

DRAFT

2015 coral disease epizootic

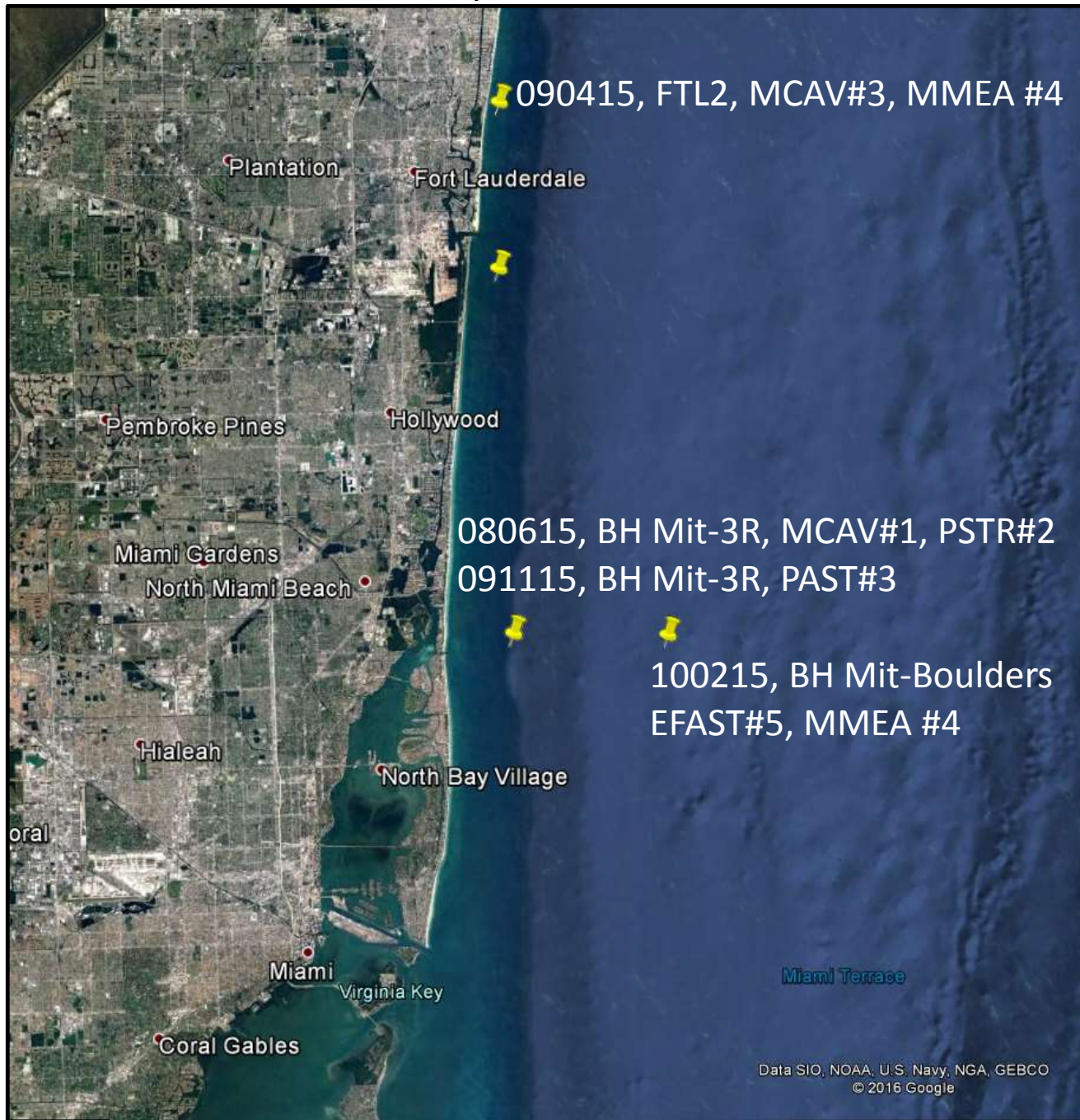
Photos and preliminary interpretation by Jan Landsberg and Yasu Kiryu (FWRI/FWC),
in consultation with Esther Peters (George Mason University)

Histological processing by Noretta Perry, Michelle Franco, and Patrick Wilson (FWRI/FWC)

Samples collected by Karen Bohnsack (FDEP) and S. Thanner (MDC)

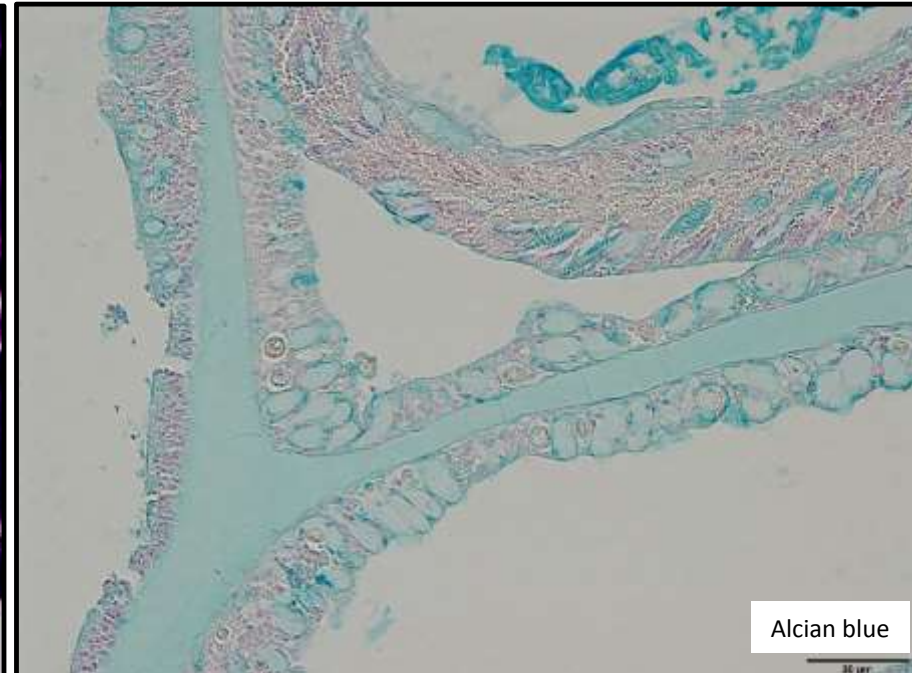
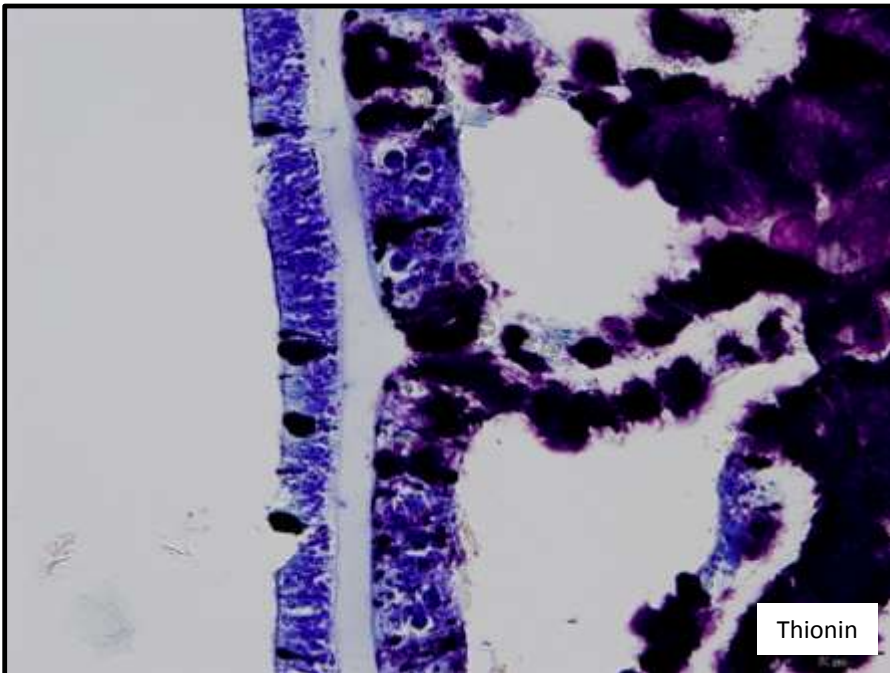
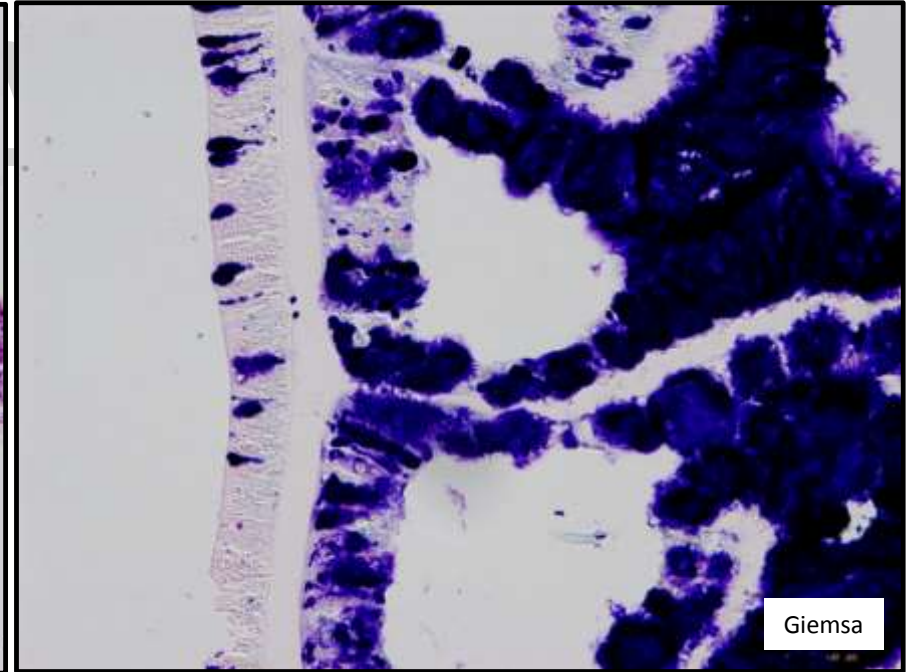
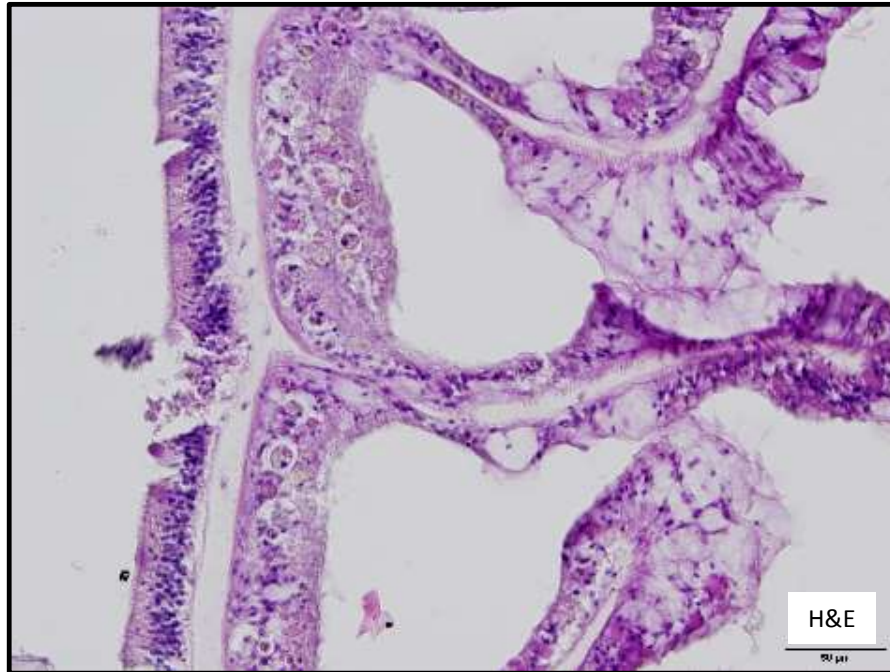


