Infection with Marteilia refringens

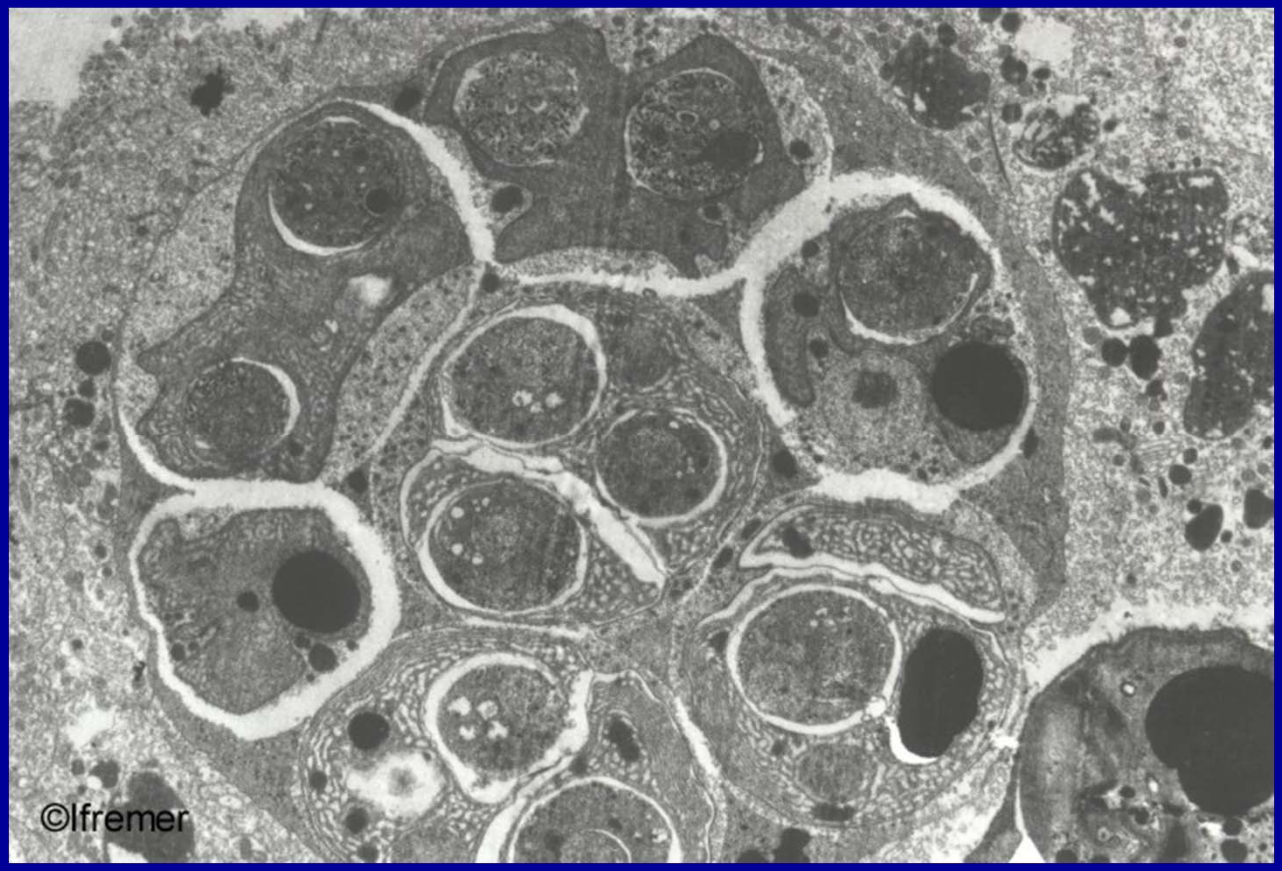


Photo: Ifremer ©

EURL for Mollusc Diseases, Laboratory of Genetic and Pathology of Marine Molluscs, La Tremblade, France (2013)

General information

➔ Category of the disease

notifiable to the OIE and listed in Directive 2006/088/EC

➔ Common, generally accepted names of the disease agent

Aber disease, Digestive gland disease of the European oyster, Marteiliosis

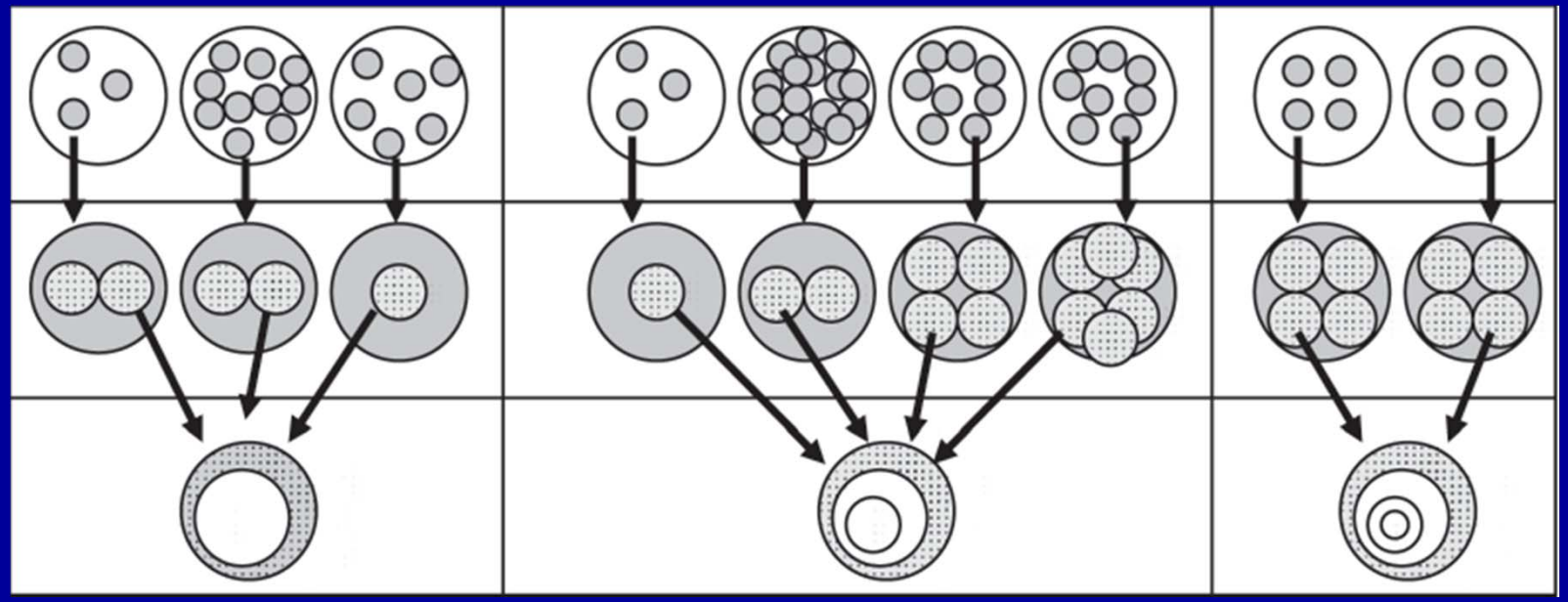
➔ Scientific name or taxonomic affiliation of the causative agent

