus

First-stage larvae (L1) of *Dictyocaulus viviparus* (316-367 µm) are unsheathed and characterized by an indistinct oesophagus, granulated midgut, and a pointed posterior end.

Dictyocaulus viviparus larvae L1

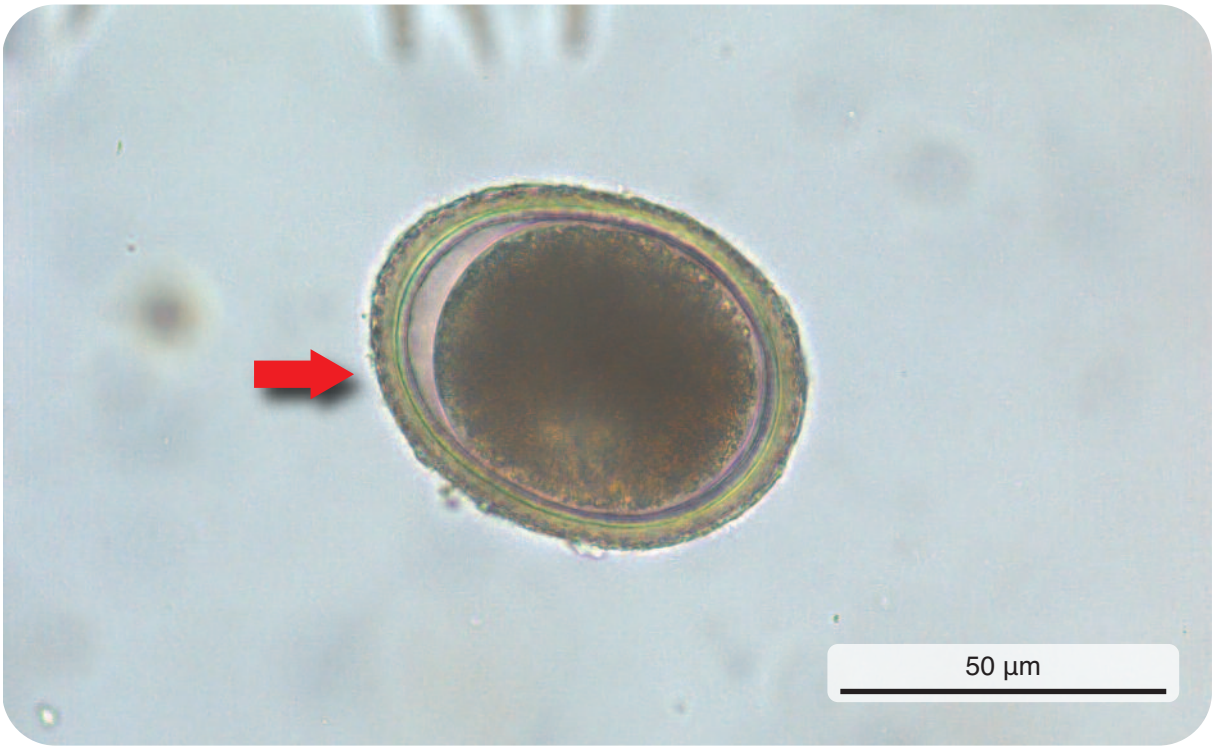
Pointed posterior end

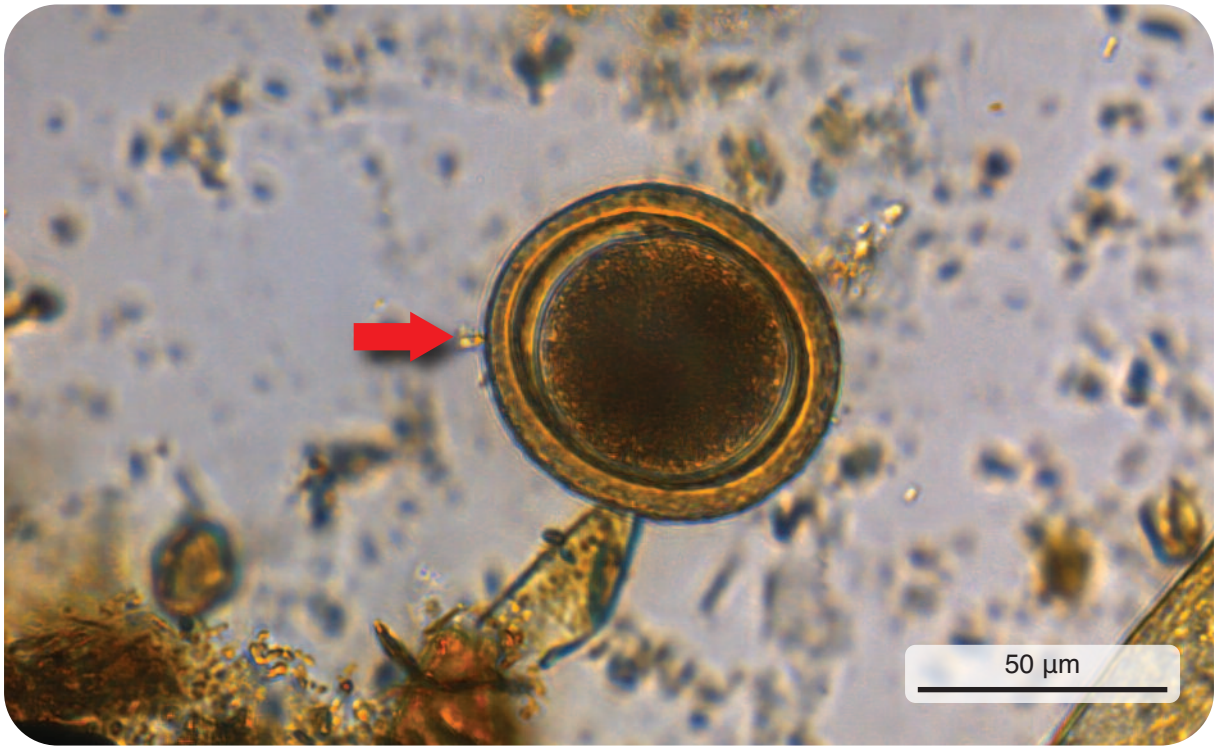


Toxocara vitulorum

Eggs of *Toxocara vitulorum* are medium-sized (70-95 x 60-77 µm), spherical, thick pitted shell (golf ball like), greyish or with white yellowish tinge, unsegmented and and granular contents.

Toxocara vitulorum eggs



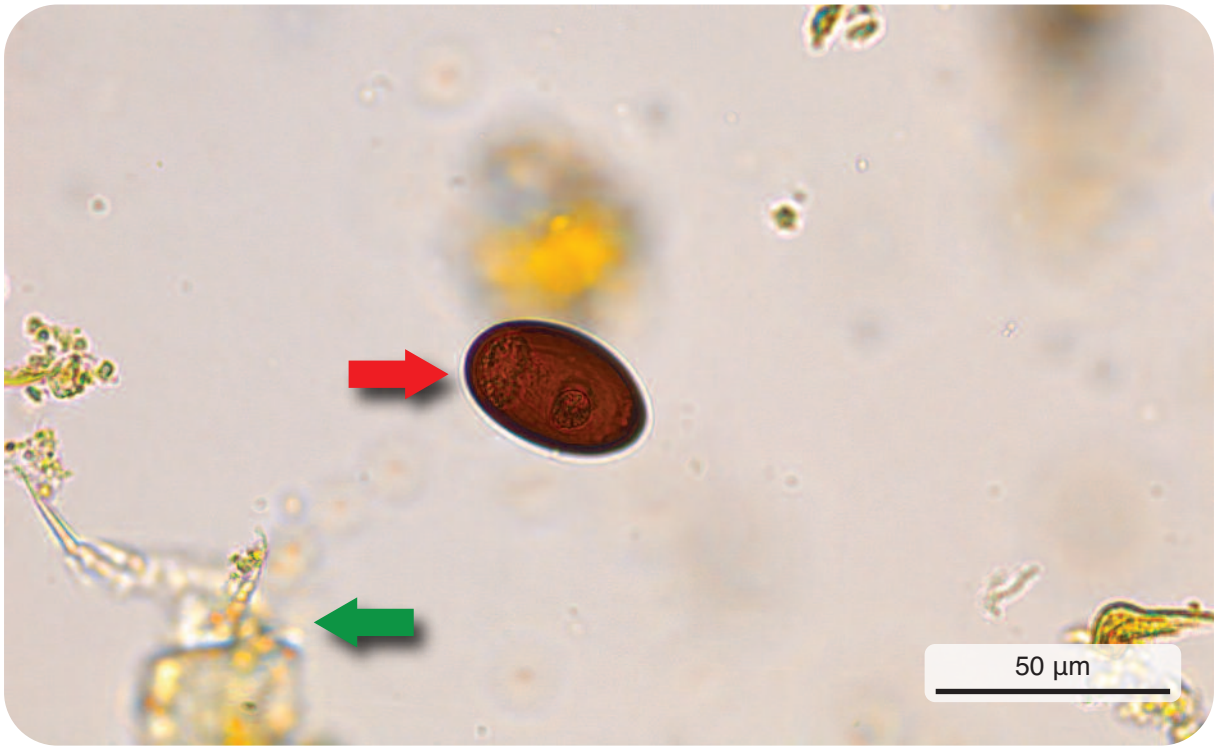


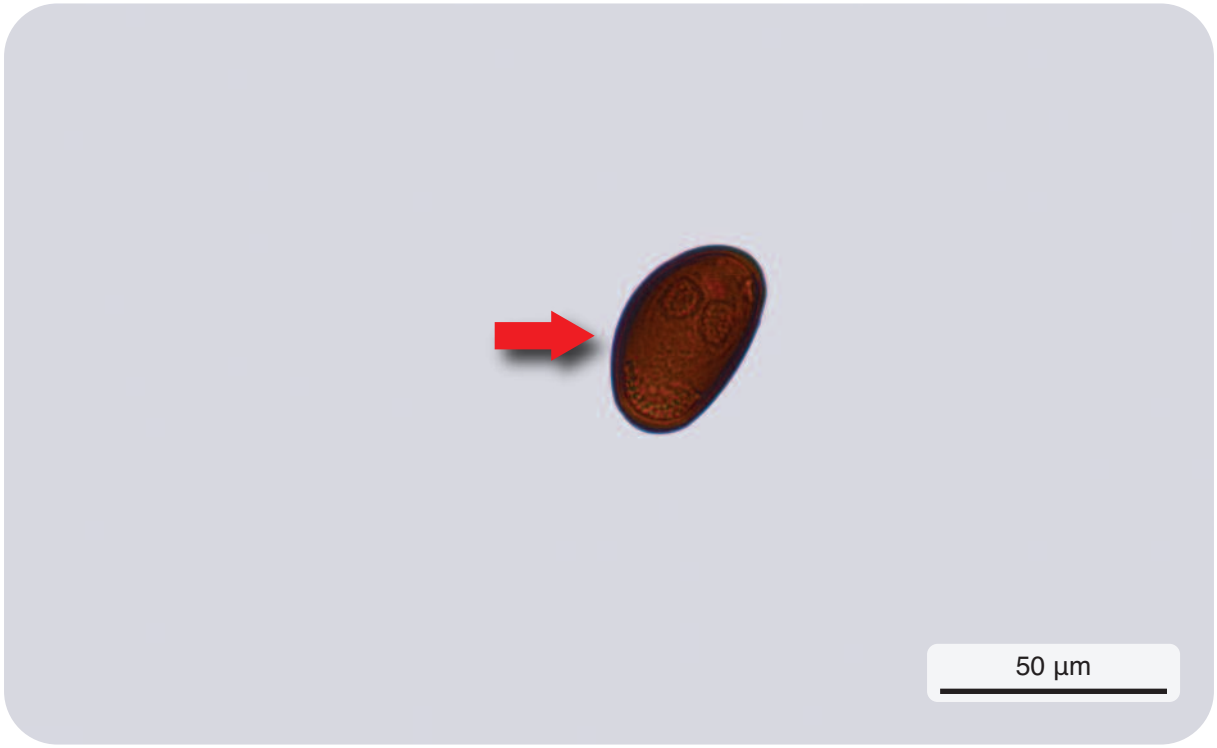
Dicrocoelium dendriticum

Eggs of *Dicrocoelium dendriticum* are small (35-40 x 39-30 µm), dark brown and operculated, usually with a flattened side. It contains a miracidium when passed in the faeces.