2015 sample locations





MCAV #3, *Montastrea cavernosa*, 090415, FTL5



Active infection (putative rickettsia-like organisms, RLOs)

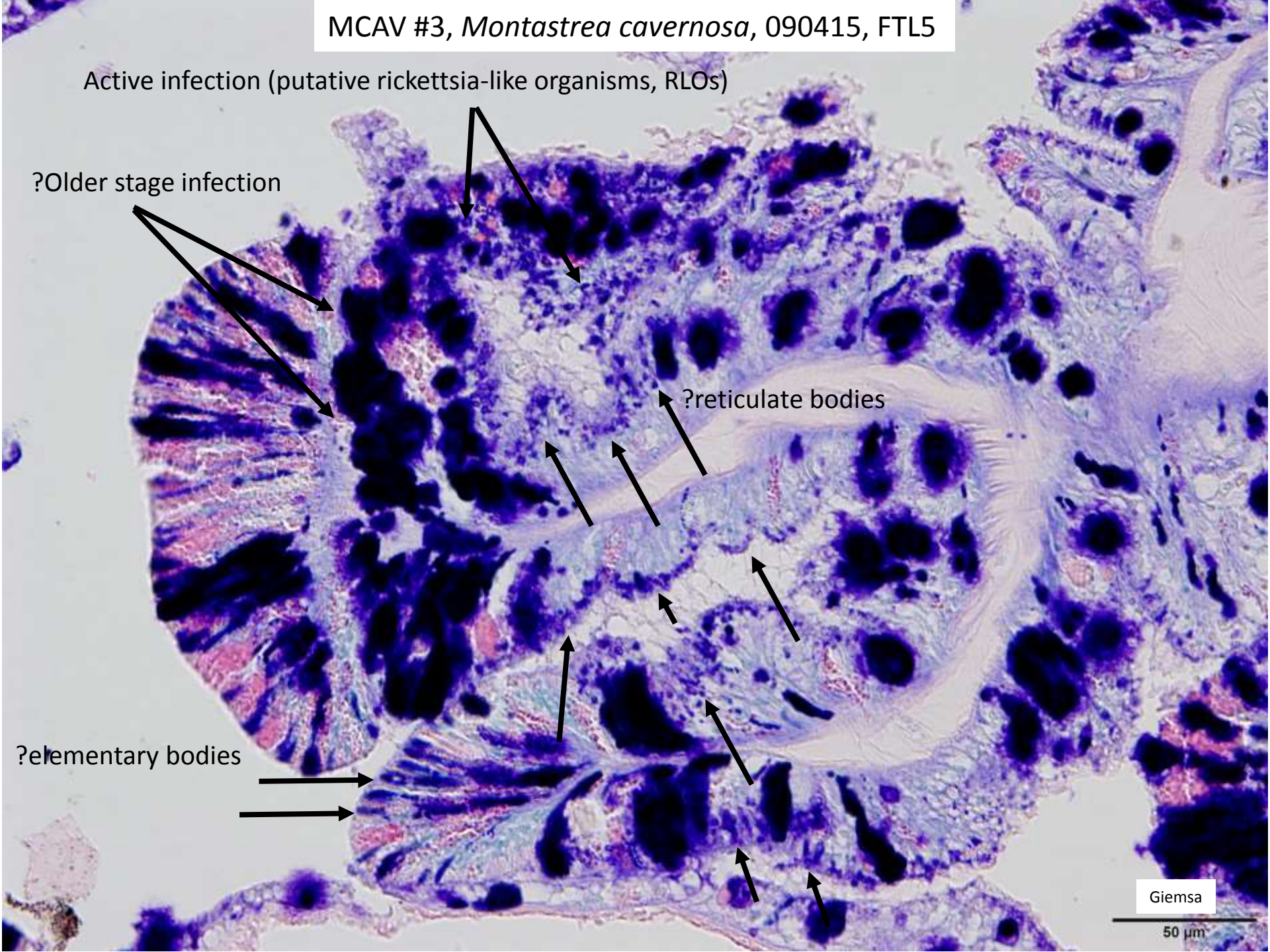
?Older stage infection

?reticulate bodies

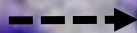
?elementary bodies

Giemsa

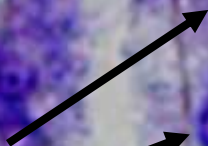
50 μ m



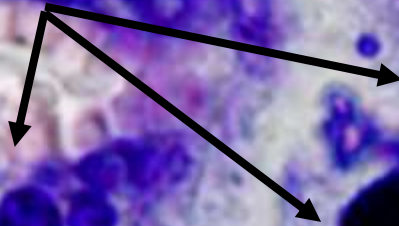
? elementary bodies



? reticulate bodies



? reticulate bodies



? elementary bodies

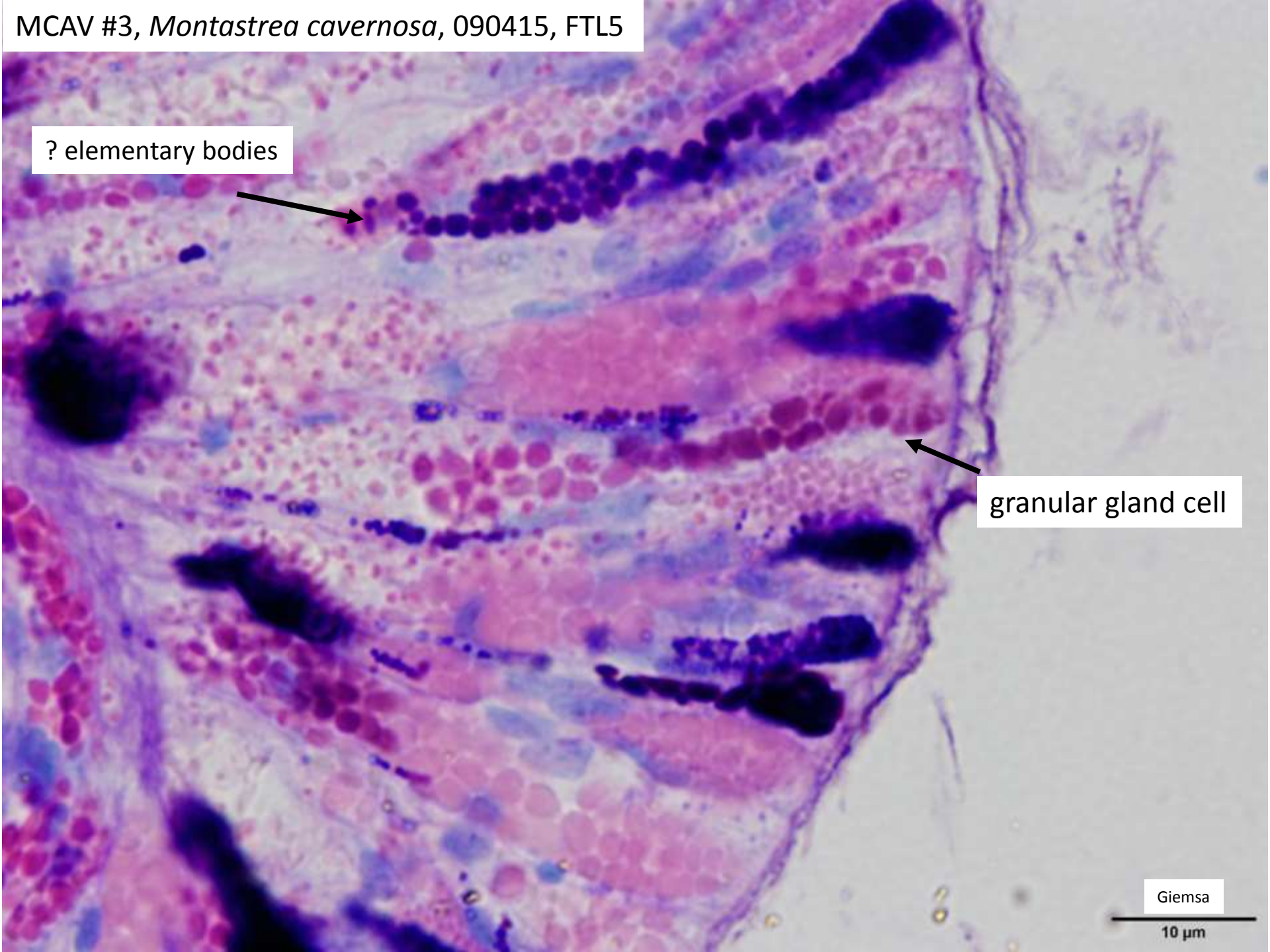


granular gland cell



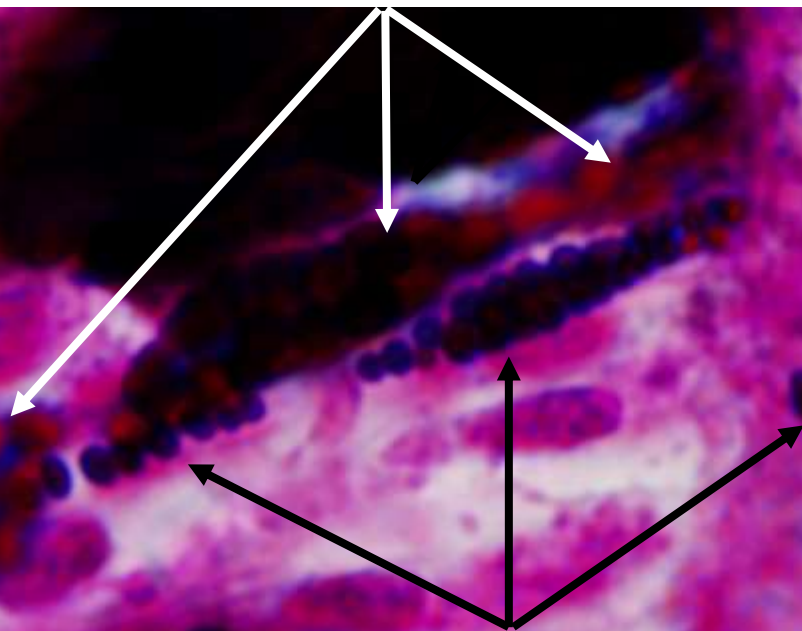
Giemsa

10 μm



MCAV #3, *Montastrea cavernosa*, 090415, FTL5

? Reticulate bodies, presumptive positive in chlamydia-specific stain (Gimenez; still testing for corals), overstained



? Elementary bodies, presumptive positive in chlamydia-specific stain (Gimenez; still testing for corals)