Marteilia refringens, (Grizel 1974) of the phylum Cercozoa and order Paramyxida (Cavalier-Smith & Chao 2003; Feist et al., 2009)

Phylum Cercozoa, order Paramyxida

Classification (Feist et al. 2009):

Genus	*Paramarteilia*			*Marteilia*				*Paramyxa*	
	P. canceri	*P. orchestiae*	*P. branchialis*	*M. chungmuensis*	*M. sydneyi*	*M. refringens*	*Marteilia sp.**	*P. paradoxa*	*P. nephtys*
Hosts	**Crab Amphipod Oyster**			**Bivalves**				**Polychaeta**	



Infection with *M. refringens*

Wide host range

Host species (fully demonstrated)	Possible host species (partly demonstrated)	Other species
Ostrea edulis	*Ostrea angasi, O. Puelchana, O. chilensis, O. denselamellosa* *Crassostrea virginica*	*Crassostrea gigas : mature stages not visible = no release of the parasite?*
Mytilus edulis *Mytilus galloprovincialis* *Xenostrobus securis*	*Ruditapes decussatus, R. philippinarum* *Tapes rhomboides, T. pullastra* *Ensis minor, E. siliqua*	*Other Marteilia species ?*
Solen marginatus *Chamelea gallina*	*Argopecten gibbus* *Saccostrea forskali* *Tridacna maxima* *Pinctada margaritifera*	*Cardium edule* *Saccostrea cucullata* *Scropicularia piperata*

General information

➔ Other *Marteilia* species :

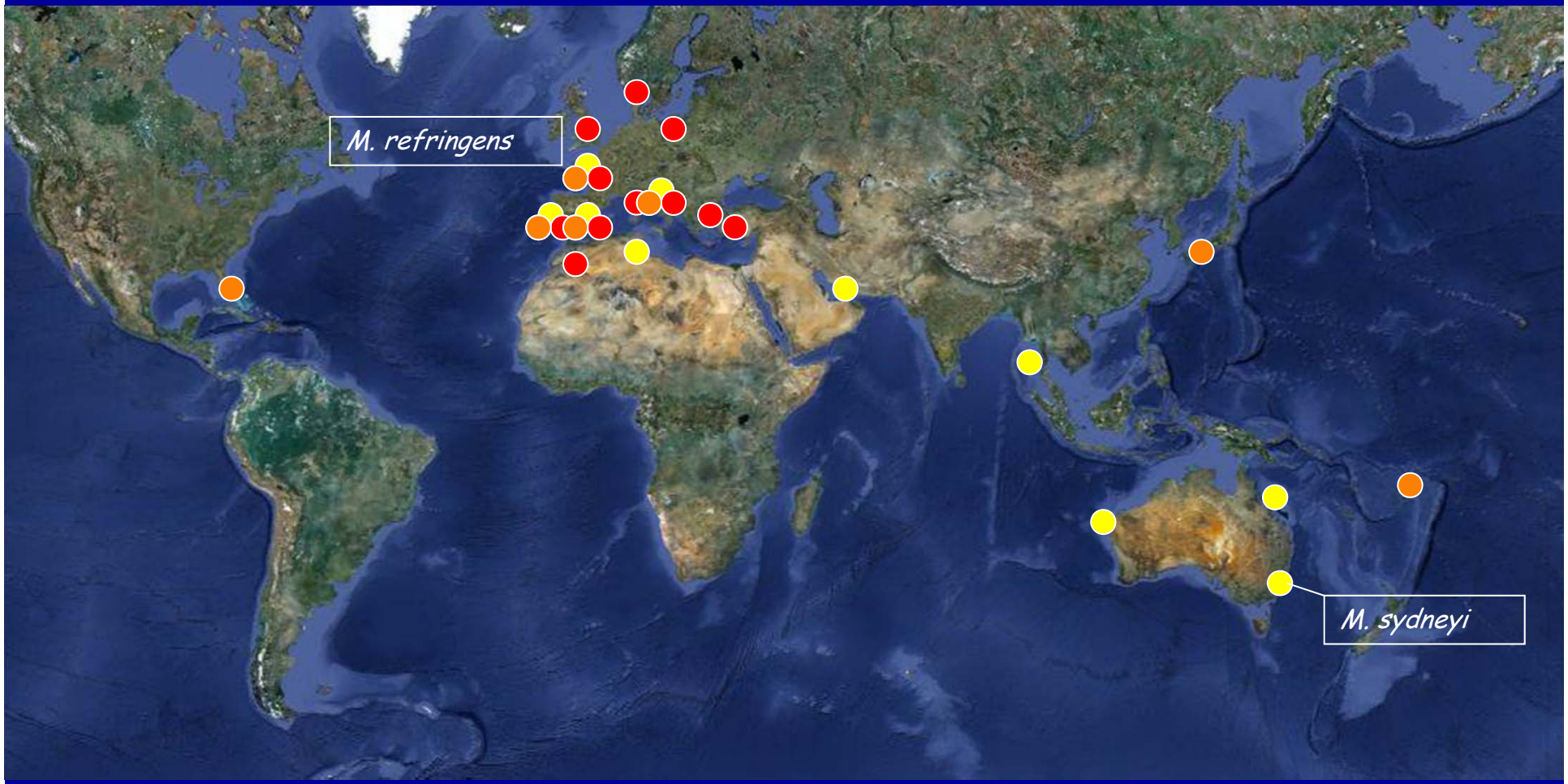
➔ *Marteilia sydneyi* infects *Saccostrea glomerata* (= commercialis) and possibly *Saccostrea echinata*.

➔ *Marteilia maurini* considered as synonymous of *M. refringens* (Lopez-Florez et al. 2004; Novoa et al. 2005) in *Mytilus galloprovincialis* and *M. edulis* in France, Spain and Adriatic sea (Italy and Croatia)

➔ *Marteilia lengehi* in *Saccostrea cucullata* reported from Persian Gulf and Western Australia

➔ *Marteilia christenseni* in *Scrobicularia plana* reported from France

Geographical distribution



- *Marteilia* in mussels
- *Marteilia* in oysters
- *Marteilia* in other species

Impact on the host

- Since 1968, *M. refringens* has caused serious recurring mortalities with a significant negative impact on the European *O. edulis* industry.

- Infection causes a poor condition index with glycogen loss (emaciation), discolouration of the digestive gland, cessation of growth, tissue necrosis, and mortalities.

- However, *Marteilia* can occur in some oysters without causing disease.