Dicrocoelium dendriticum eggs

Plant matters

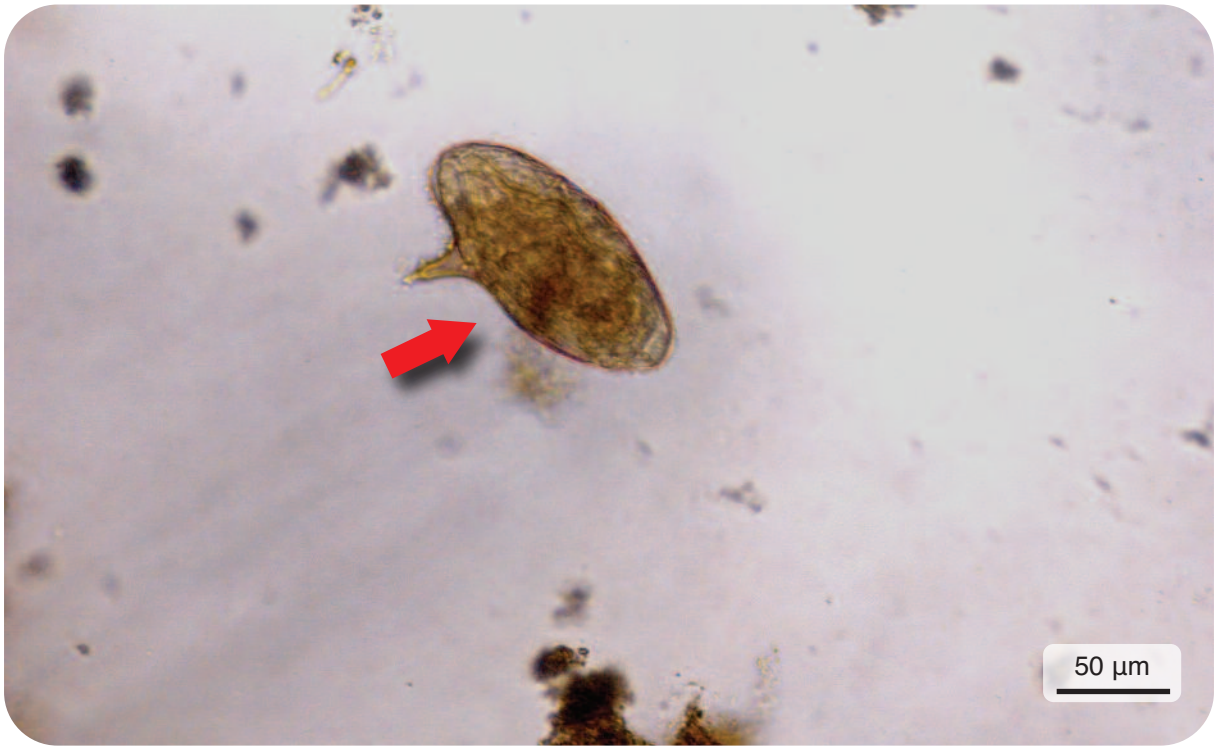




***Schistosoma* spp.**

Eggs of *Schistosoma* spp. are large (114-180 x 45-70 µm), oval or spindle-shaped, without operculum, some with terminal or lateral spine, contain a fully developed miracidium when released from the host.

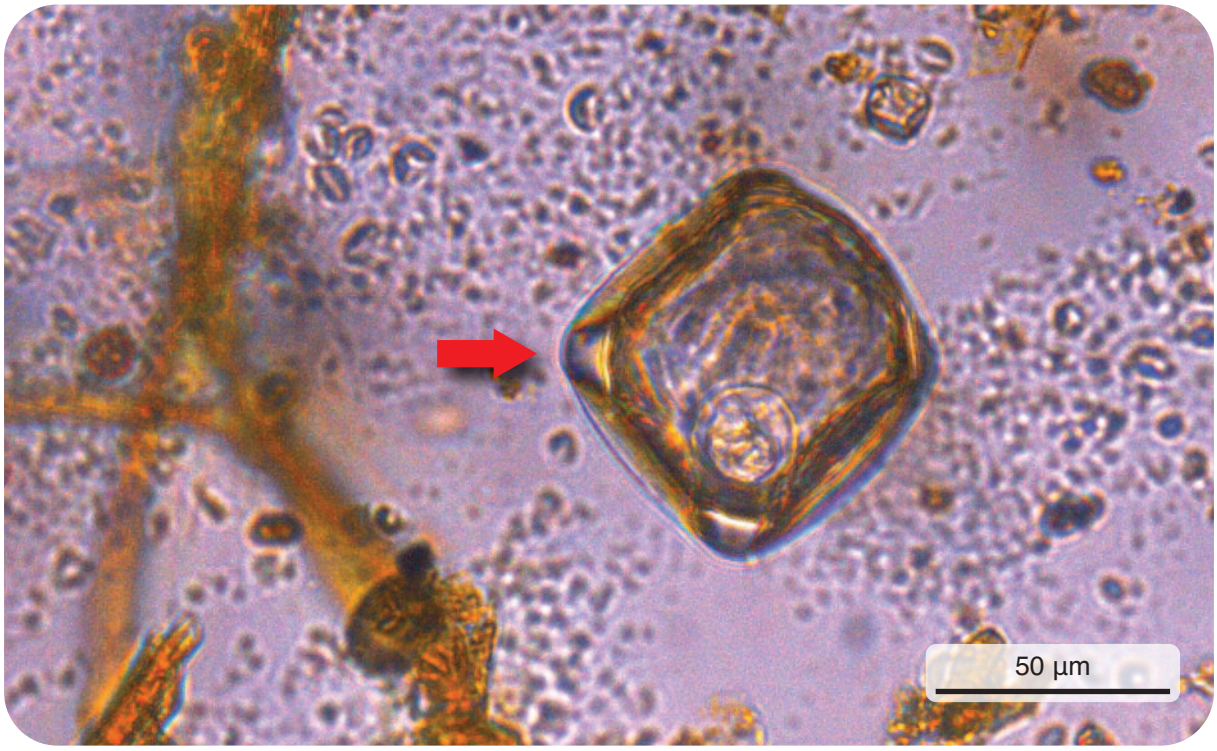
➡ *Schistosoma* eggs

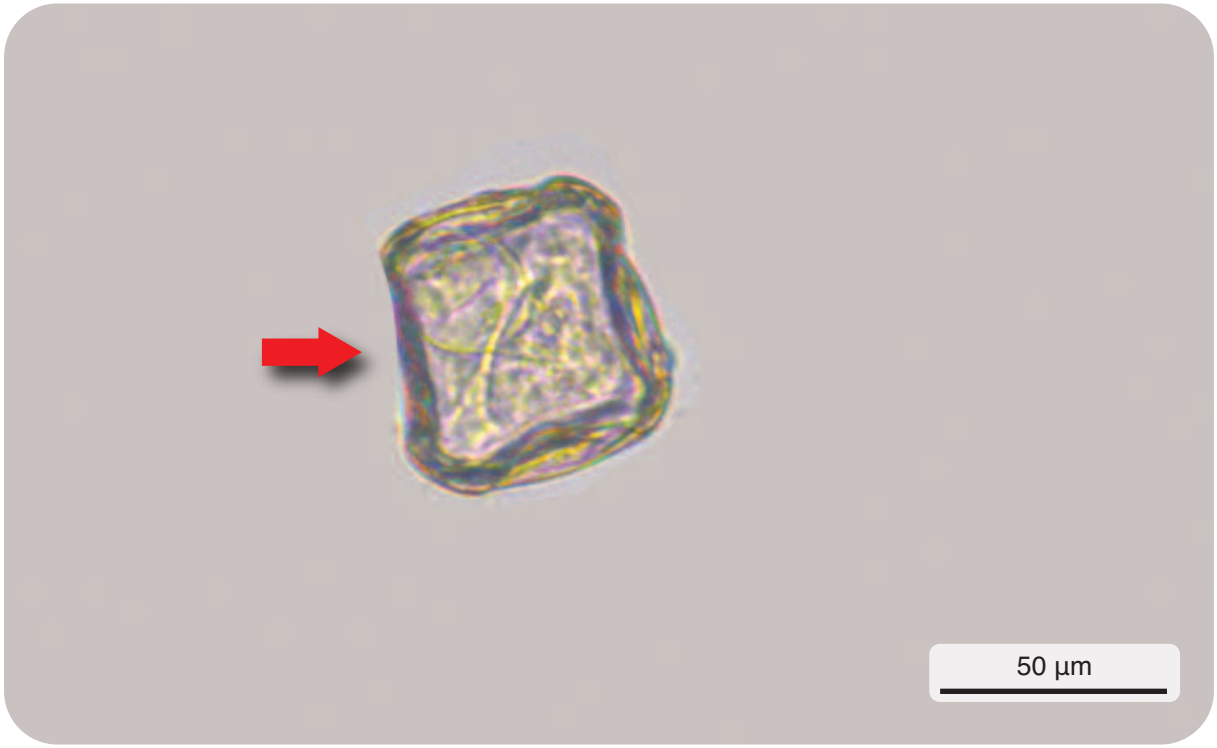


Moniezia benedeni

Moniezia benedeni eggs are medium-sized (70-90 µm), rectangular-shaped, whick-walled, contains an oncosphere, surrounded by pear-shaped embryophore (pear-shaped apparatus).