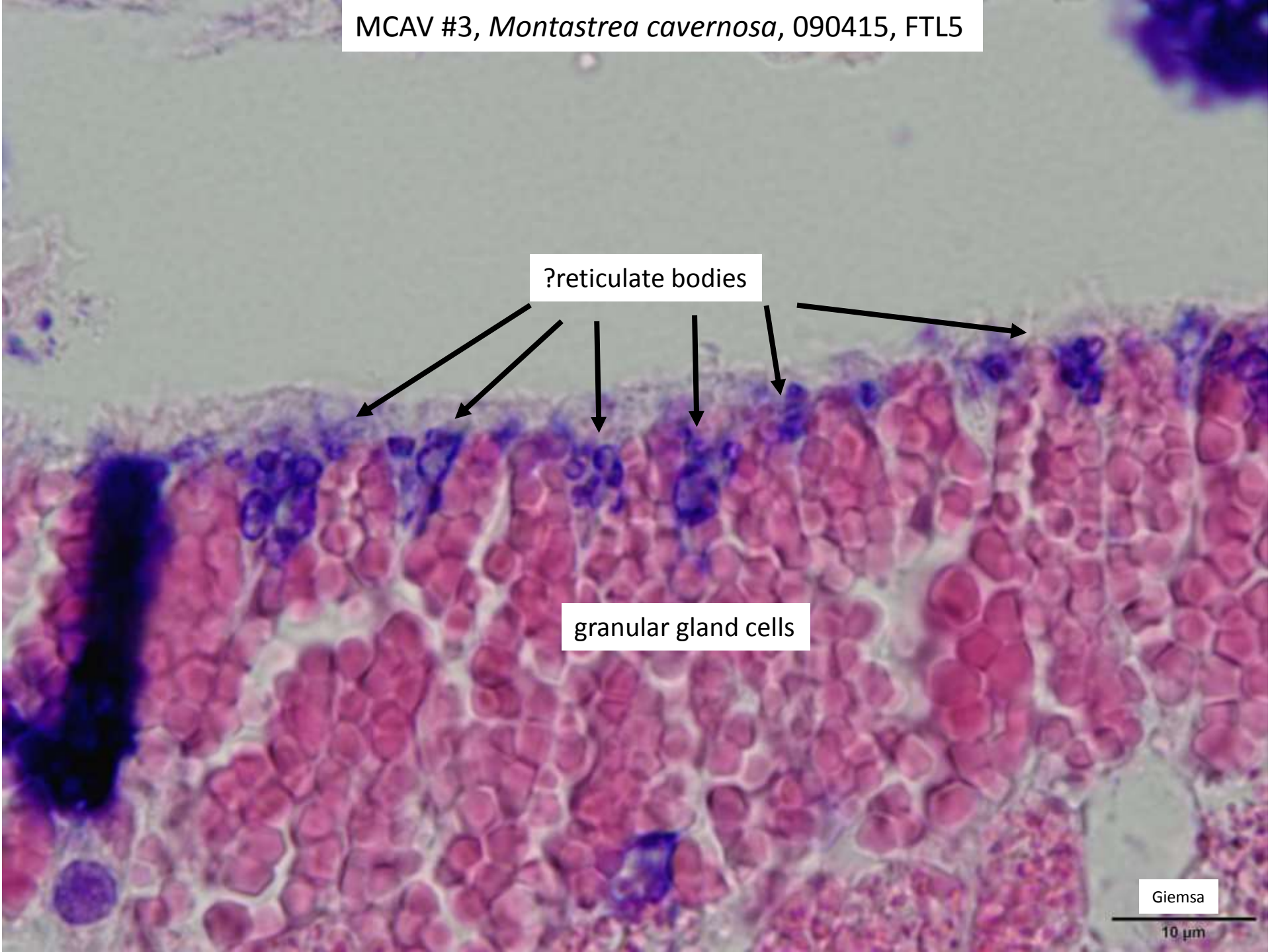
MCAV #3, *Montastrea cavernosa*, 090415, FTL5