Infection with *M. refringens*

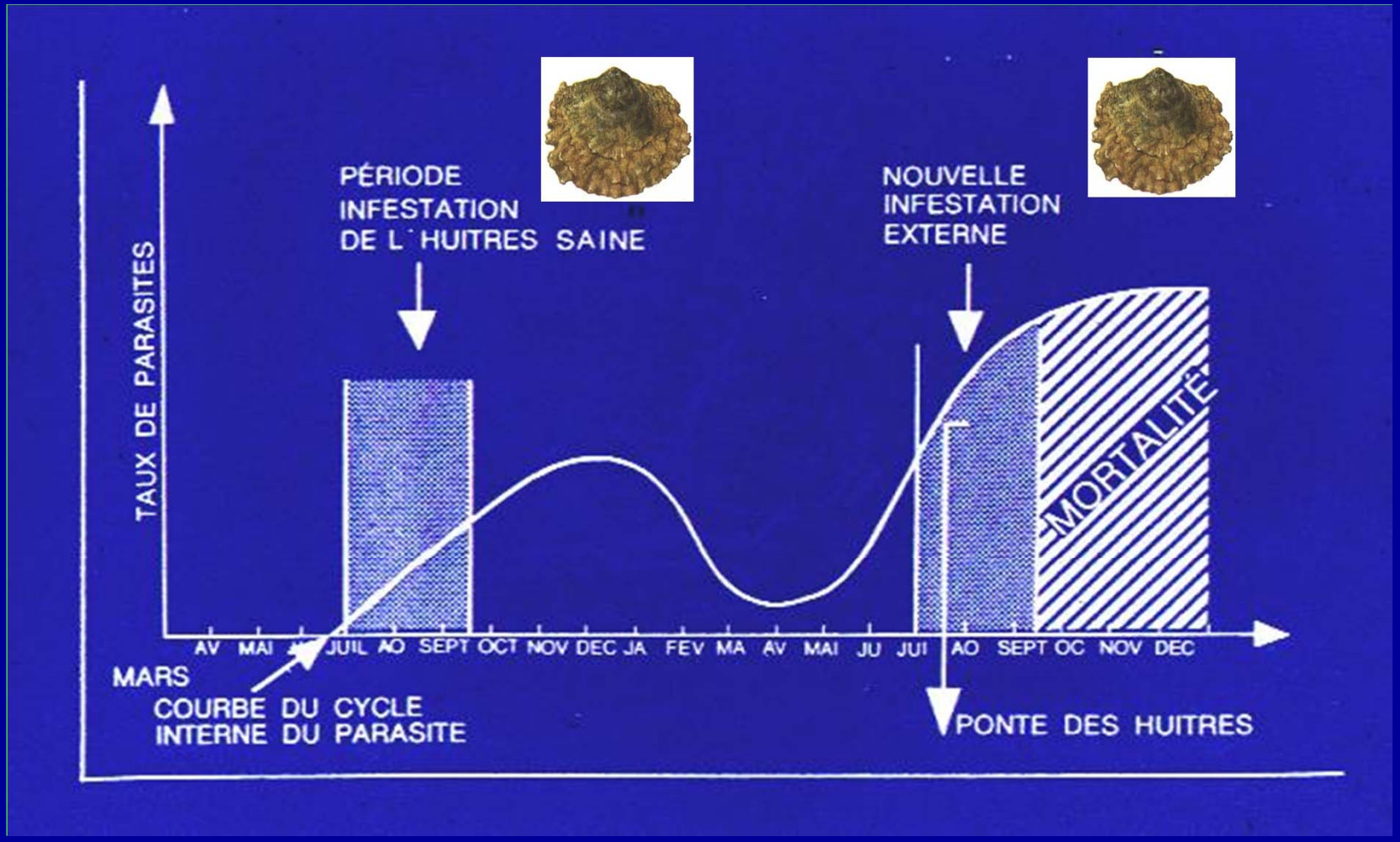
Impact on the host

Healthy oyster

Diseased oyster

Infection with *M. refringens*

Impact on the host

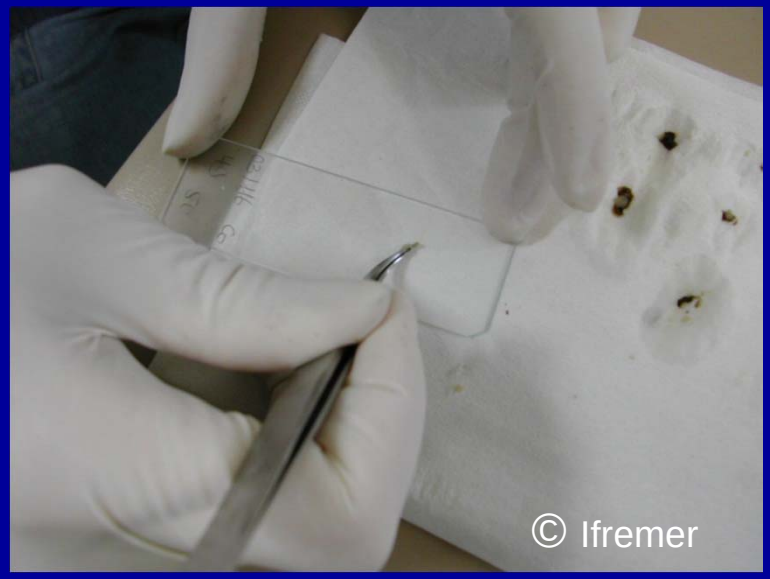


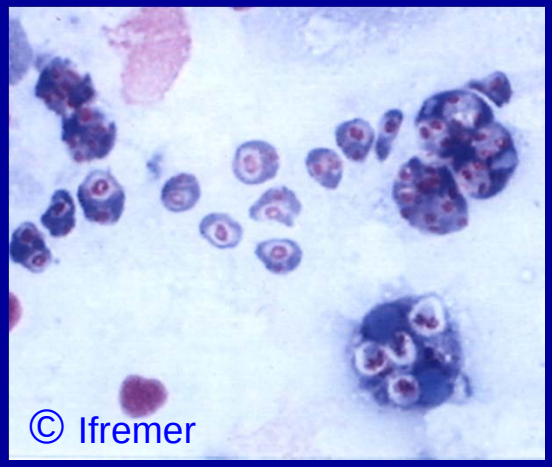
Infection with *M. refringens*

Diagnostic techniques

➔ Tissue Imprint:

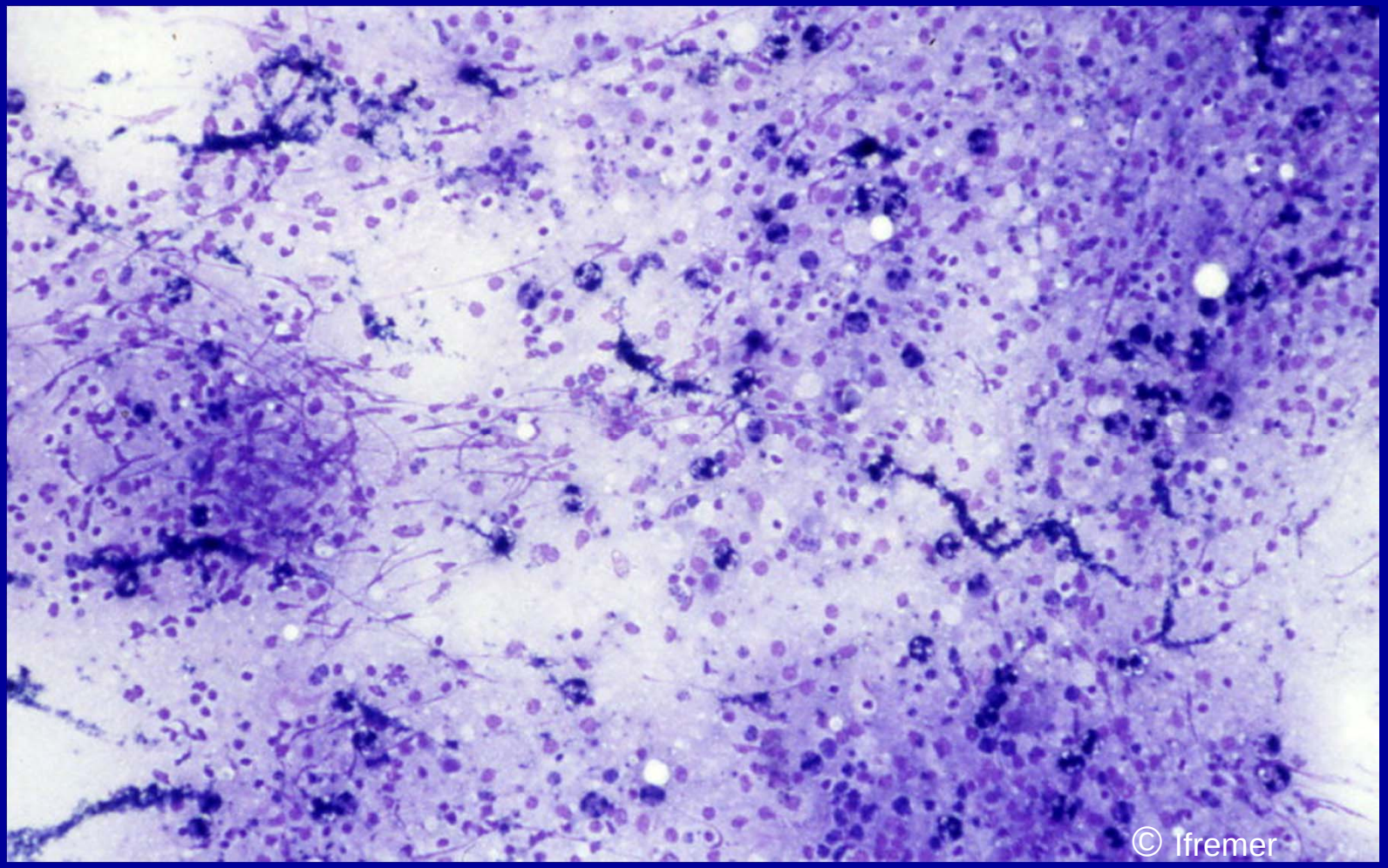
- ➔ Make acetone- (or methanol-) fixed impression smears from digestive gland tissue. Stain with Wright, Wright-Giemsa or equivalent stain (e.g. Hemacolor, Merck; Diff-QuiK, Baxter).
- ➔ The parasite is 5–8 µm in size in the early stages and may reach up to 40 µm during sporulation. The cytoplasm of the cells stains basophilic, the nucleus is eosinophilic. The secondary cells or sporoblasts are surrounded by a bright halo (colour may vary slightly with the stain used)