Moniezia benedeni eggs

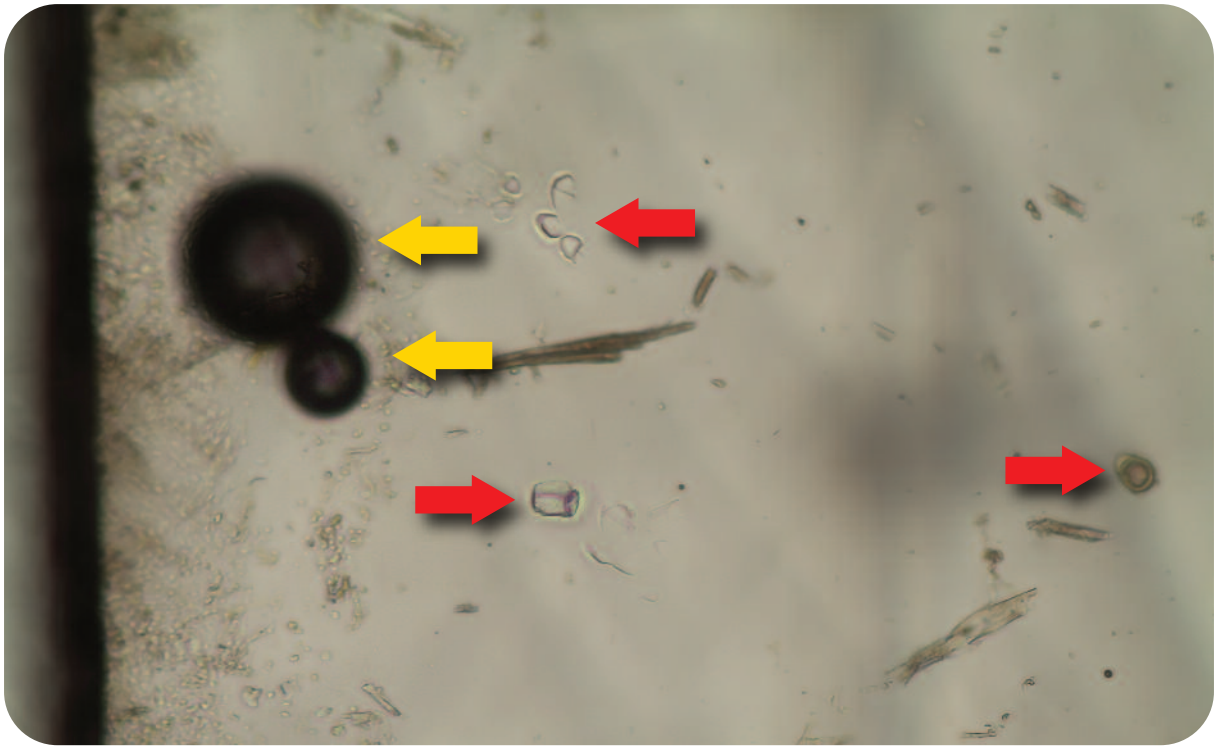




Many elements can be confused with parasite eggs such as: plant matters, pollens, plant cells, plant fibers, artifacts caused by crystal, soap and oil drops.