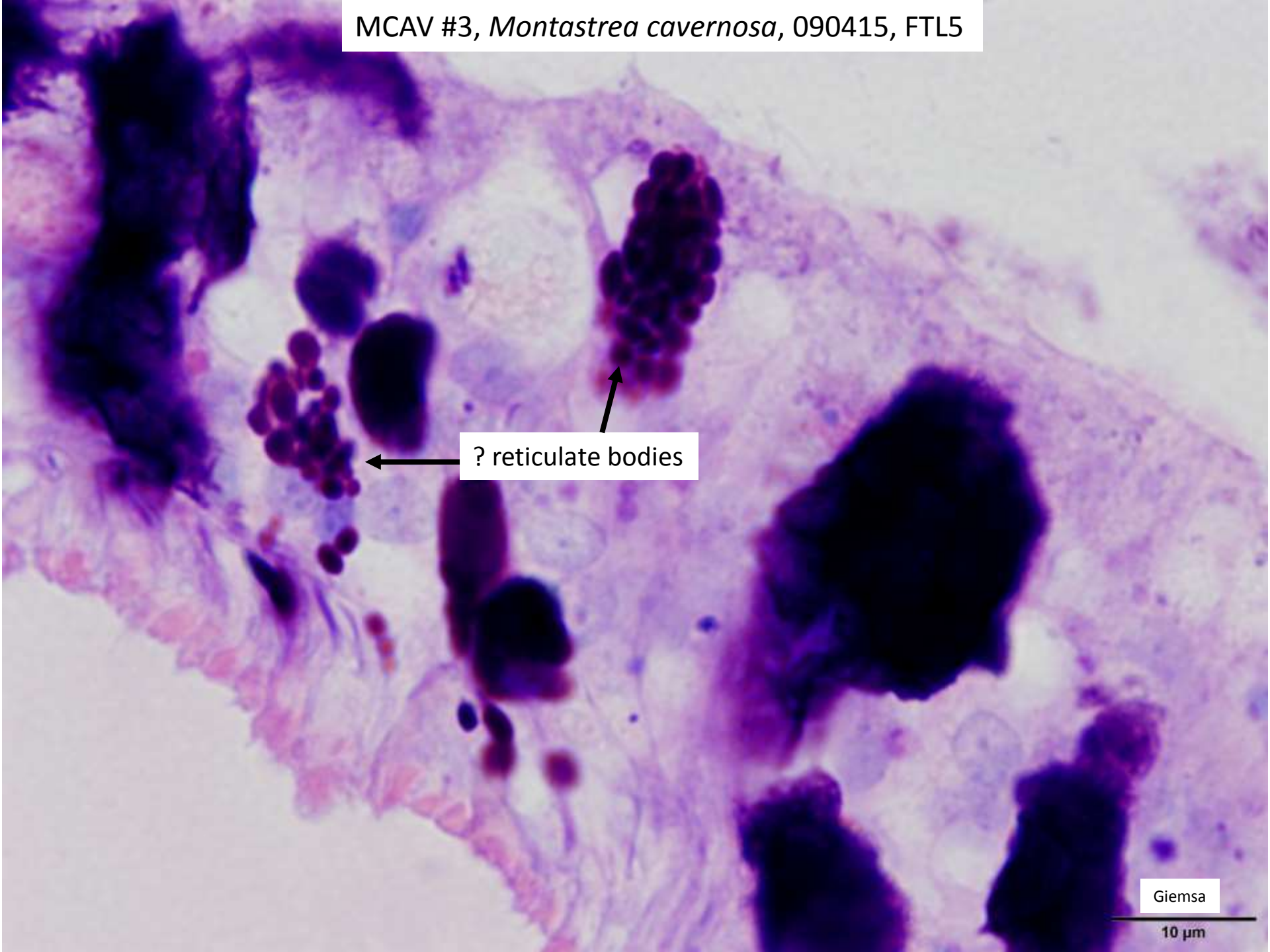
?reticulate bodies

granular gland cells

Giemsa

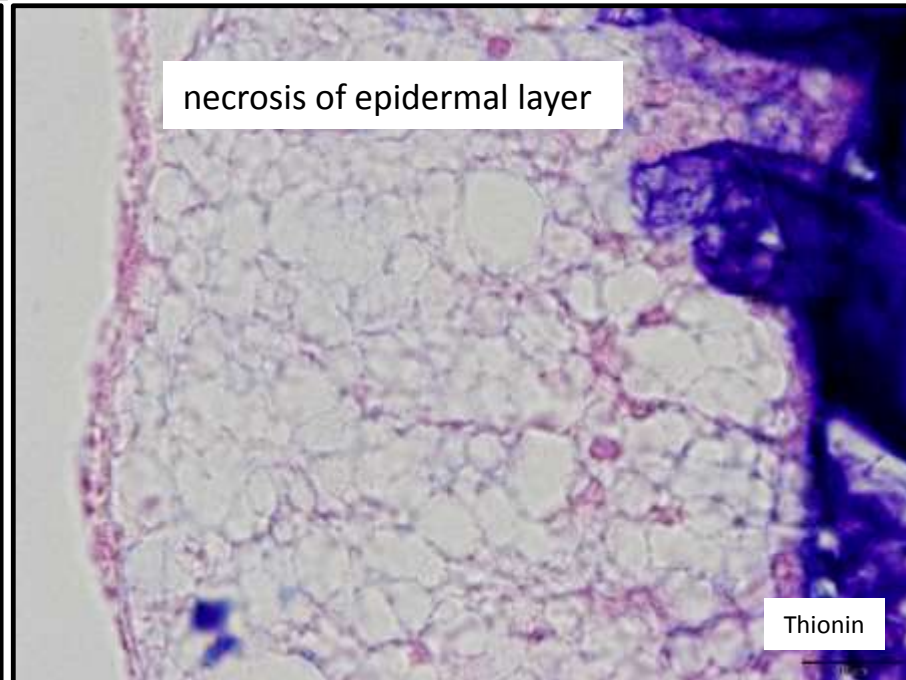
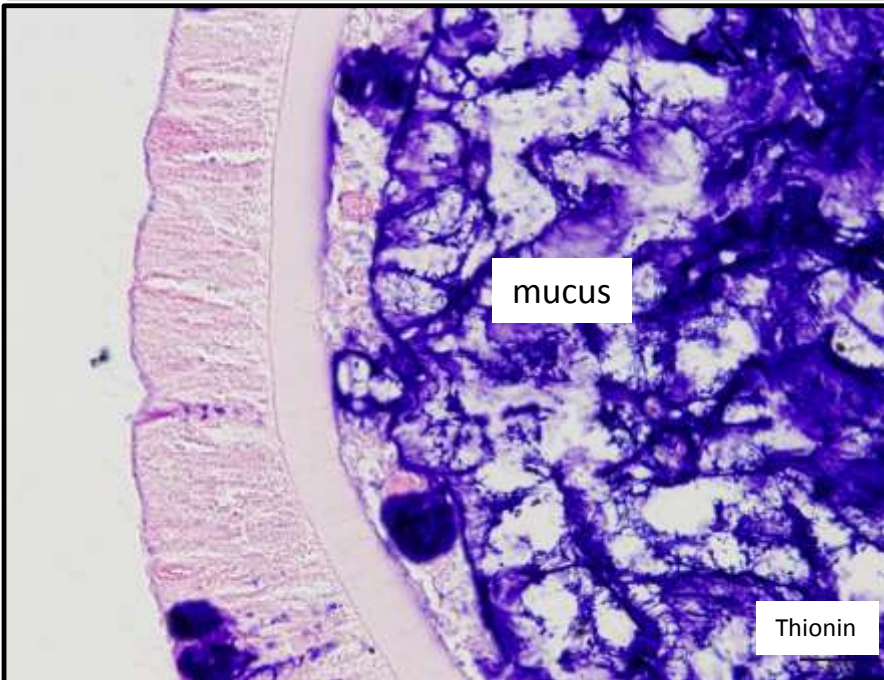
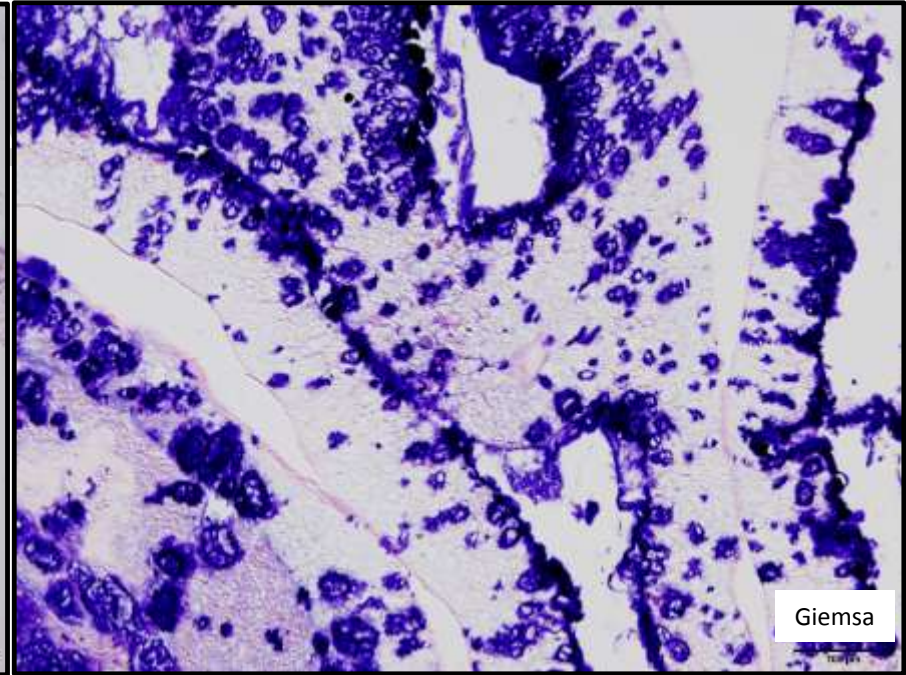
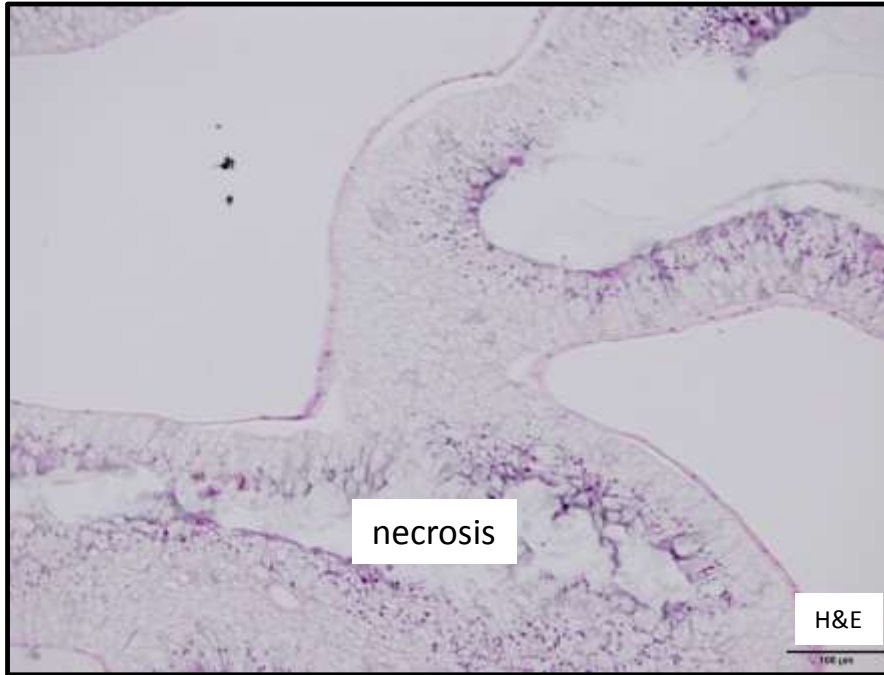
10 μ m



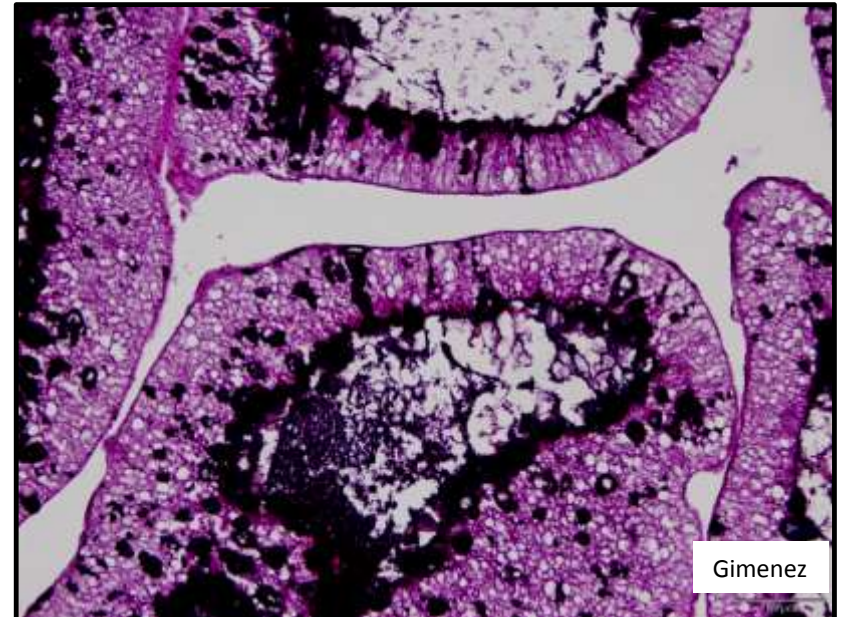
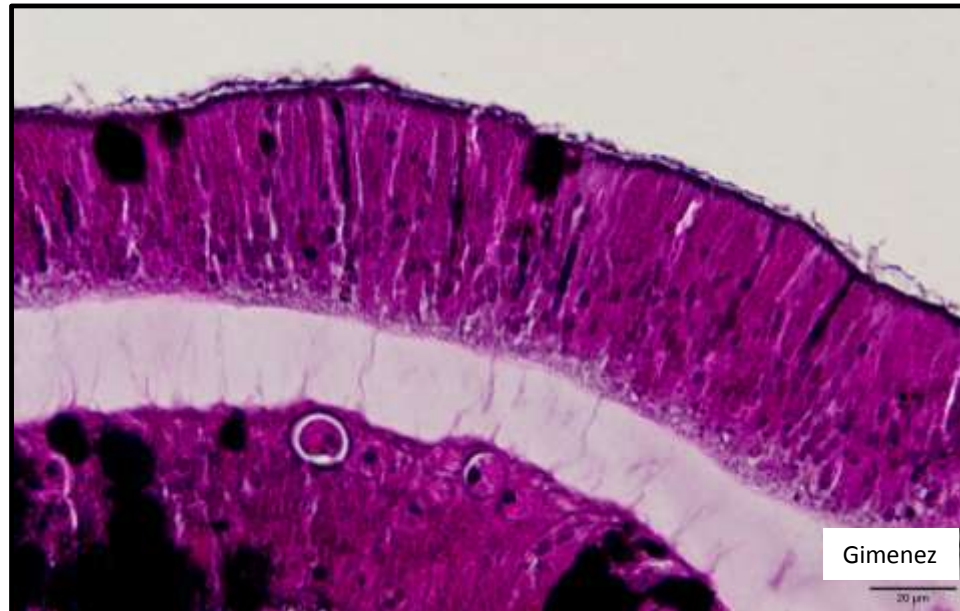
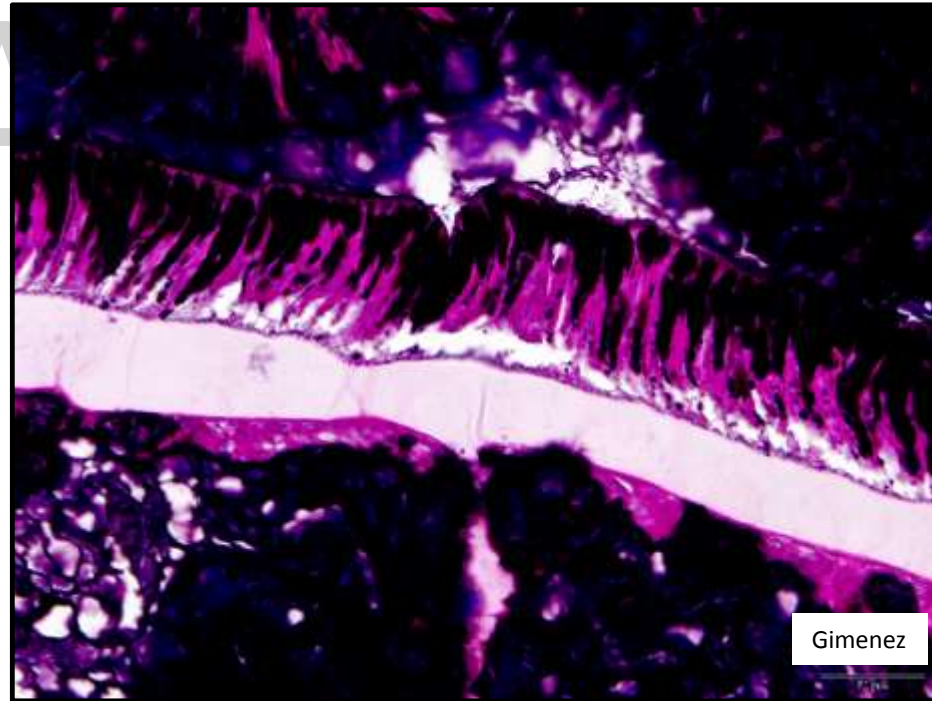
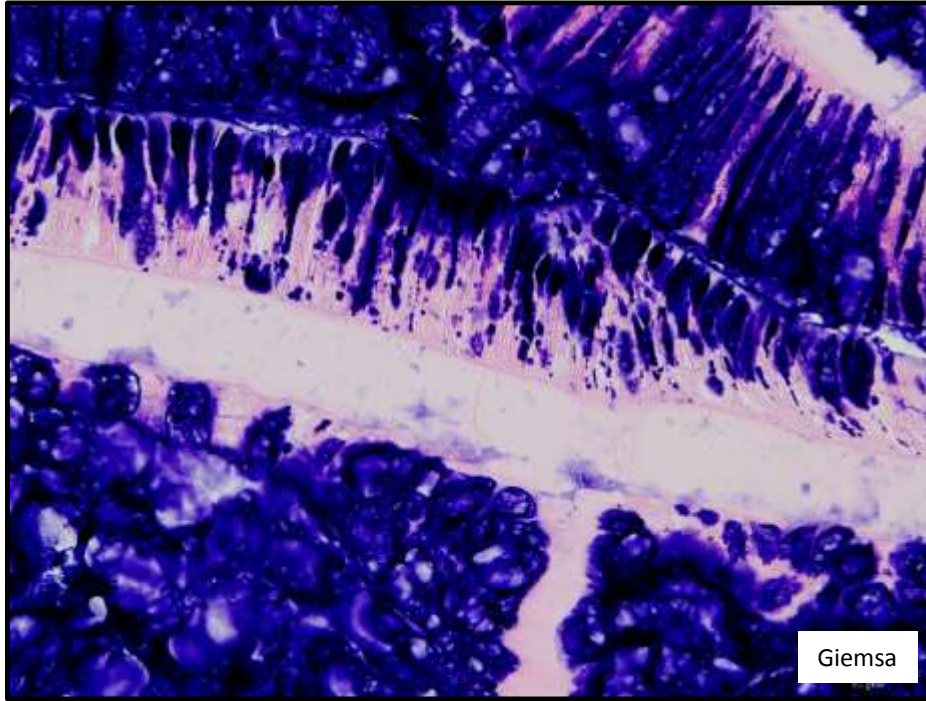