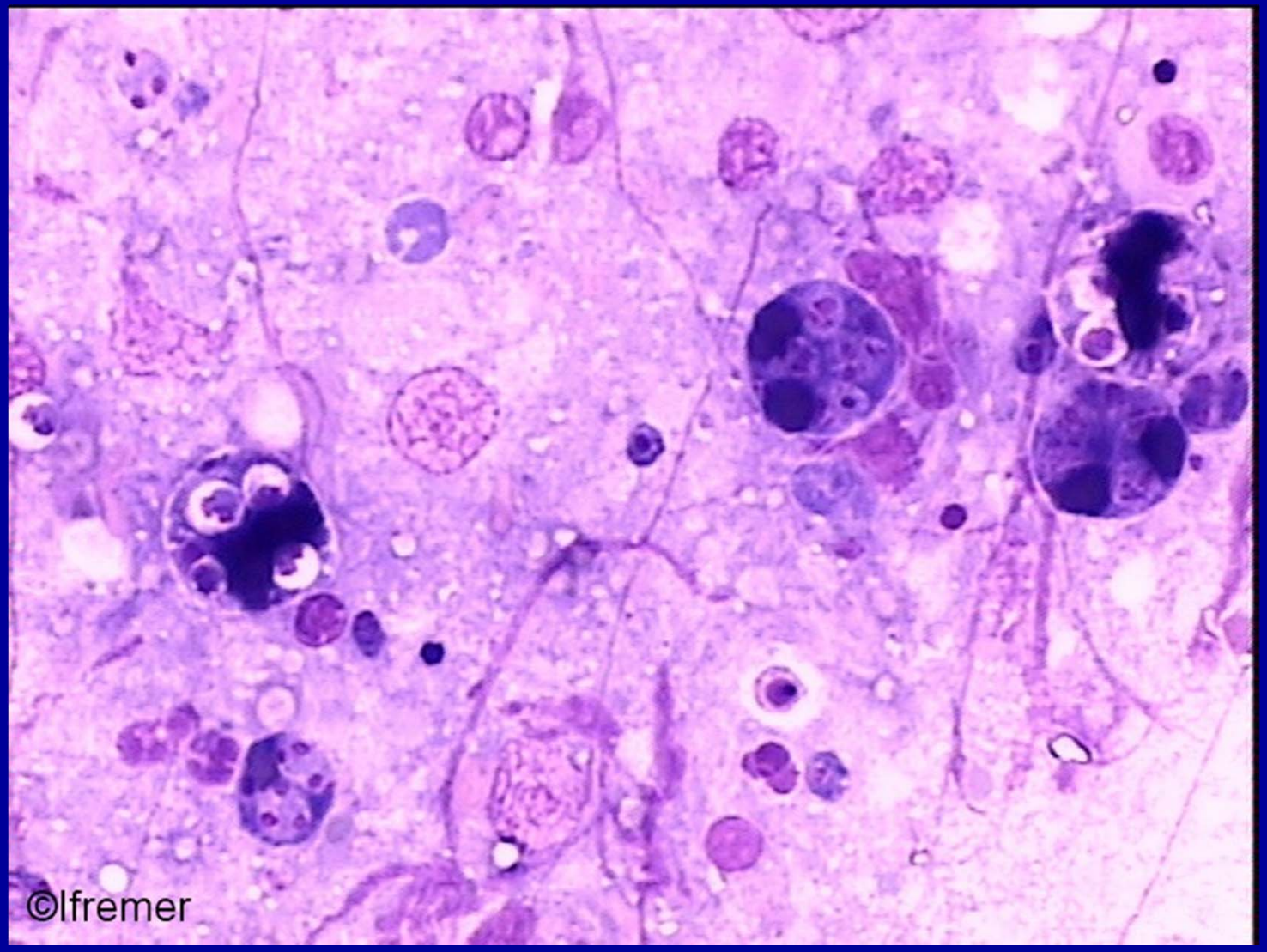
Infection with *M. refringens*

Digestive gland imprints



Infection with *M. refringens*

Digestive gland imprints



Infection with *M. refringens*

Diagnostic techniques

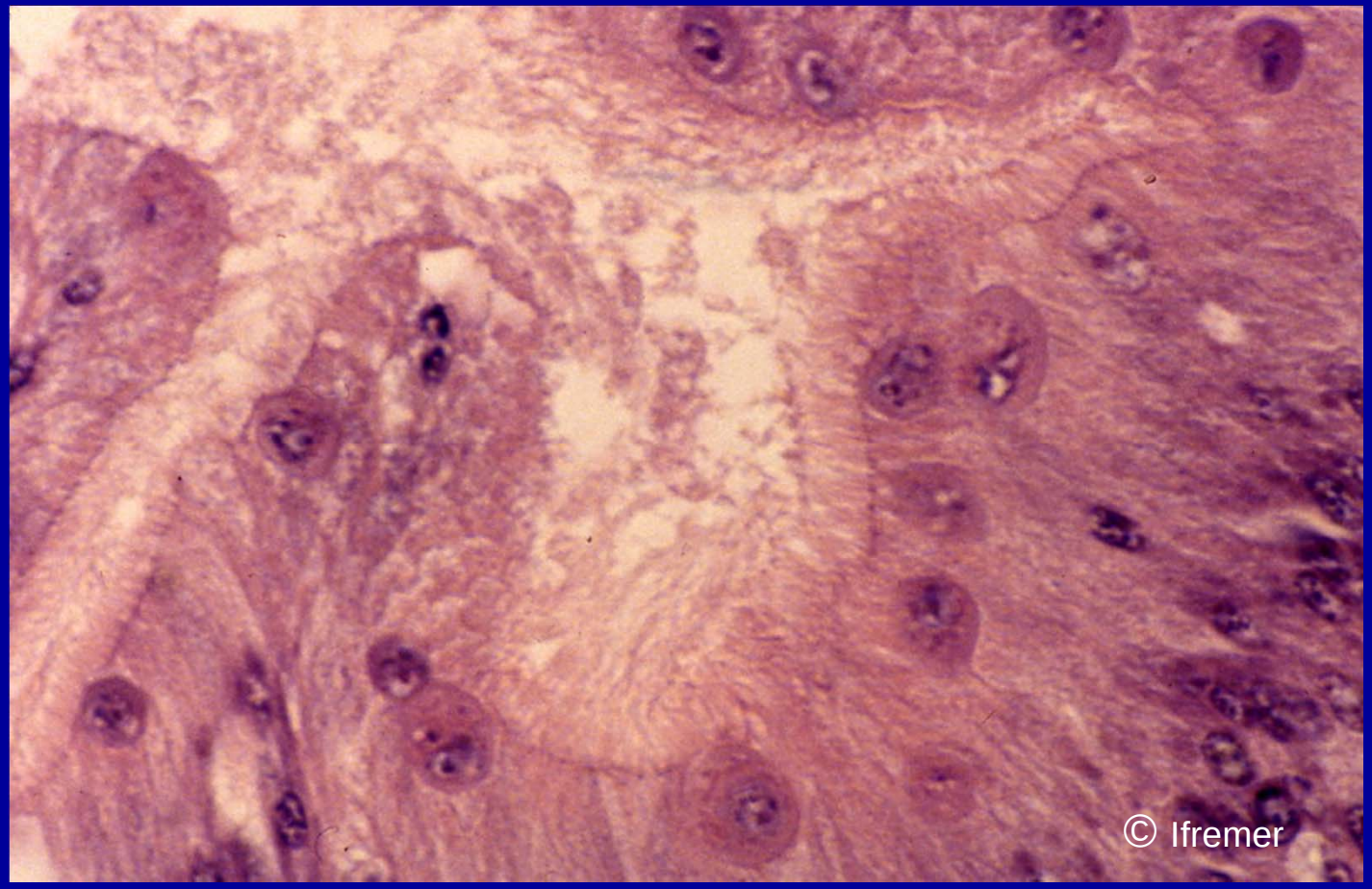
➔ Histology:

- ➔ Cross-sections of the digestive gland show the parasite in the epithelial cells of the digestive ducts (basophilic stages) and the epithelial cells of the digestive tubules (acidophilic stages). The unique feature of internal cleavage to produce cells within cells during sporulation differentiates *Marteilia* spp. from all other protista.

- ➔ A modified staining technique described by Gutiérrez (1977) may enhance the detection of the parasite in paraffin embedded histological sections.