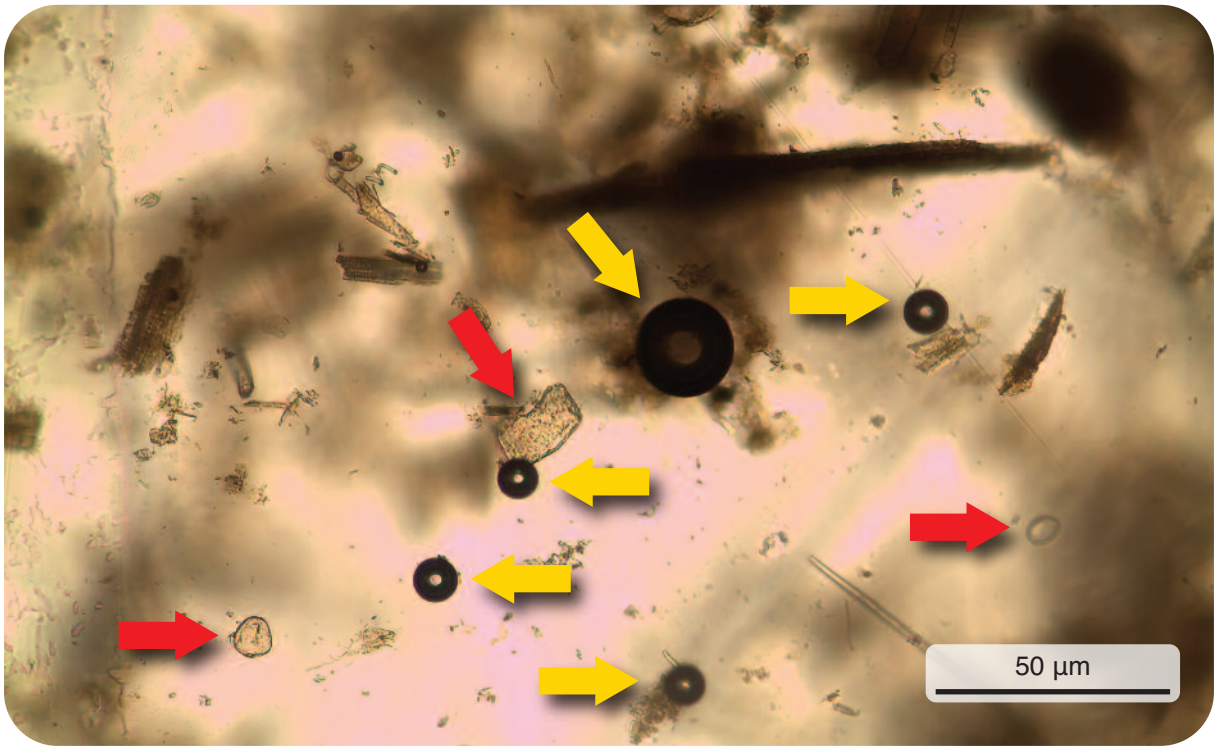
Artifacts

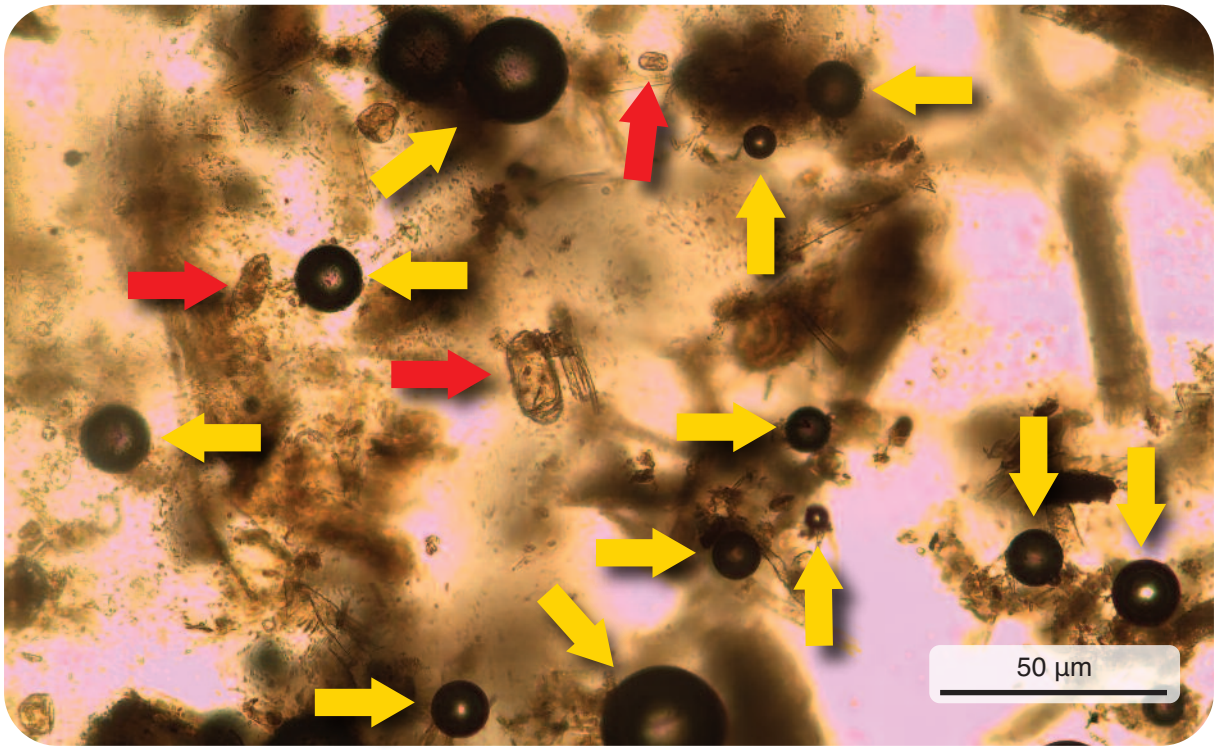
Air bubbles



Artifacts

Air bubbles



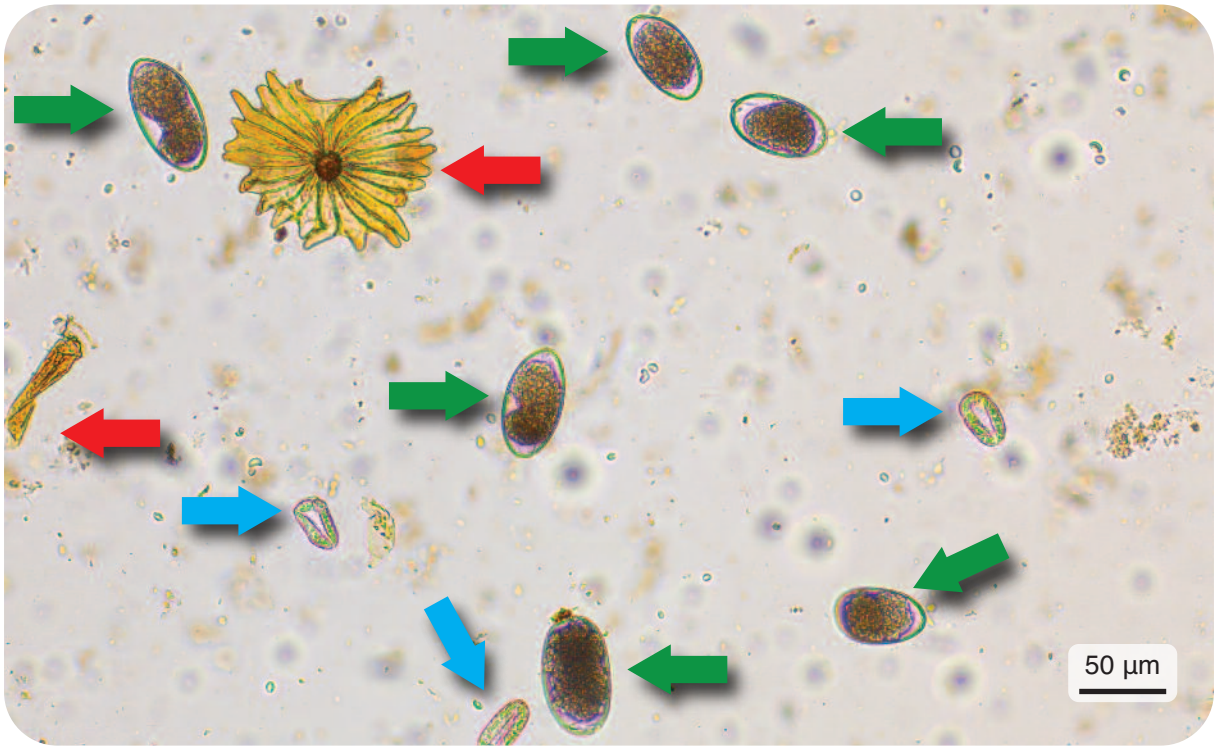


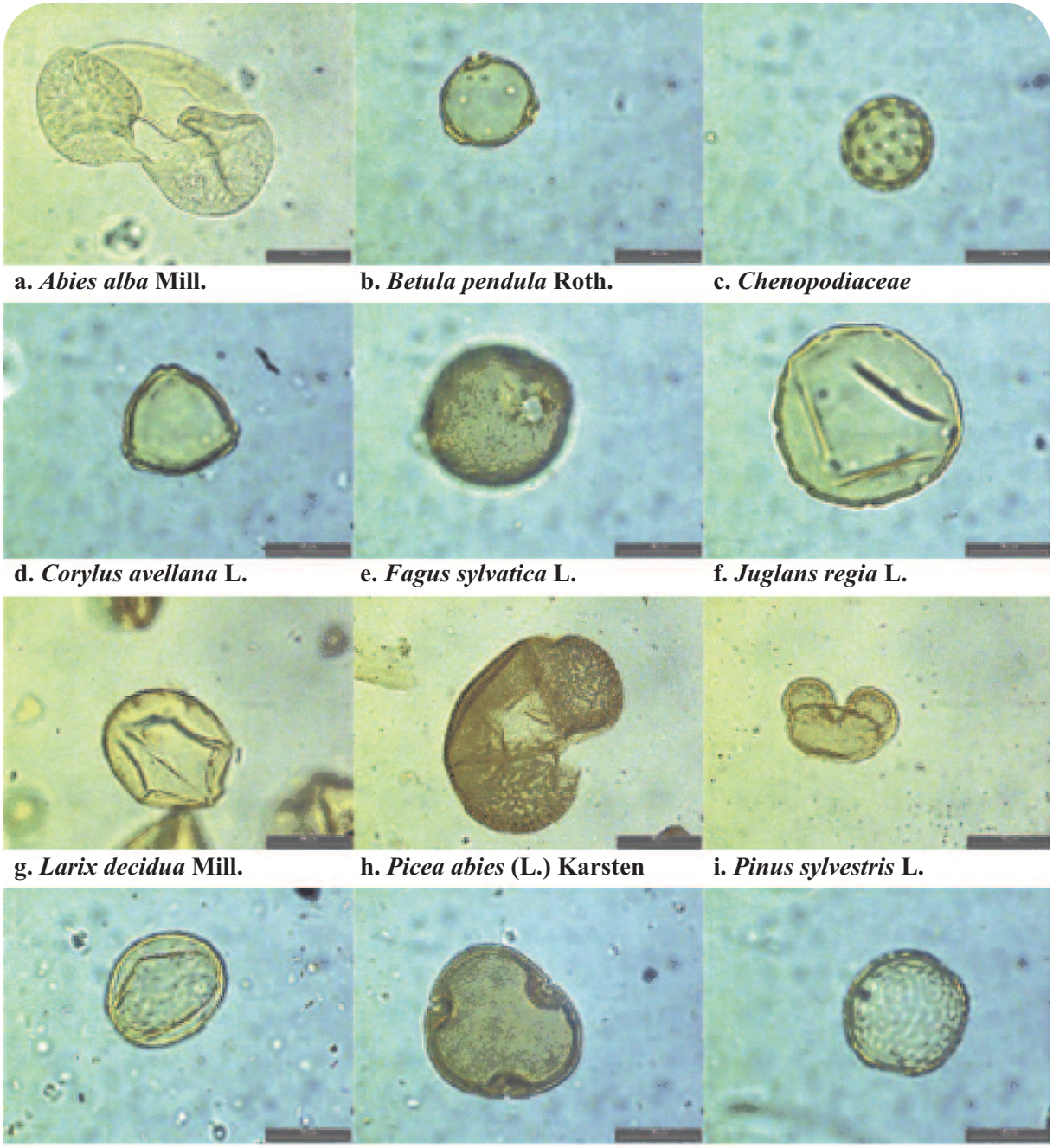
Artifacts

Air bubbles

GIN eggs

Strongyloides eggs





a. *Abies alba* Mill. b. *Betula pendula* Roth. c. *Chenopodiaceae*

d. *Corylus avellana* L. e. *Fagus sylvatica* L. f. *Juglans regia* L.

g. *Larix decidua* Mill. h. *Picea abies* (L.) Karsten i. *Pinus sylvestris* L.

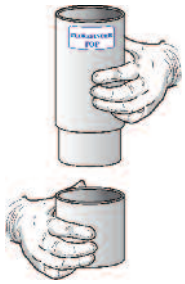
l. *Quercus robur* L. m. *Tilia cordata* Mill. n. *Ulmus glabra* Hud.

Filippi ML, Festi D, "Paleolimnology in Trentino: methods and state of the art." (2005)

FLUKEFINDER Technique

FLUKEFINDER
Technique

STEP 1



Fit the two units together. Wet the screens by running water through the top

STEP 2

Mix 2 grams of faeces with 30 ml of tap water

STEP 3

Tap the Flukefinder against the side of the sink to expedite the passing of water through the screens and hold the column under the cold running tap until about half full (repeat this step for 4-5 times)

STEP 4

Invert the bottom unit over a cup and backwash the eggs and debris from the screen into the cup

STEP 5

Swirl suspension and pour into a tube and allow to settle for 3-4 minutes

STEP 6

Slowly pour supernatant from the tube without disturbing the sediment

STEP 7

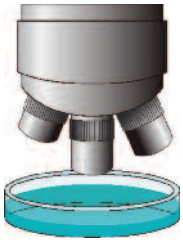
Refill the tube with tap water up to 9-10 ml and allow to settle for 2 minutes

Repeat steps 6 and 7 for two/three times more

STEP 8

Discard the supernatant and transfer the sediment in a Petri plate and add a drop of methylene blue dye

STEP 9



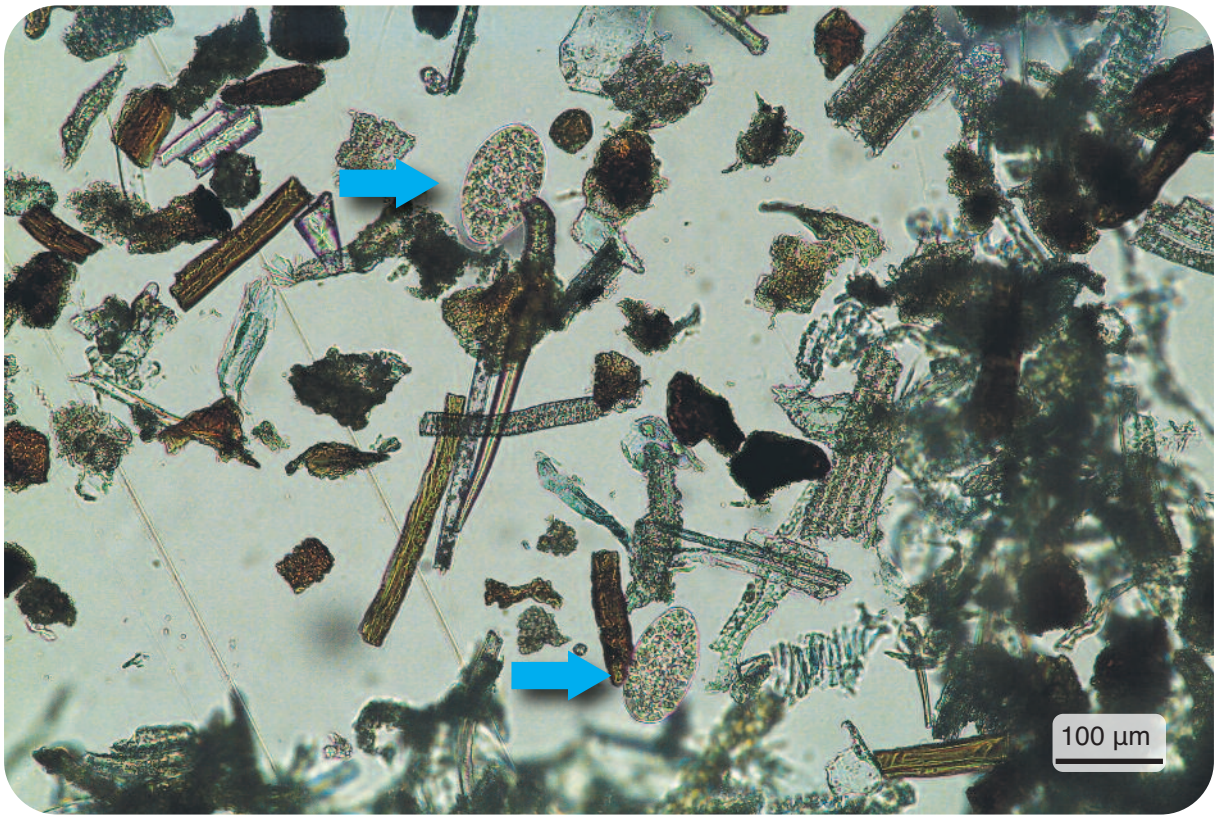
Examine under the microscope

Eggs of *Fasciola hepatica* (red arrow) and *Calicophoron daubneyi* in aqueous suspension with a drop of methylene blue dye (100× magnification): *F. hepatica* eggs are golden-yellow, whereas *C. daubneyi* eggs are light grey in colour.