? reticulate bodies

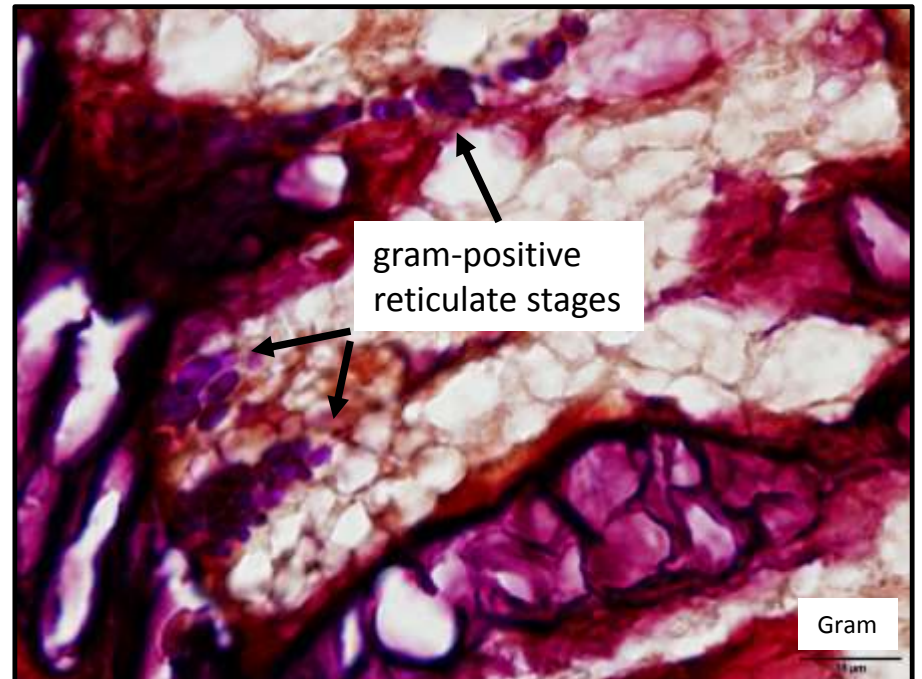
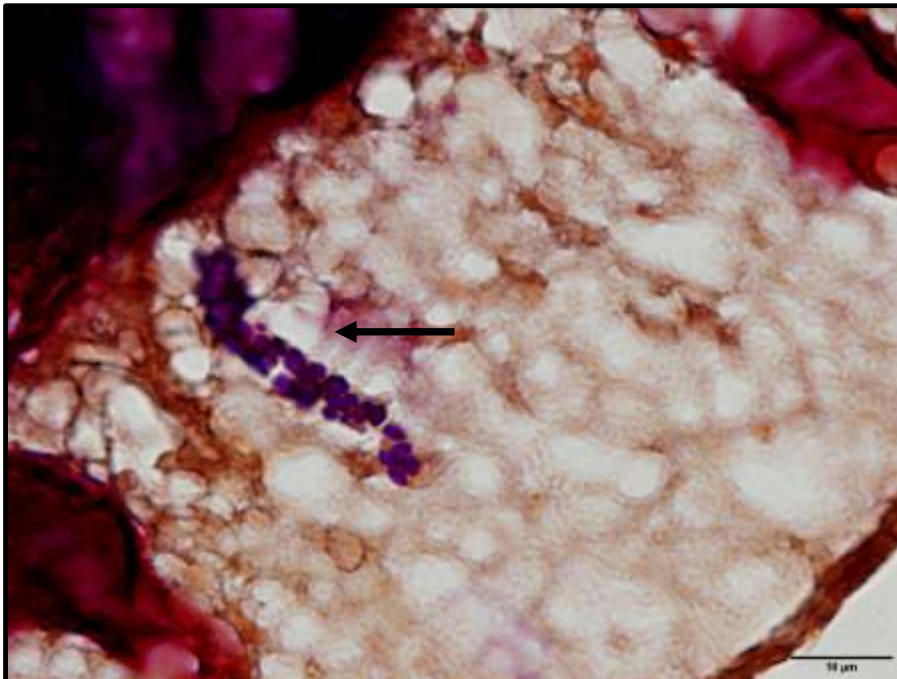
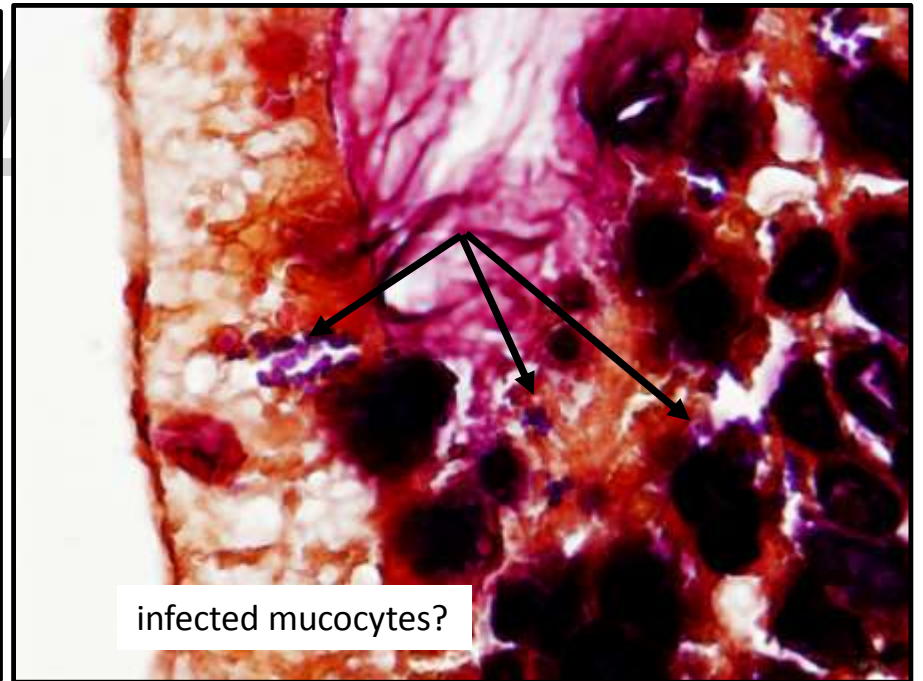
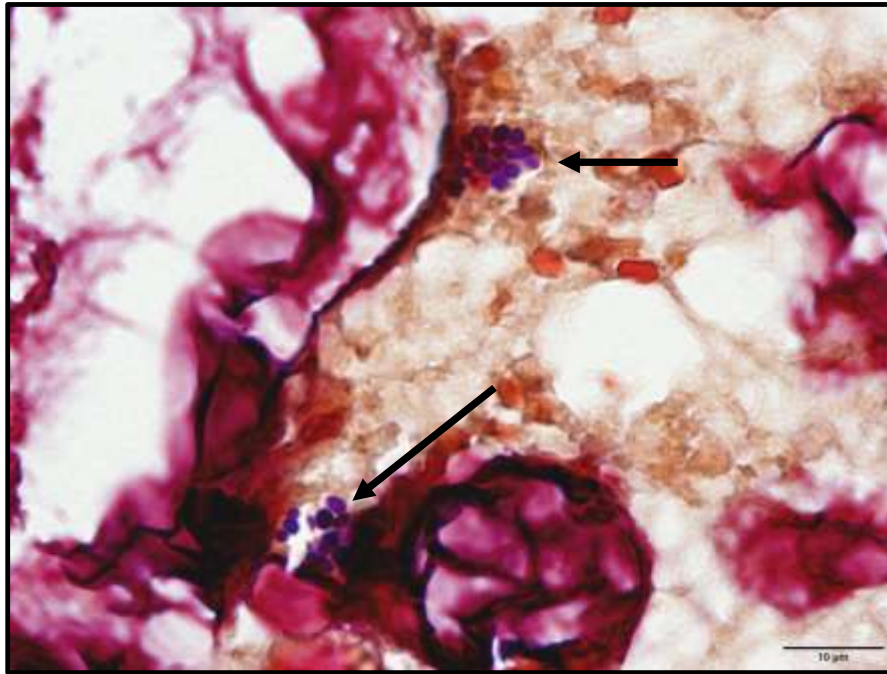
MCAV #1, *Montastrea cavernosa*, 080615, BH Mit-3R