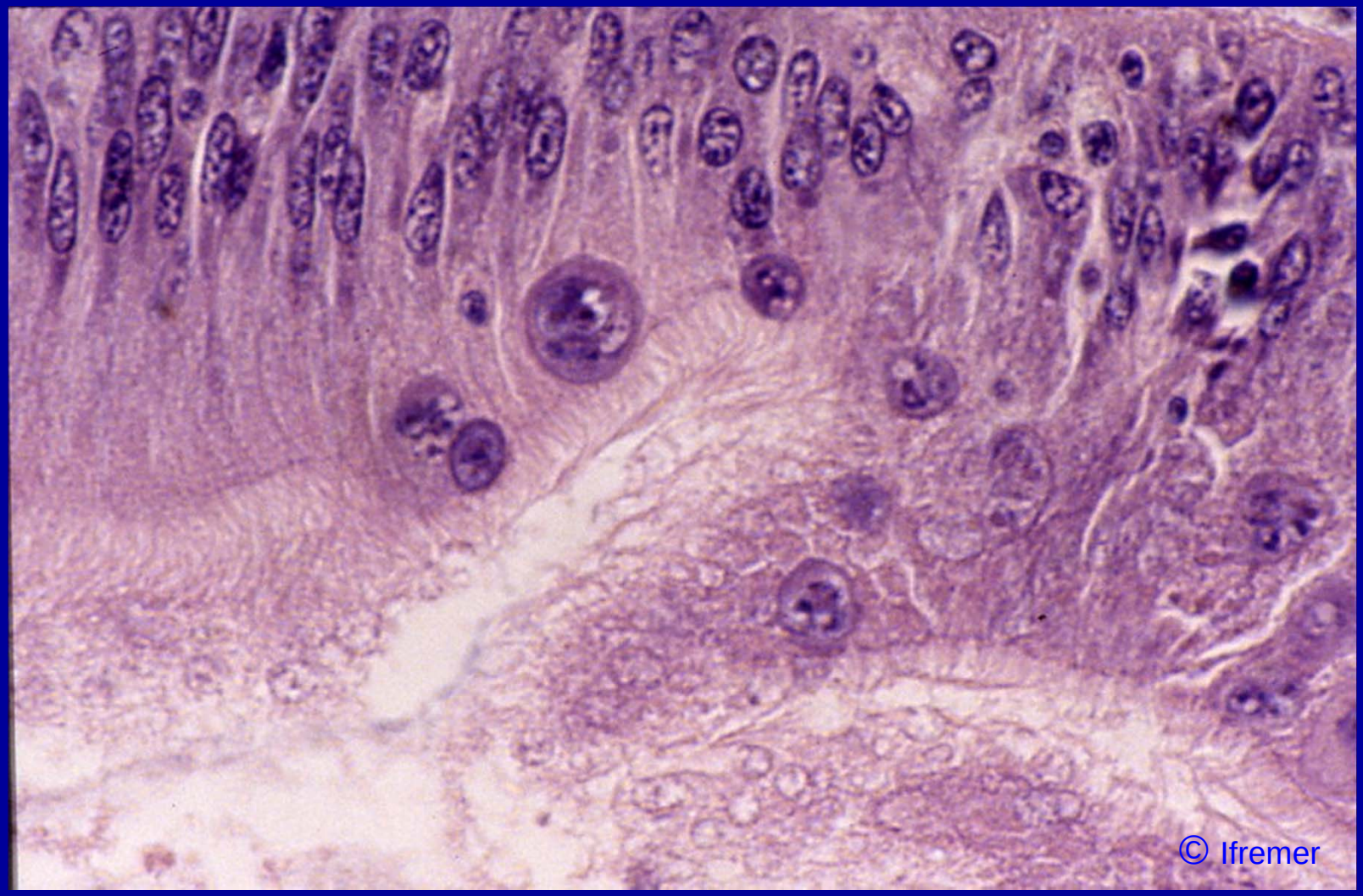
Infection with *M. refringens*

Histology



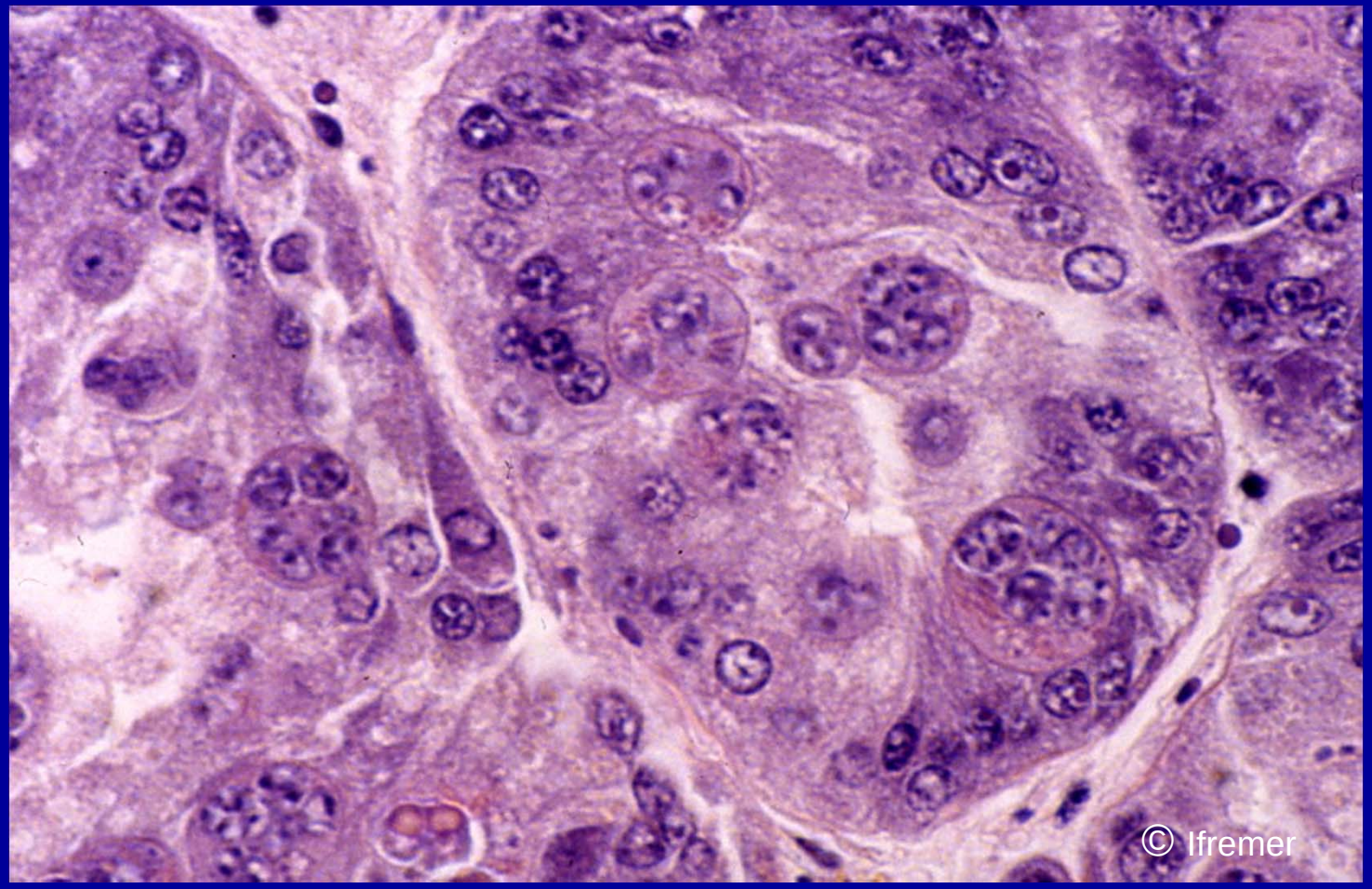
Infection with *M. refringens*

Histology



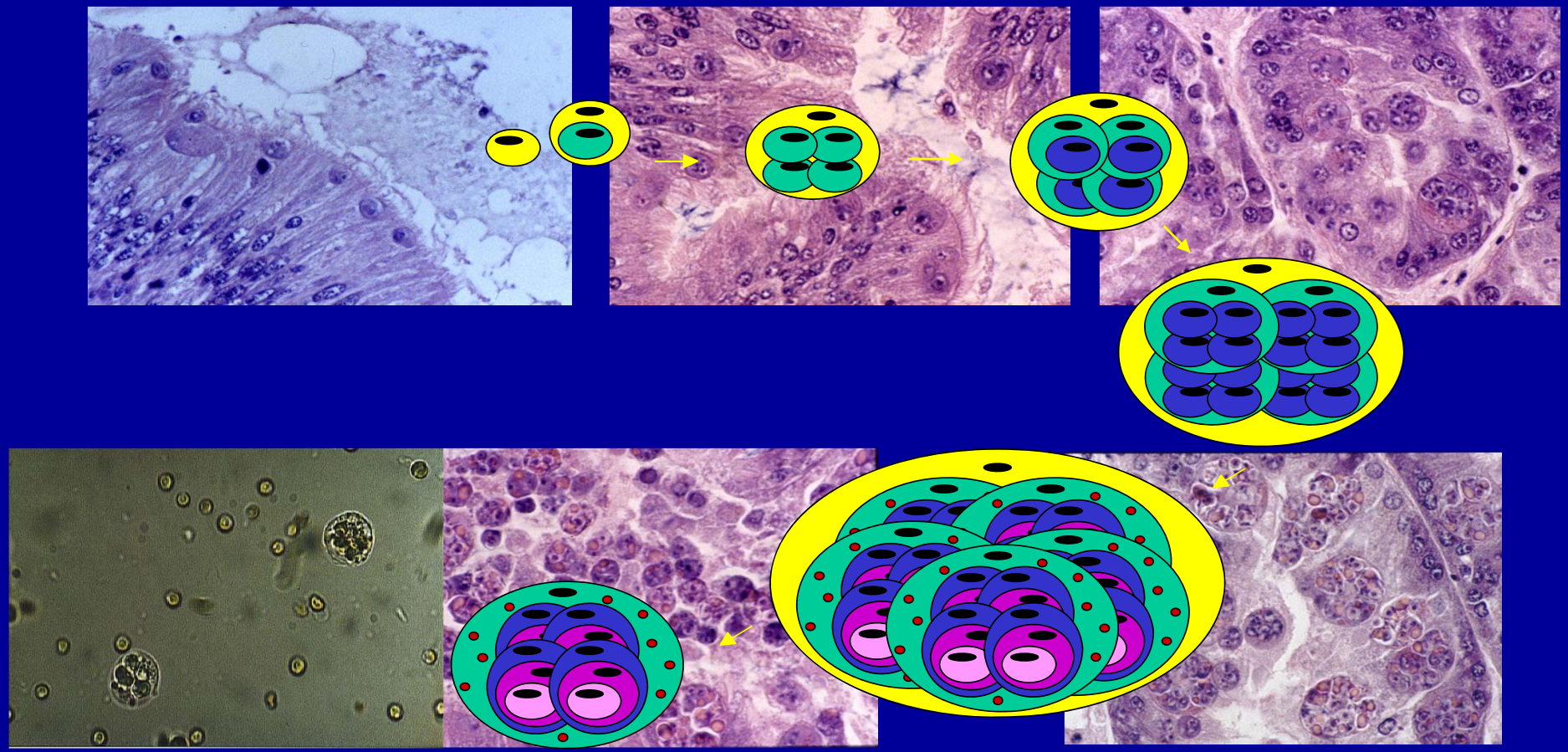
Infection with *M. refringens*

Histology



Infection with *M. refringens*

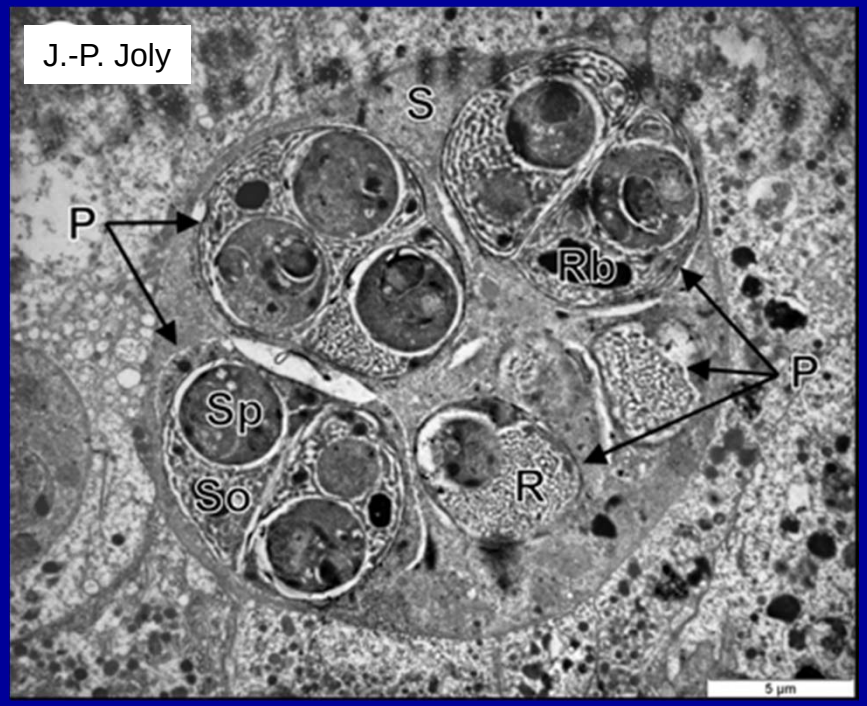
Sporulation process



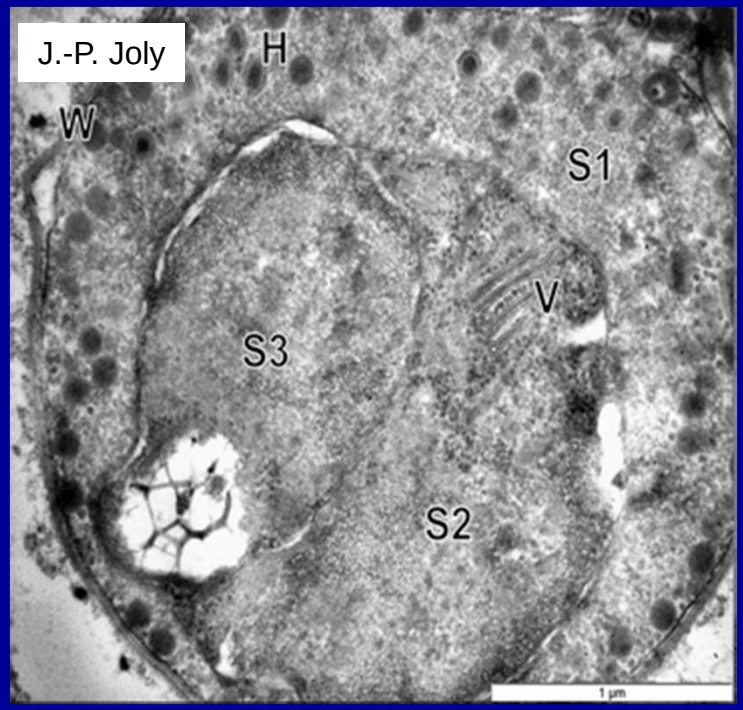
Infection with *M. refringens*

Electron Microscopy