FLUKEFINDER Technique
(100x magnification)

F. hepatica eggs

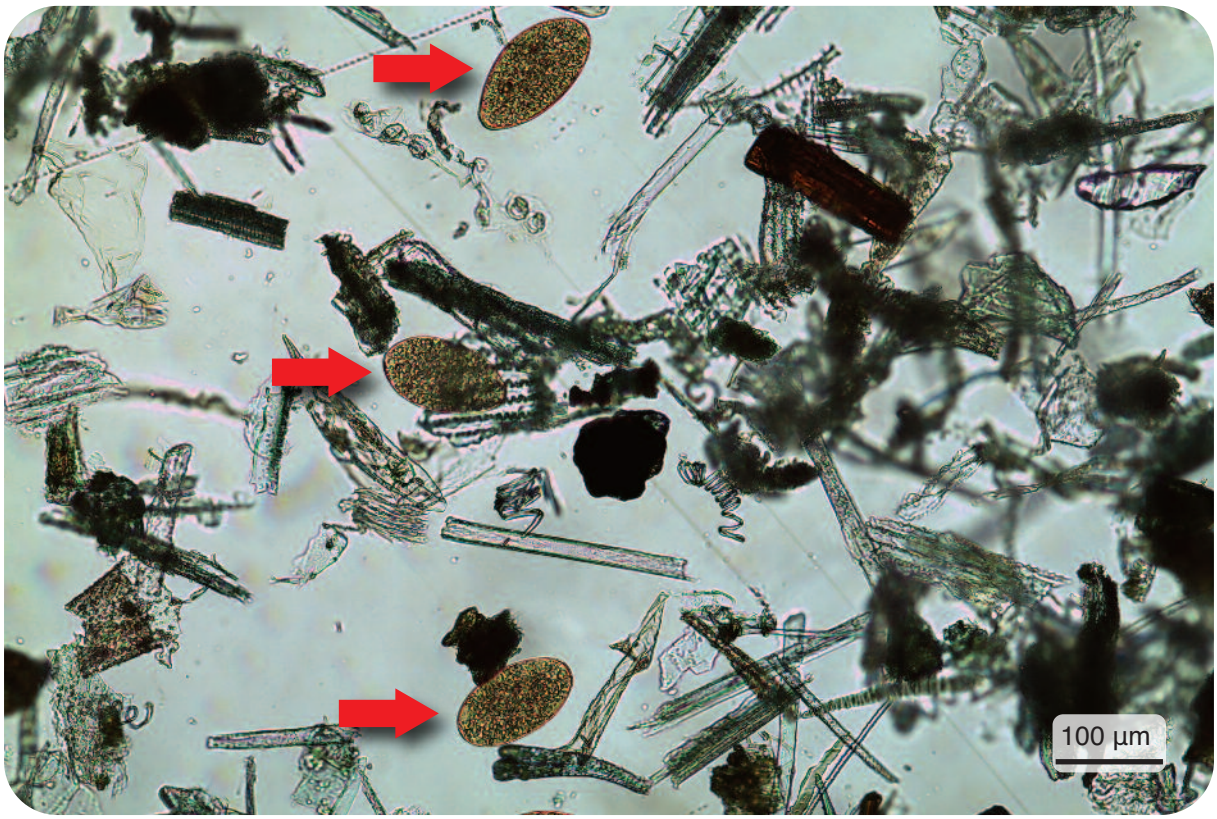
C. daubneyi eggs

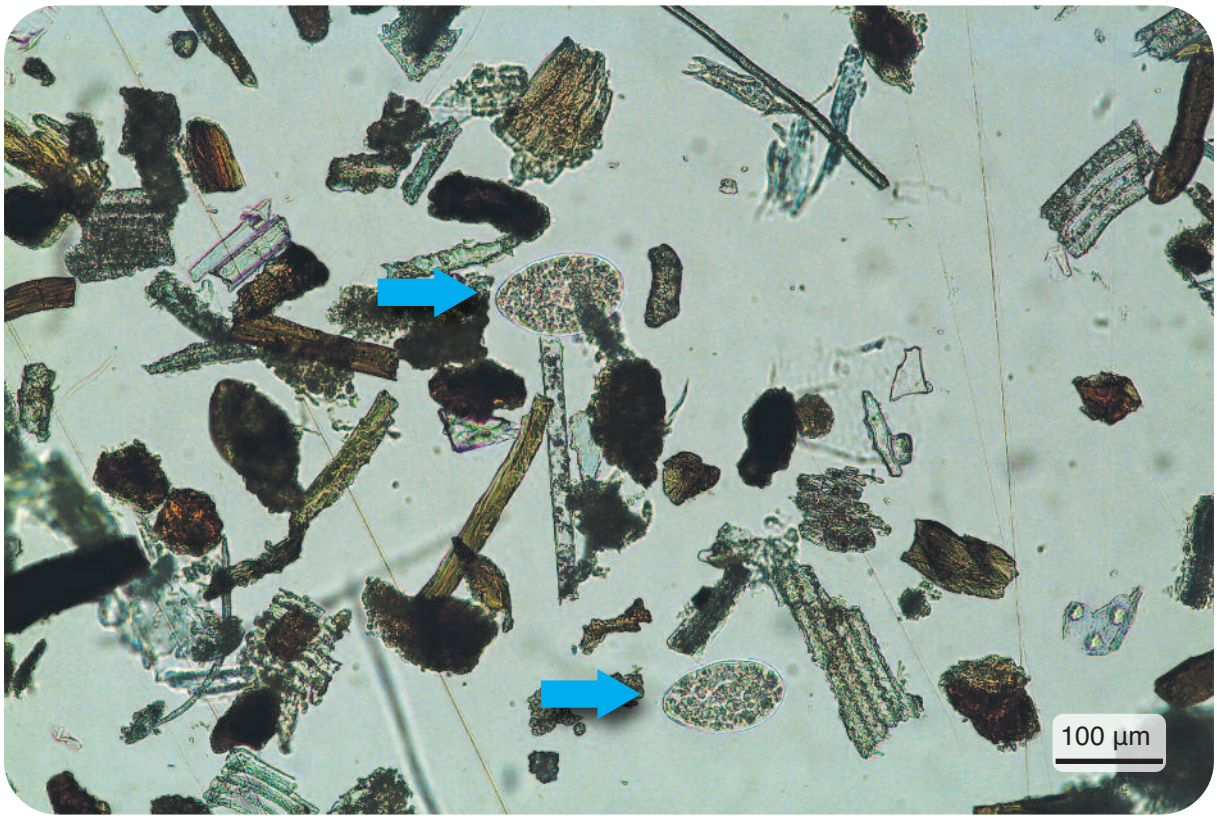


FLUKEFINDER Technique
(100x magnification)

F. hepatica eggs

C. daubneyi eggs



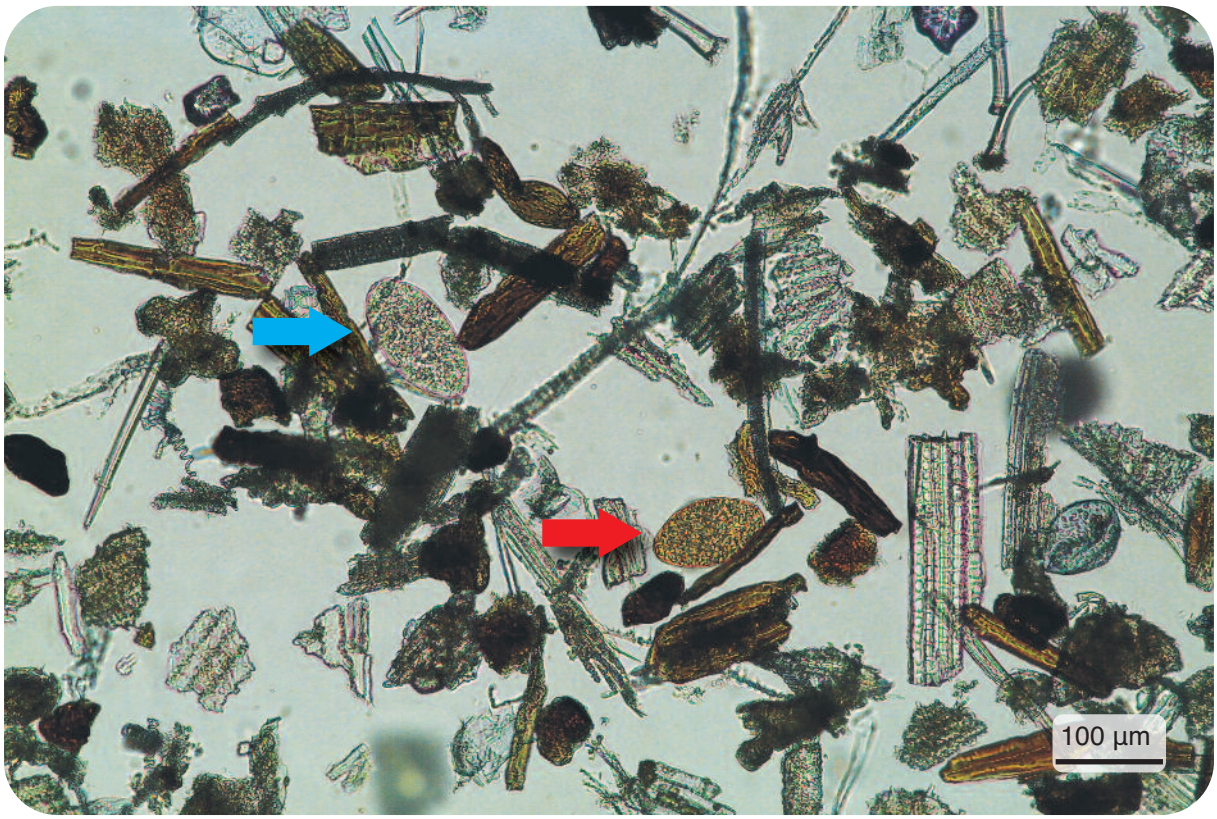


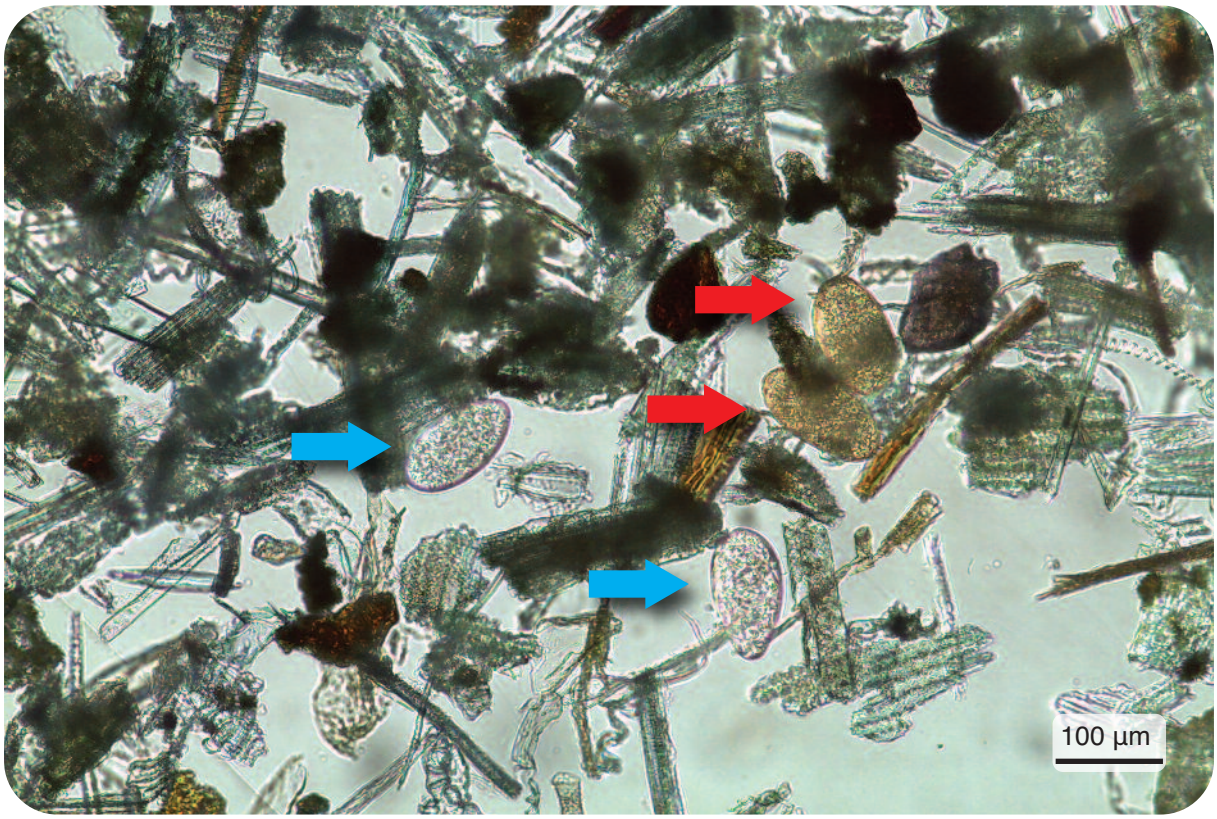
FLUKEFINDER Technique

(100x magnification)