MCAV #1, *Montastrea cavernosa*, 080615, BH Mit-3R

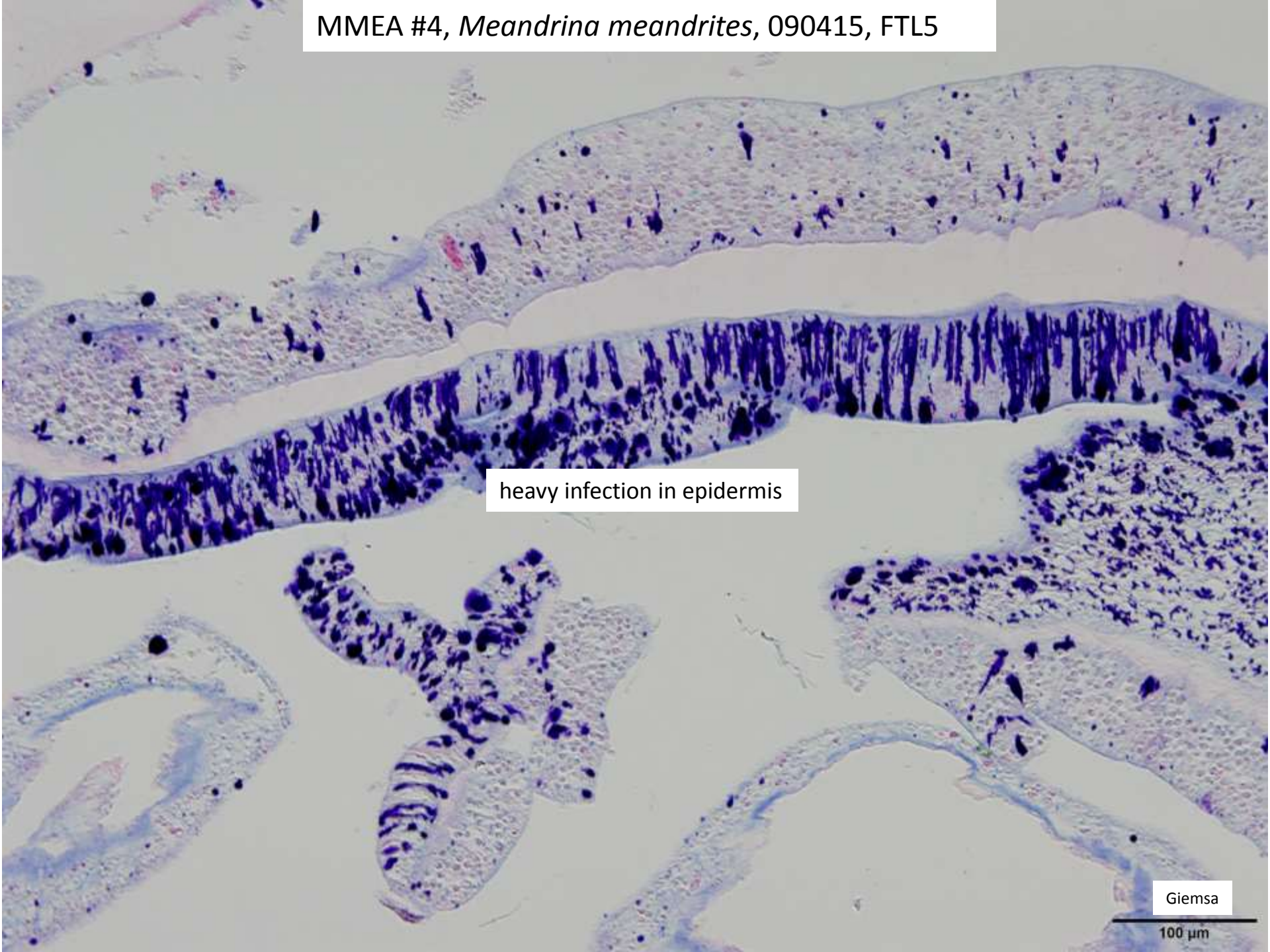


MCAV #1, *Montastrea cavernosa*, 080615, BH Mit-3R



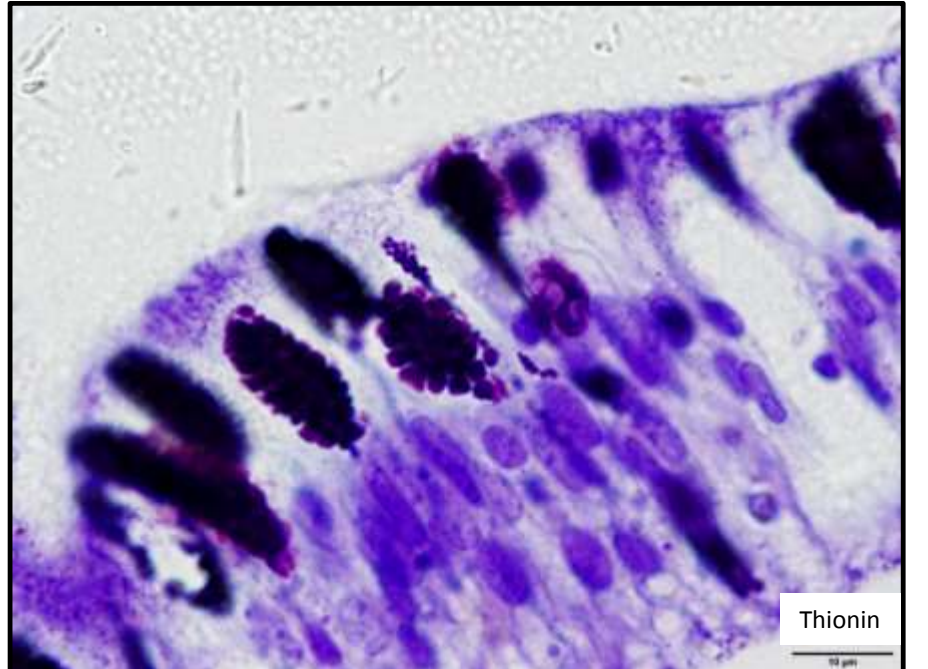
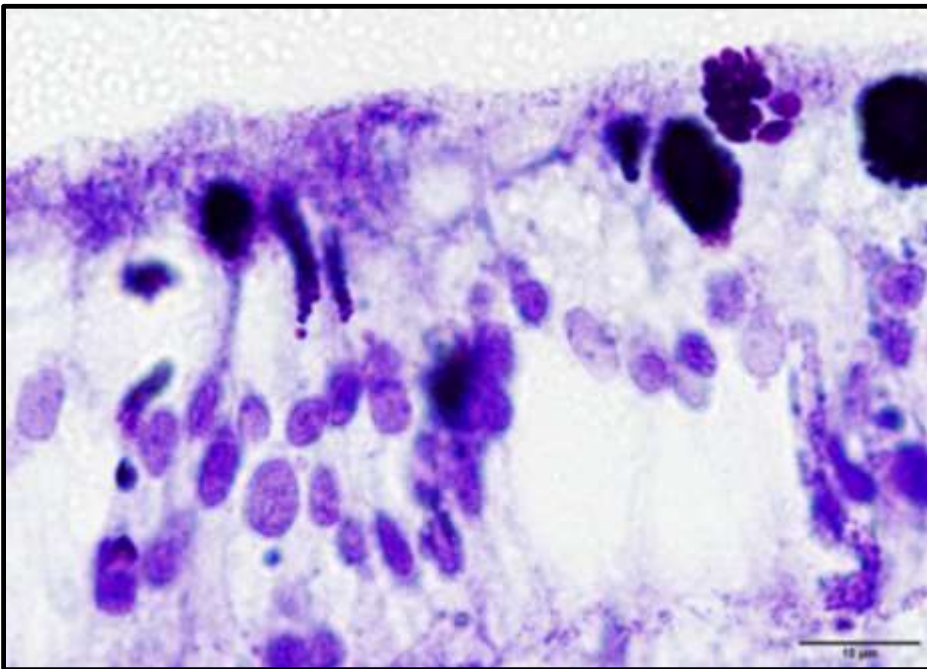
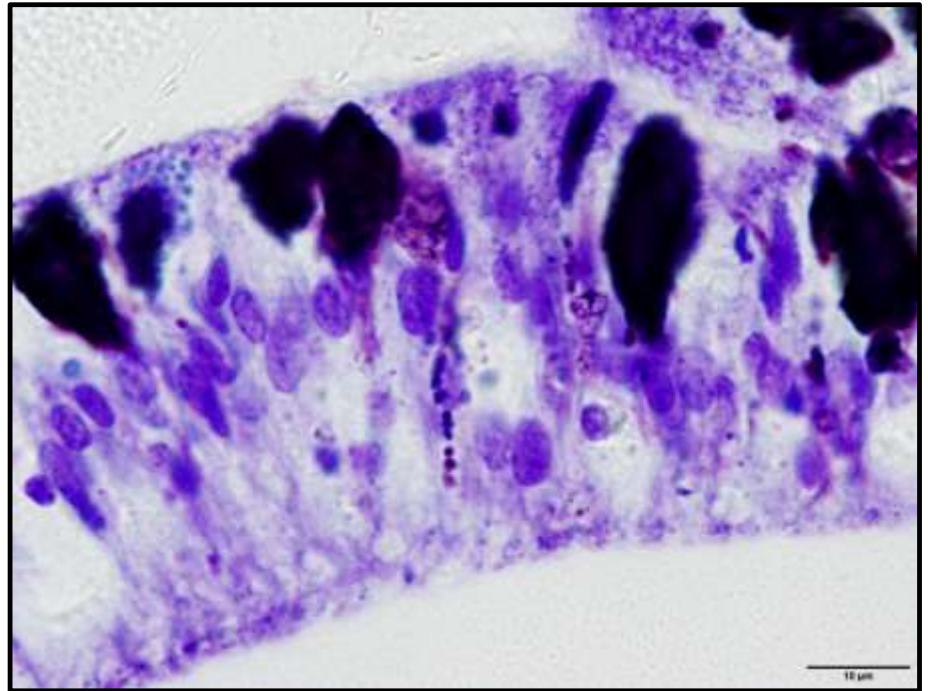
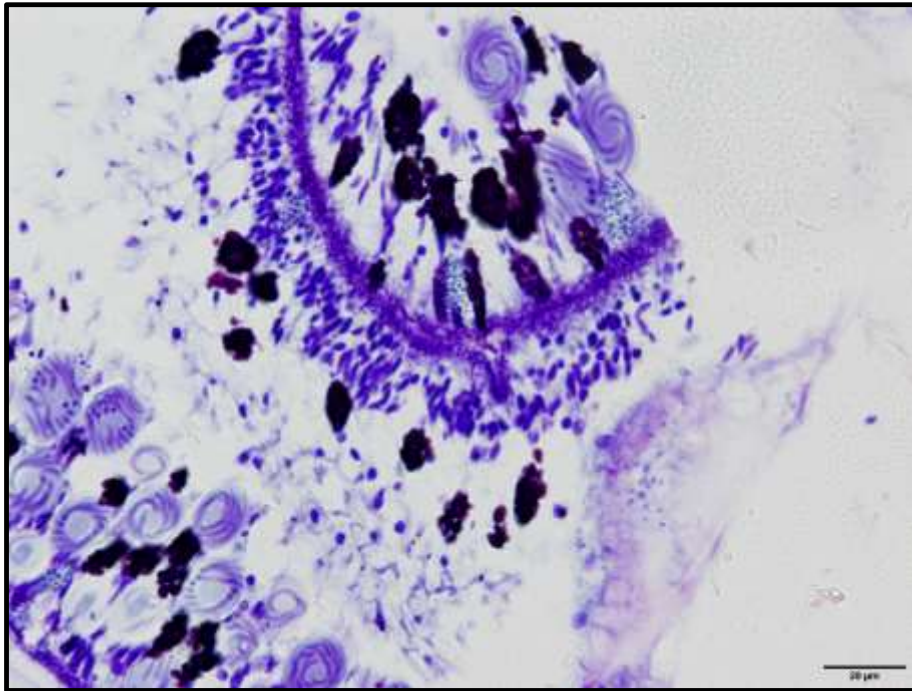