M. refringens in *Ostrea stentina* from Tunisia (Elgharsalli et al. 2013)



Sporangiosorus S containing presporongiosora P with immature spores Sp. R: reticulated cytoplasm of sporangium So; Rb: refringent body



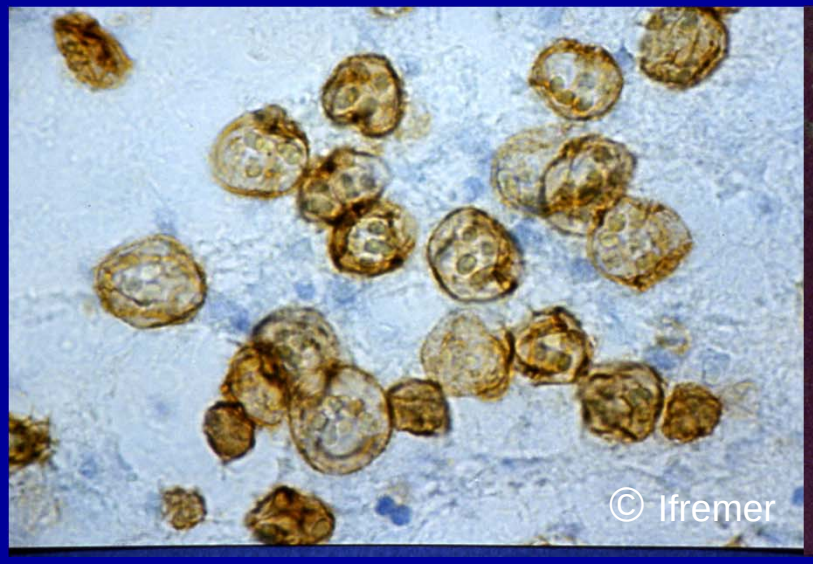
Almost mature spore with intermediate sporoplasm S2 and innermost sporoplasm S3. S1: outermost sporoplasm containing numerous haplosporosomes H; V: flattened vesicles in the intermediate sporoplasm; W: spore wall