F. hepatica eggs

C. daubneyi eggs

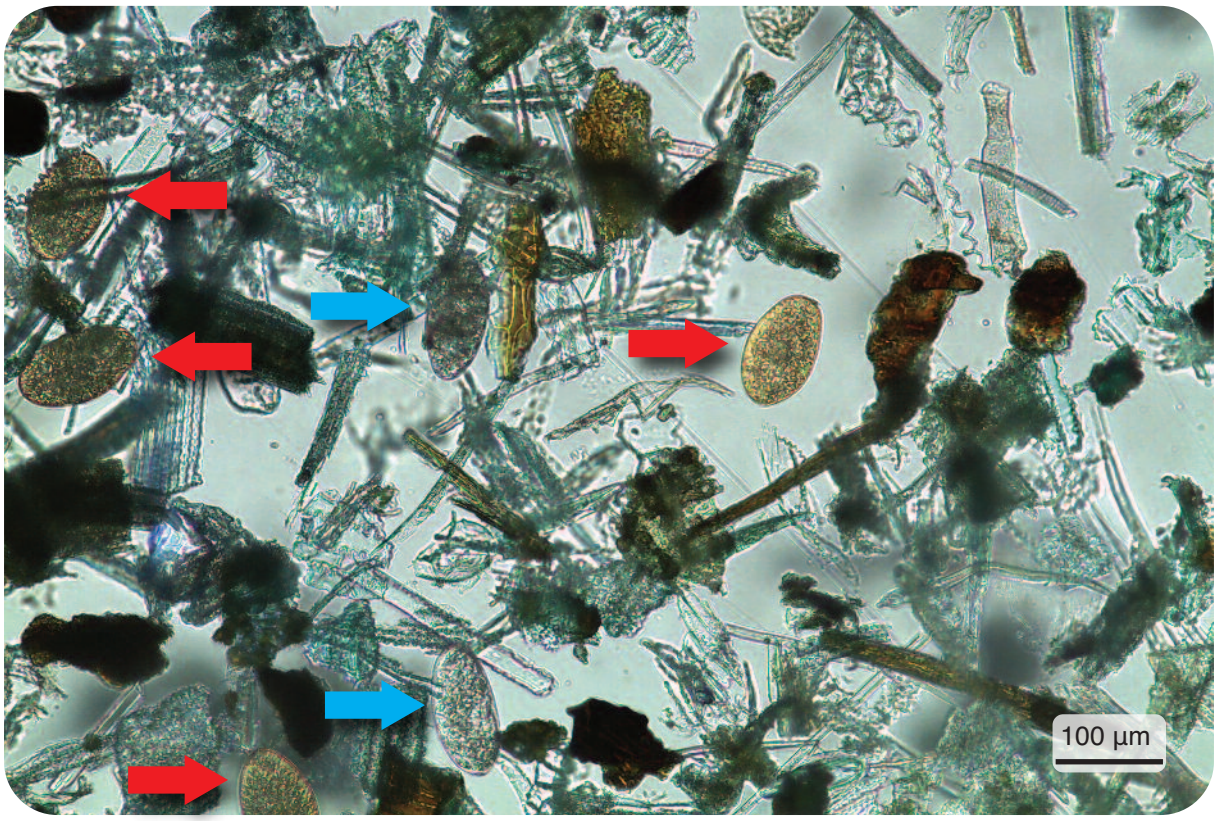


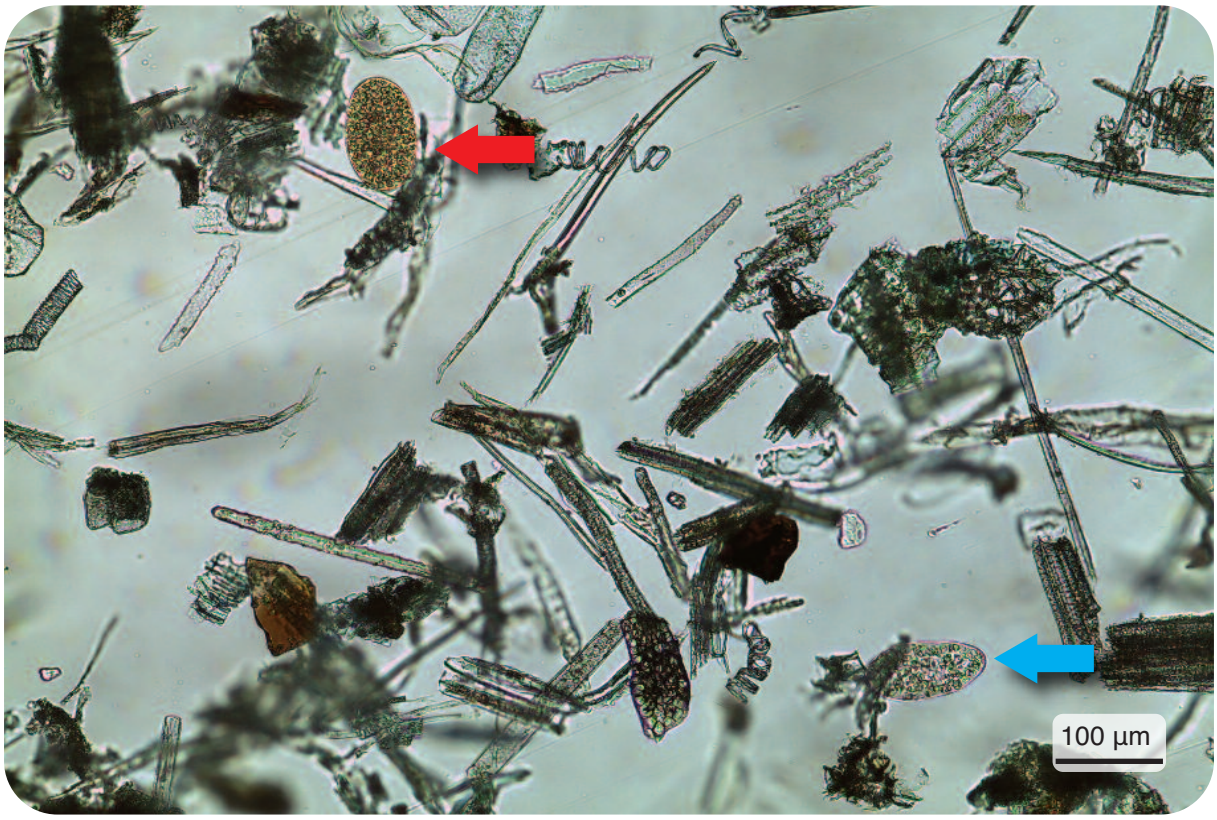


FLUKEFINDER Technique
(100x magnification)

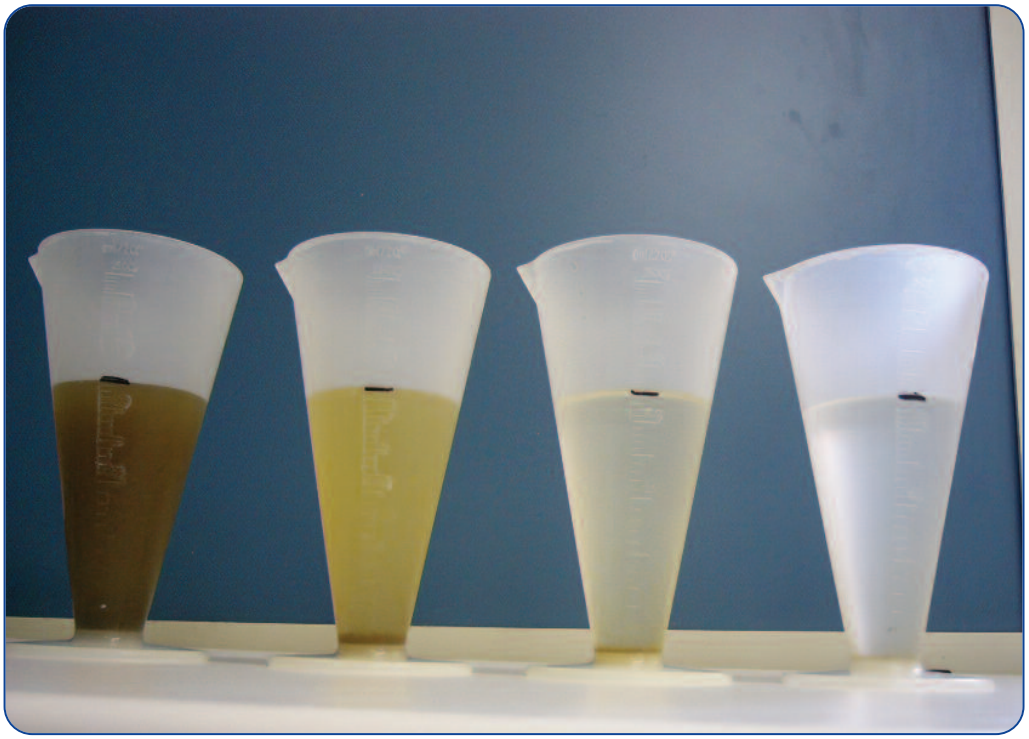
F. hepatica eggs

C. daubneyi eggs





SEDIMENTATION Technique



SEDIMENTATION
Technique

STEP 1

Homogenize the sample thoroughly

STEP 2

Weight 10 g of faeces

STEP 3

Add 90 ml of tap water

STEP 4

Homogenize

STEP 5

Filter the faecal suspension using a wire mesh (aperture of 250 µm) and add tap water up to 250 ml