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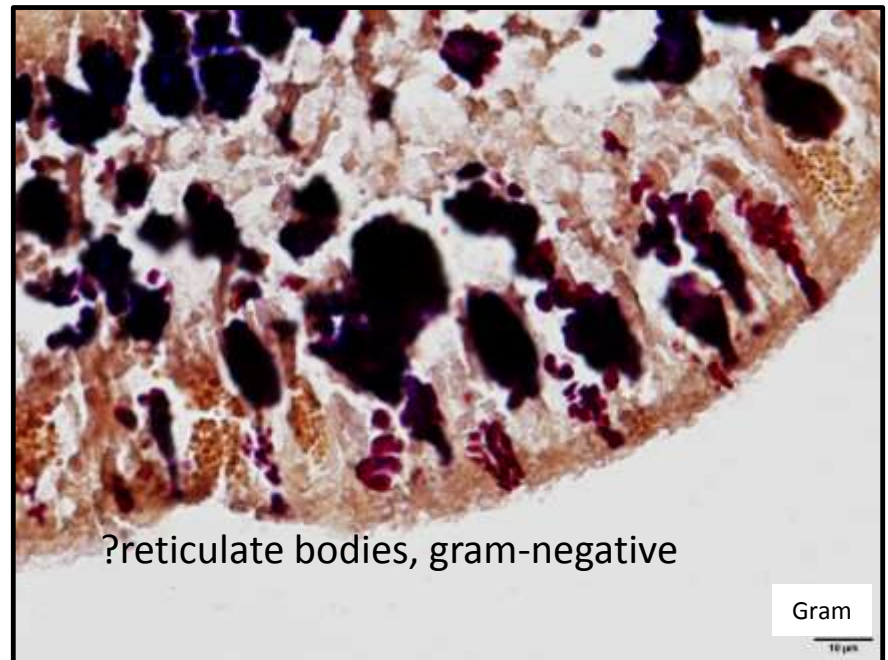
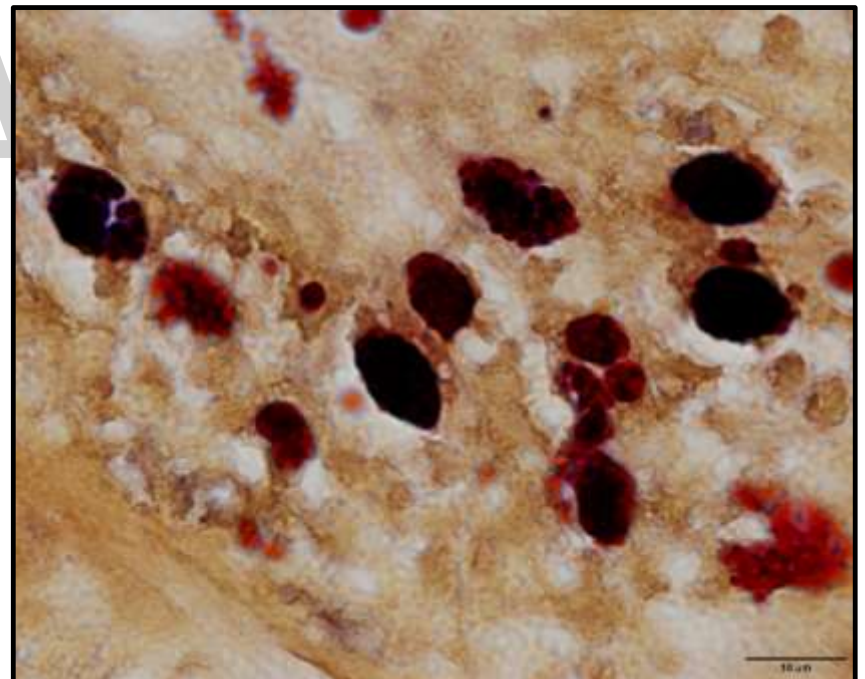
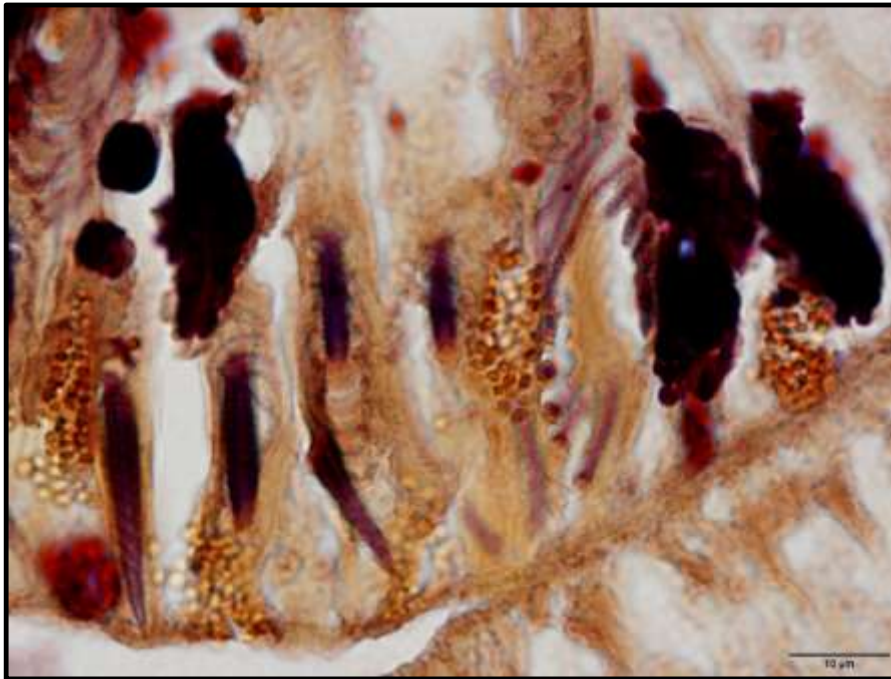


heavy infection in epidermis

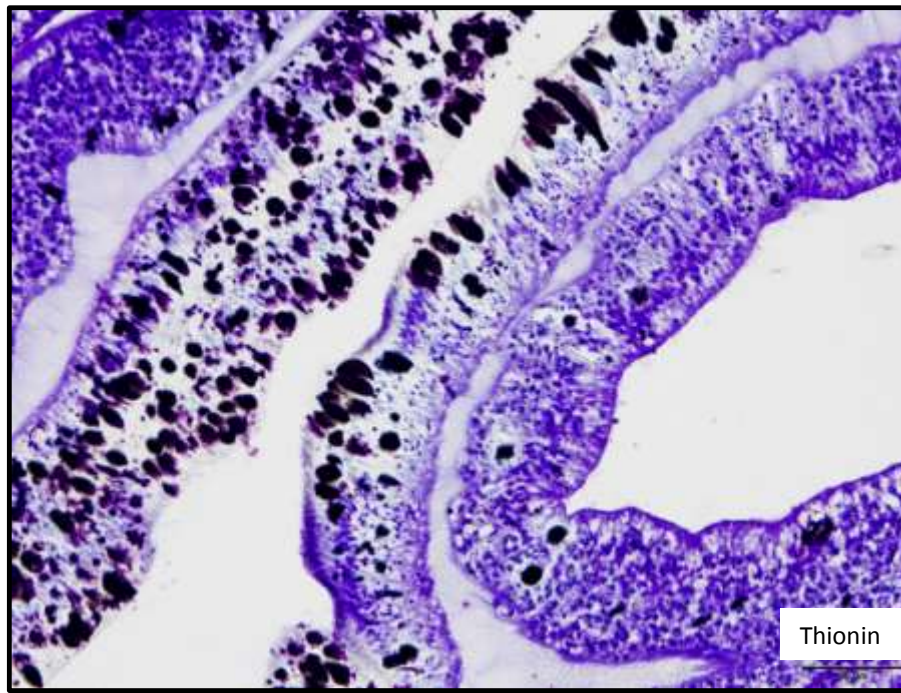
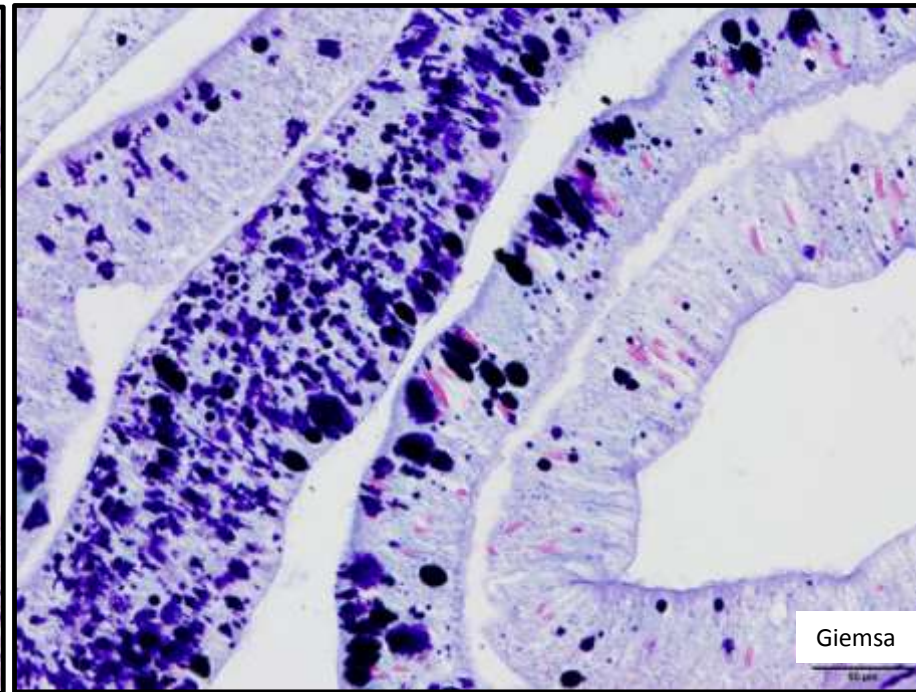
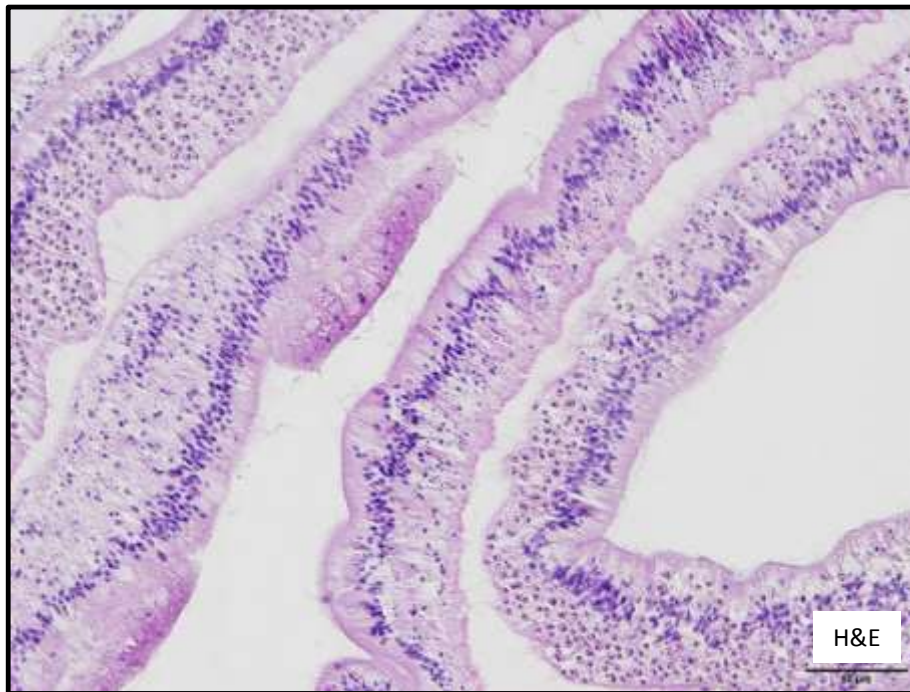
MMEA #4, *Meandrina meandrites*, 090415, FTL5