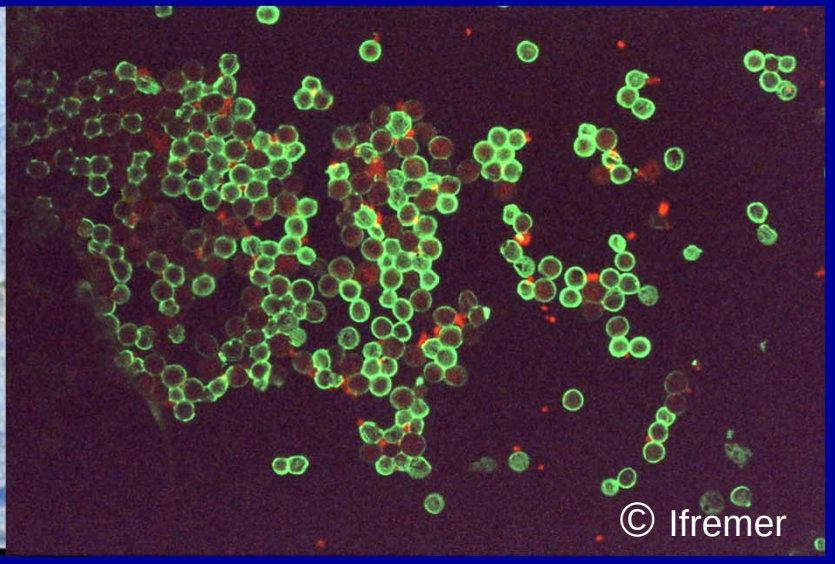
Diagnostic techniques

➔ Immunological Assay:

- ➔ An immunohistochemistery technique based on monoclonal antibodies was developed by Robledo et al. (1994). However, this technique is very rarely used in diagnostic laboratories.
- ➔ Two clones are of particular interest for their stage specificity: 4/1-1 (sporangia) and 9/1-1 (young plasmodia).
- ➔ No cross reaction with M. sydneyi (Anderson et al., 1994)
- ➔ However, there is a lack of specificity for European isolates (Pernas et al., 2000).

Immunological Assay





Infection with *M. refringens*

Diagnostic techniques

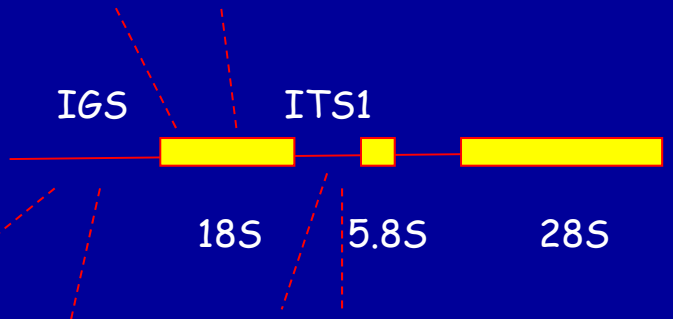
➔ DNA Probes:

- Several PCR protocols are available :
  - PCR primers that target the ITS1 (internal transcribed spacer) region (Le Roux *et al*., 2001) are recommended as they are able to amplify only *Marteilia refringens.*
  - Some primers targeting the small subunit (SSU) of the rRNA gene complex are also available and allow *M. refringens* and *M. sydneyi* to be amplified (Le Roux *et al*., 1999; Berthe et al., 2000)
  - A nested PCR assay targeting the rDNA intergene spacer (López-Flores *et al*., 2004) seems to be more sensitive than other assays

Specificity of PCR assays

Marteilia Genus

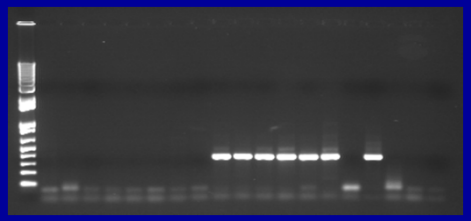
Conventional PCR (Le Roux et al. 1999)



Nested PCR
(Lopez Flores et al. 2004)

Conventional PCR
(Le Roux et al. 2001)

Marteilia refringens species

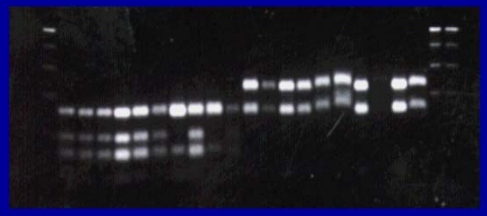


Infection with *M. refringens*

PCR RFLP

Based on a dimorphism in the locus of endonuclease *Hha*I in the ITS-1 sequence, two types O and M were defined and can be detected by PCR-RFLP (Le Roux *et al.* 2001).

	***Hha* I restriction profiles**
Marteilia refringens type M	157 bp + 156 bp + 68 bp + 31 bp
Marteilia refringens type O	226 bp + 156 bp + 31 bp



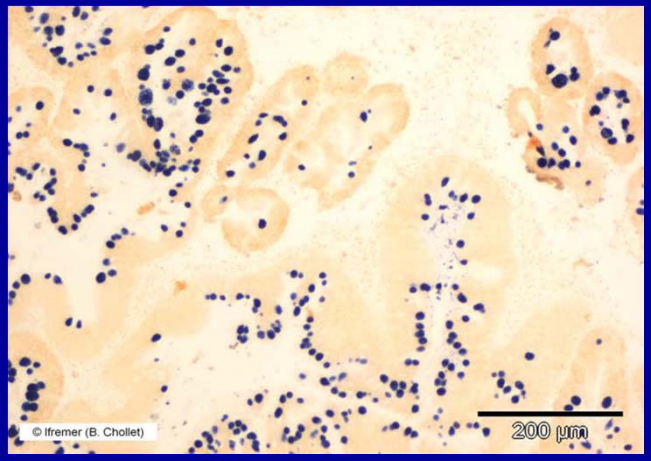
Type M Type O

Diagnostic techniques

➔ *In situ* hybridization:

- The probe named Smart 2 can detect *Marteilia refringens* and also *M. sydneyi* by *in situ* hybridisation in infected oysters (Le Roux et al. 199; Kleeman et al. 2002).

- In addition, it is possible to use primers targeting the ITS-1 to produce a probe able to detect only *M. refringens* by *in situ* hybridization (SOP available on the EURL website : http://www.eurl-mollusc.eu/SOPs)



Infection with *M. refringens*

Methods of control

- Oysters, mussels, clams … from areas known to be infected (currently or historically) should not be transferred to areas with no record of *M. refringens*.

- Results of field and experimental studies (Berthe et al. 1998, Audemard et al. 2000 & 2001, Carrasco et al. 2008, Boyer 2012) provide evidence of an intermediate hosts in the life cycle of *M. refringens*, the copepod, *P. grani*.

- In enzootic areas, control is attempted by curtailing the planting of European oyster seed during the period of transmission (July and August) and by growing European oysters in areas with high salinities (35-37 ppt) to limit the development of *M. refringens*.

The end