STEP 6

Wait for 4 minutes

STEP 7

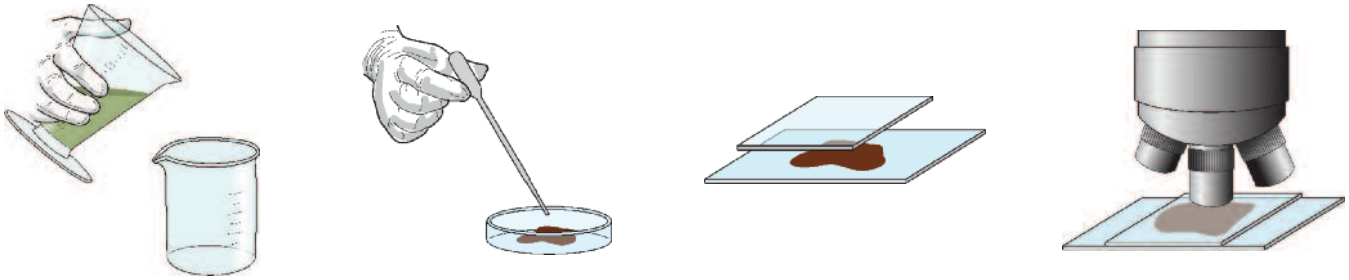
Discard the supernatant

STEP 8

Refill the becker with tap water up to 250 ml

Repeat steps 6, 7 and 8 for three times more

STEP 9



Discard the supernatant and transfer 10-15 ml of sediment in a Petri plate or add some drops of sediment on a glass slide, add a coverslip and examine under the microscope.

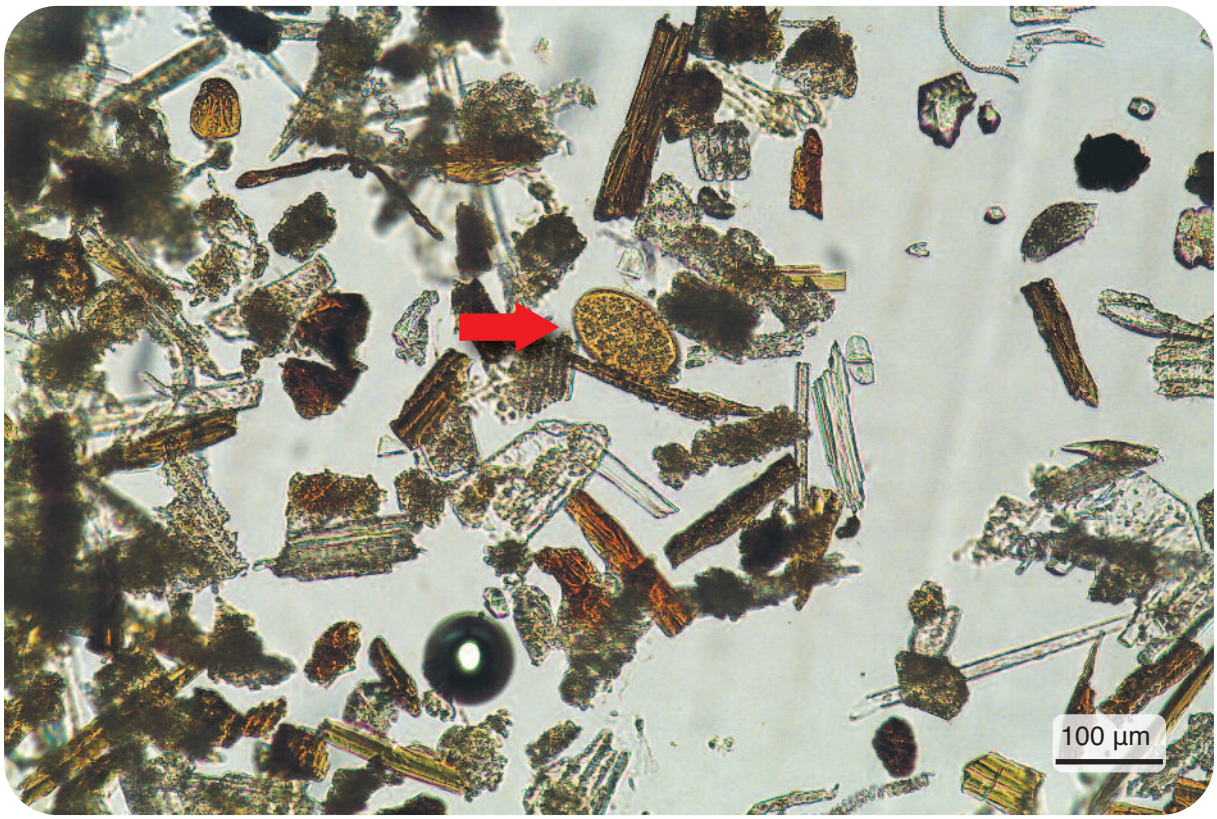
Eggs of *Fasciola hepatica* (red arrow) and *Calicophoron daubneyi* in aqueous suspension with a drop of methylene blue dye (100× magnification): *F. hepatica* eggs are golden-yellow, whereas *C. daubneyi* eggs are light grey in colour.

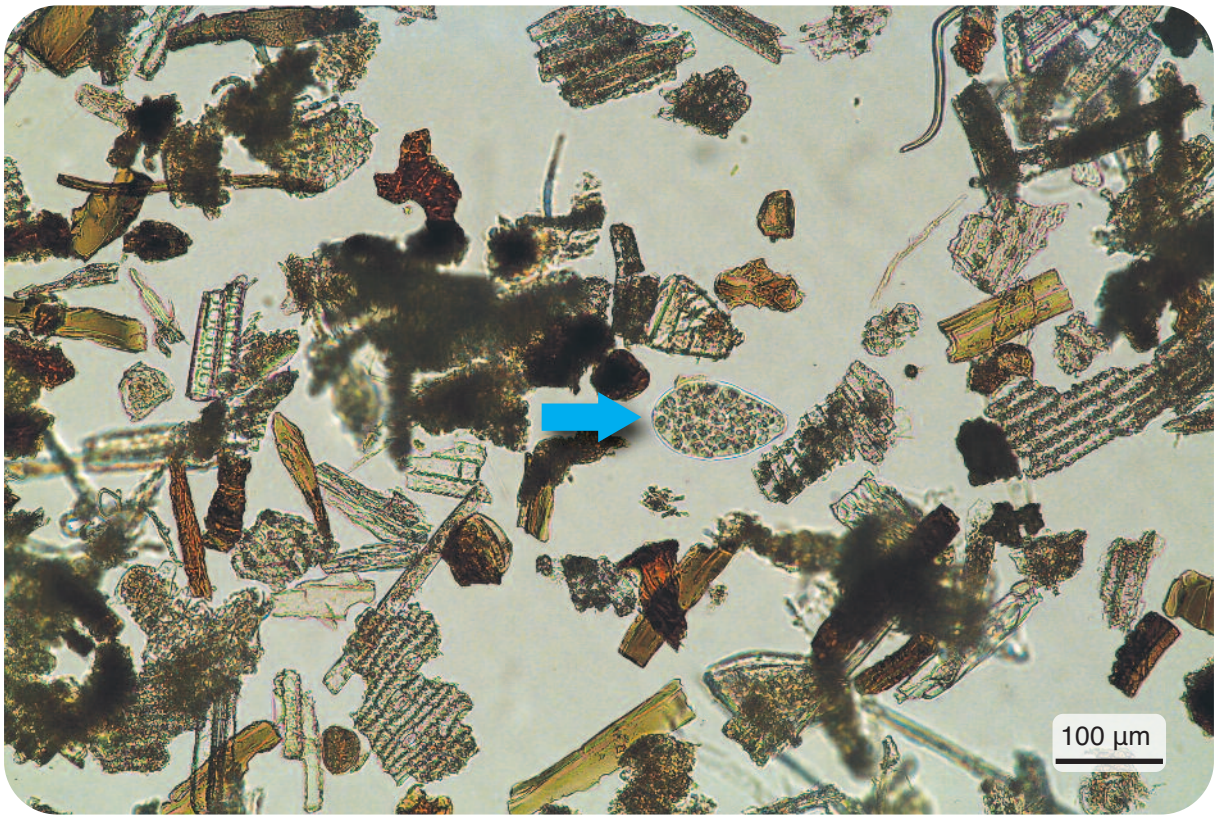
SEDIMENTATION Technique

(100x magnification)

F. hepatica eggs

C. daubneyi eggs



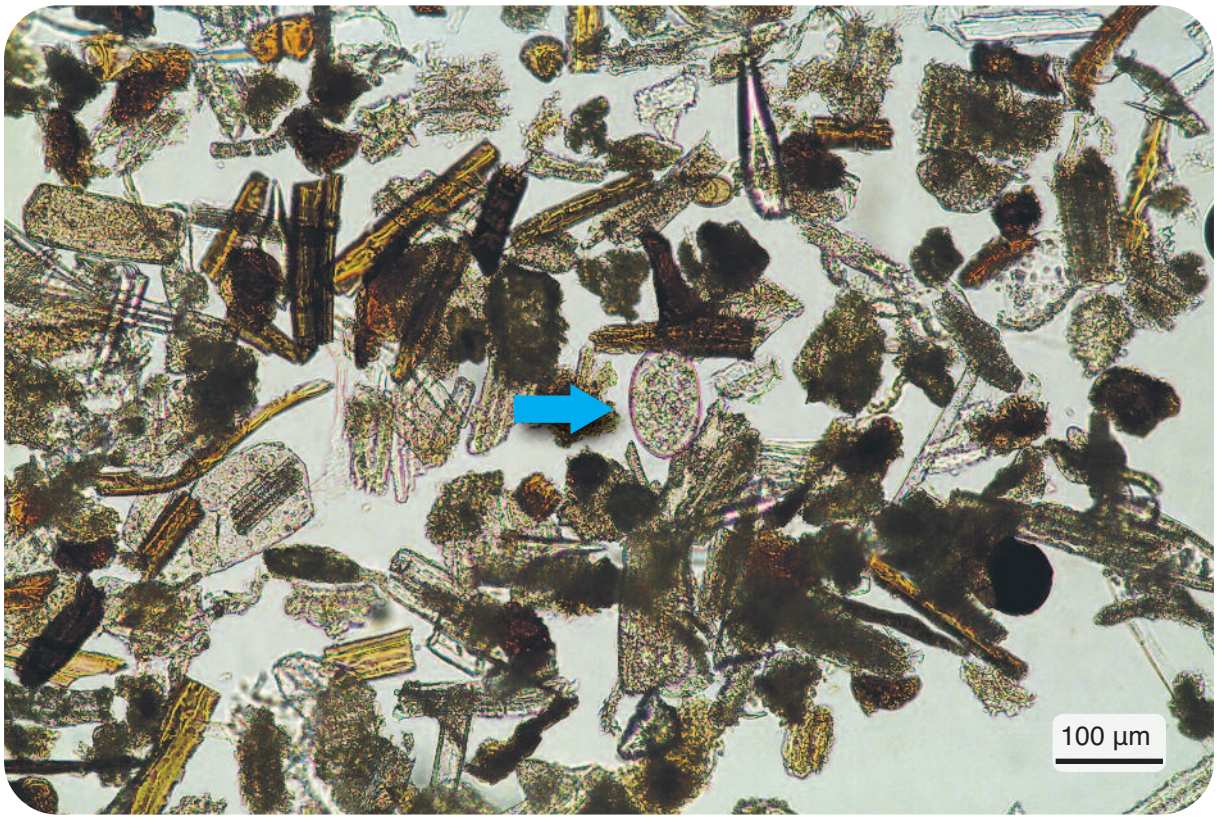


SEDIMENTATION Technique

(100x magnification)

F. hepatica eggs

C. daubneyi eggs

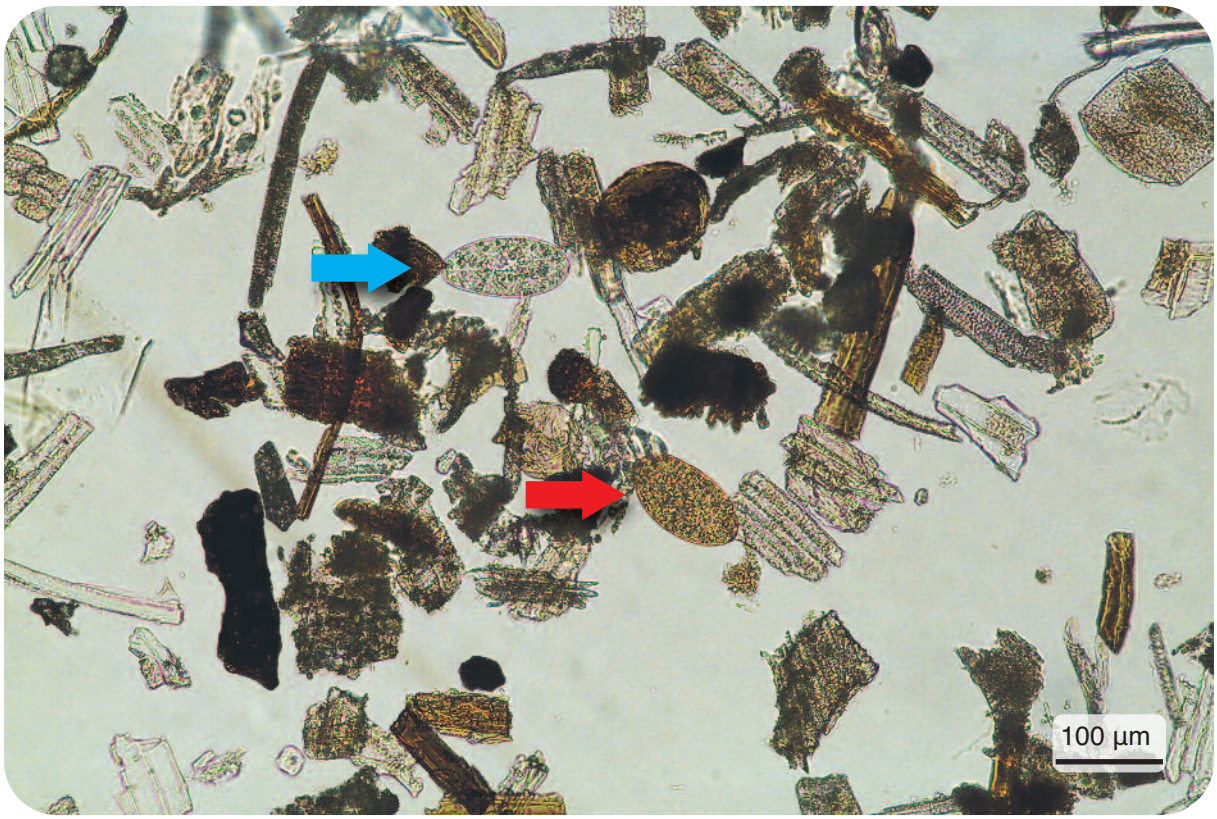


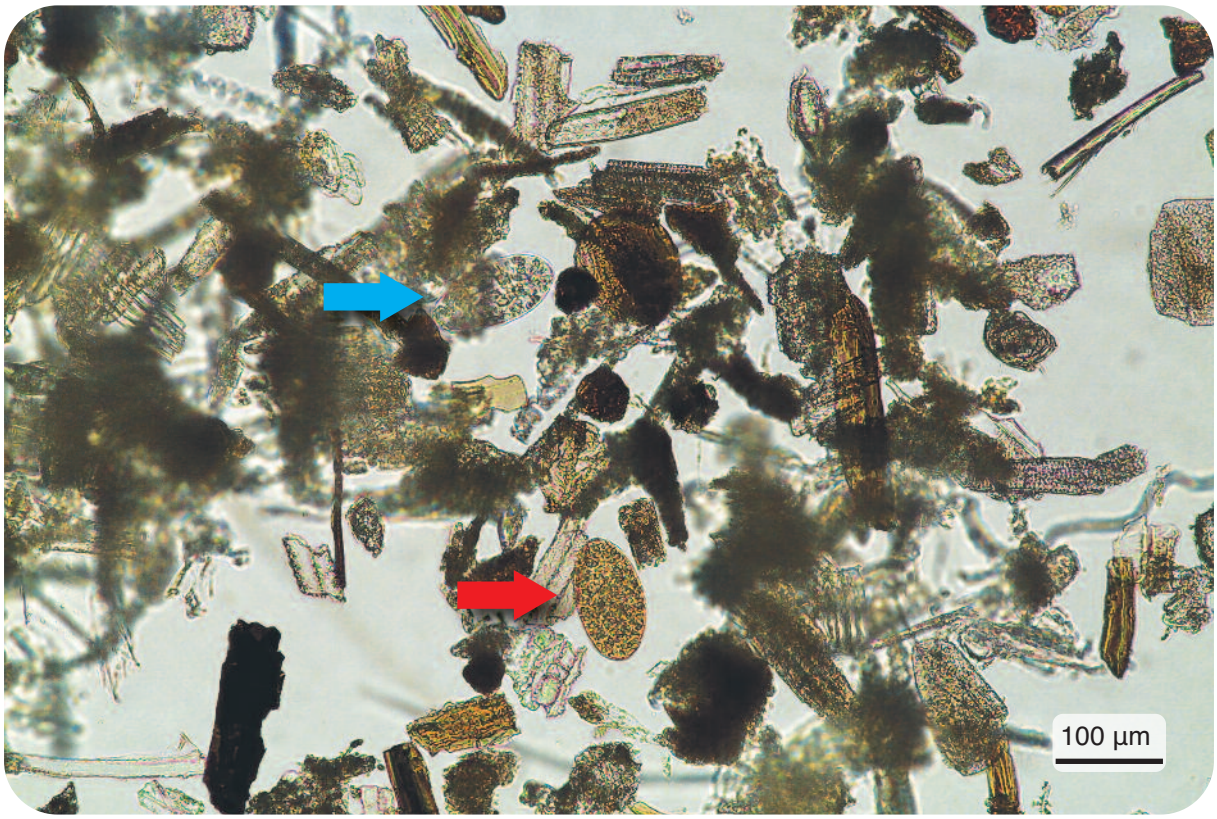
SEDIMENTATION Technique

(100x magnification)

F. hepatica eggs

C. daubneyi eggs



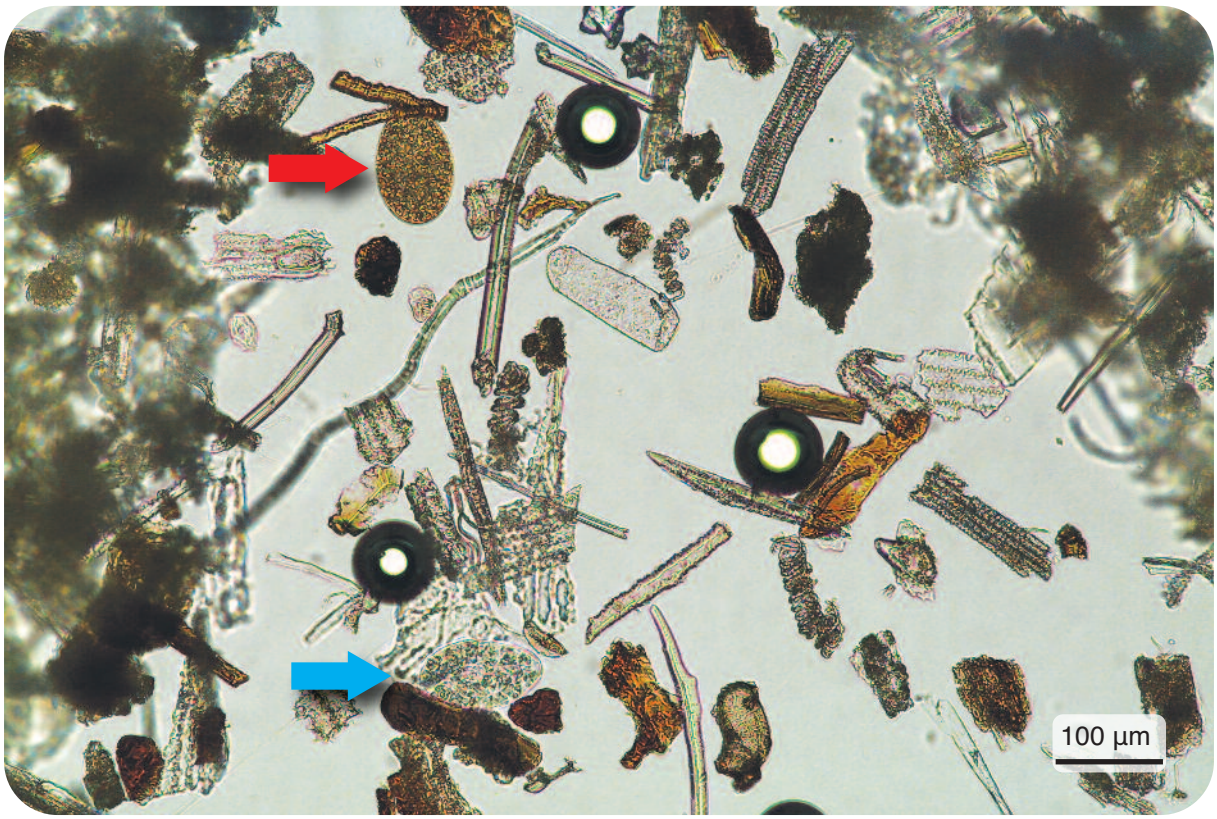


SEDIMENTATION Technique

(100x magnification)

F. hepatica eggs

C. daubneyi eggs



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