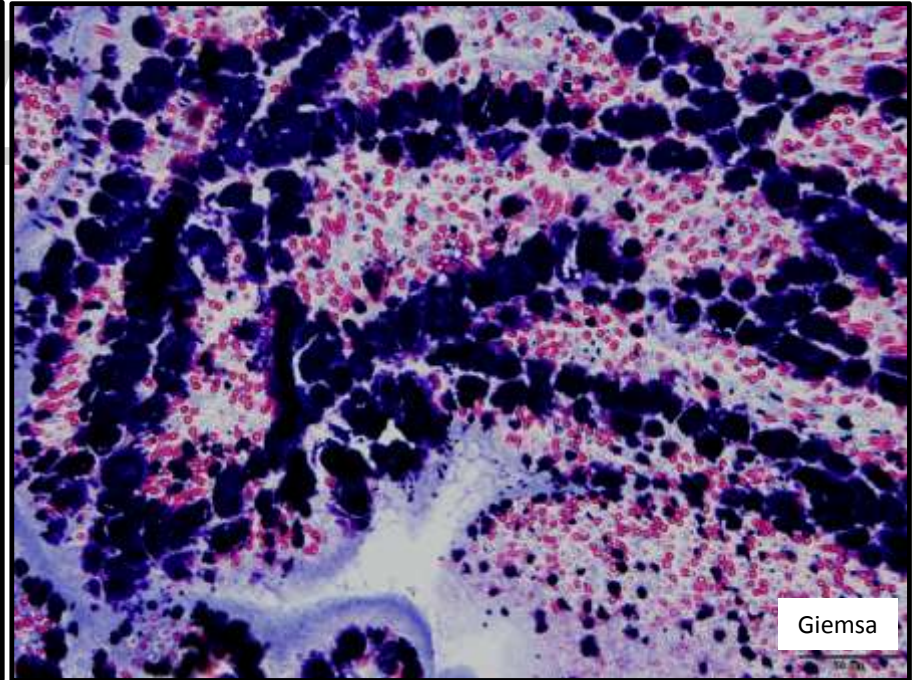
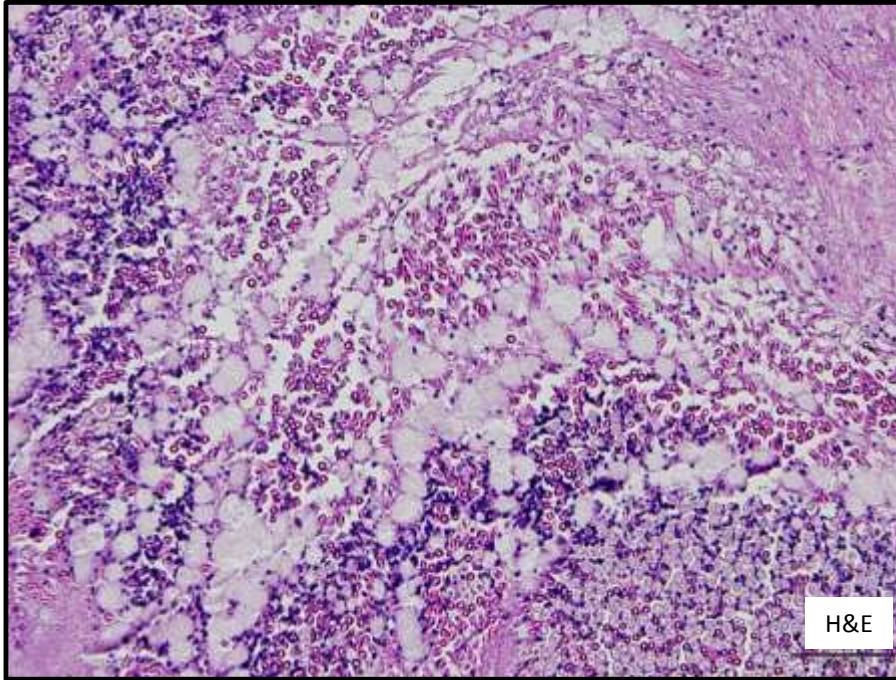
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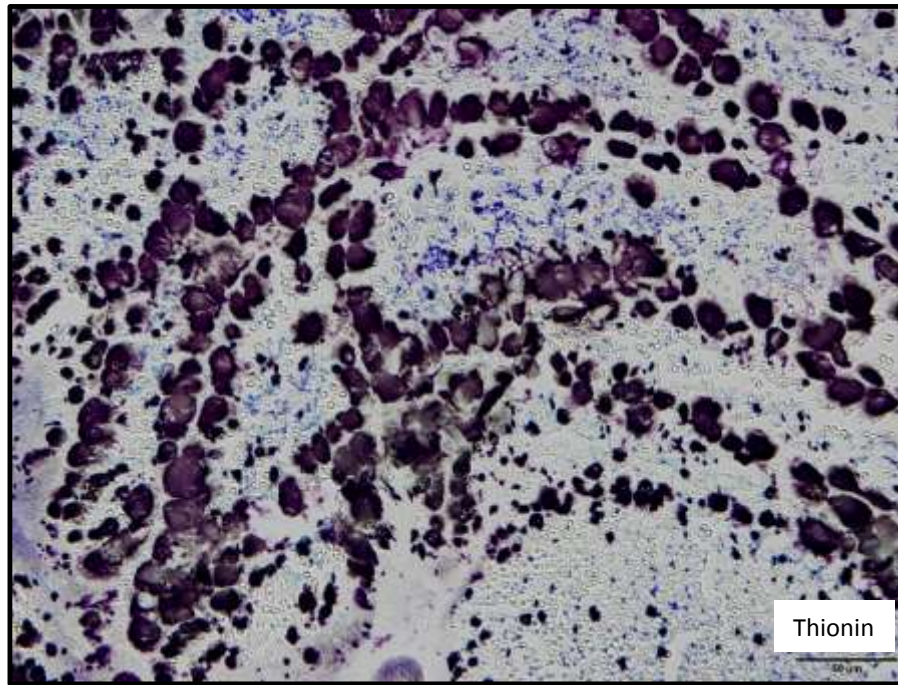
MMEA #4, *Meandrina meandrites*, 100215, BH Mit-Boulders



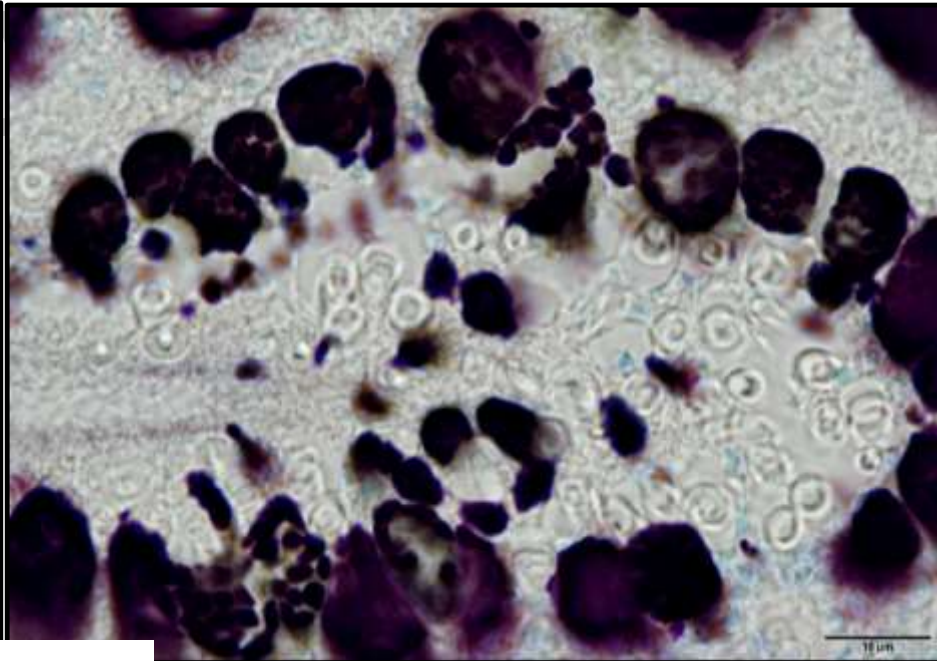
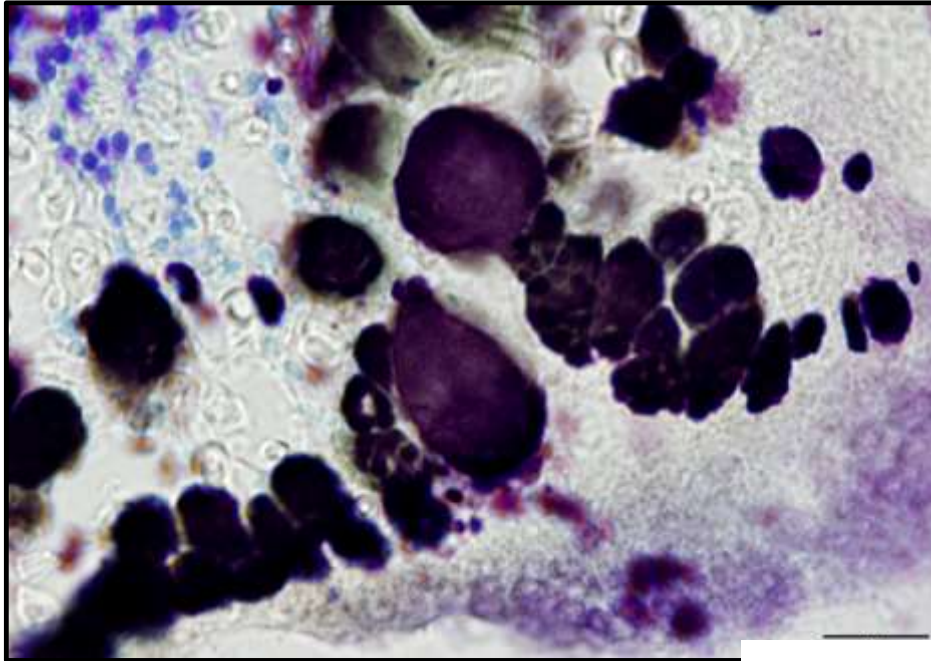
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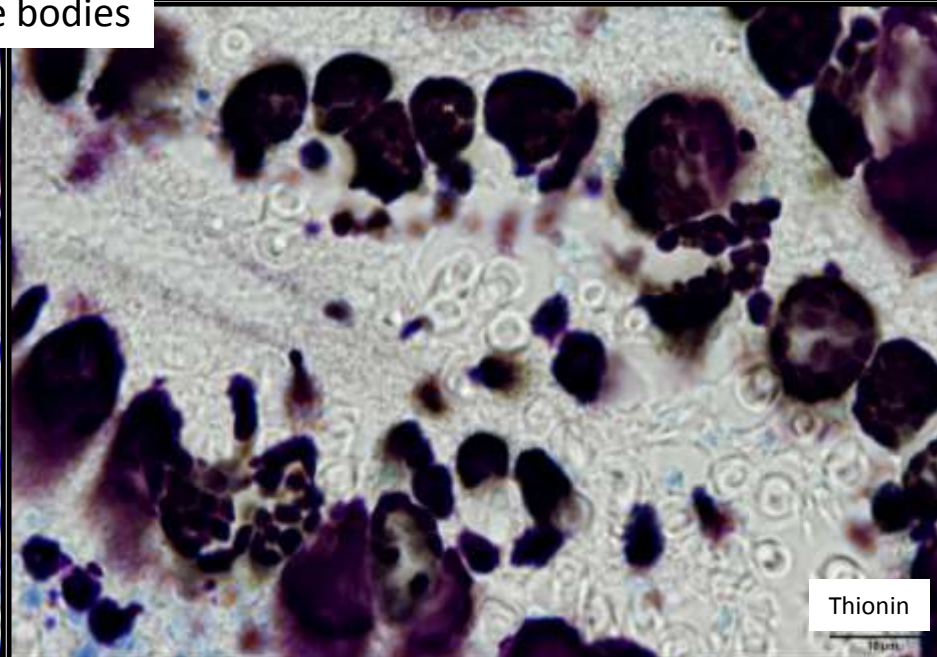
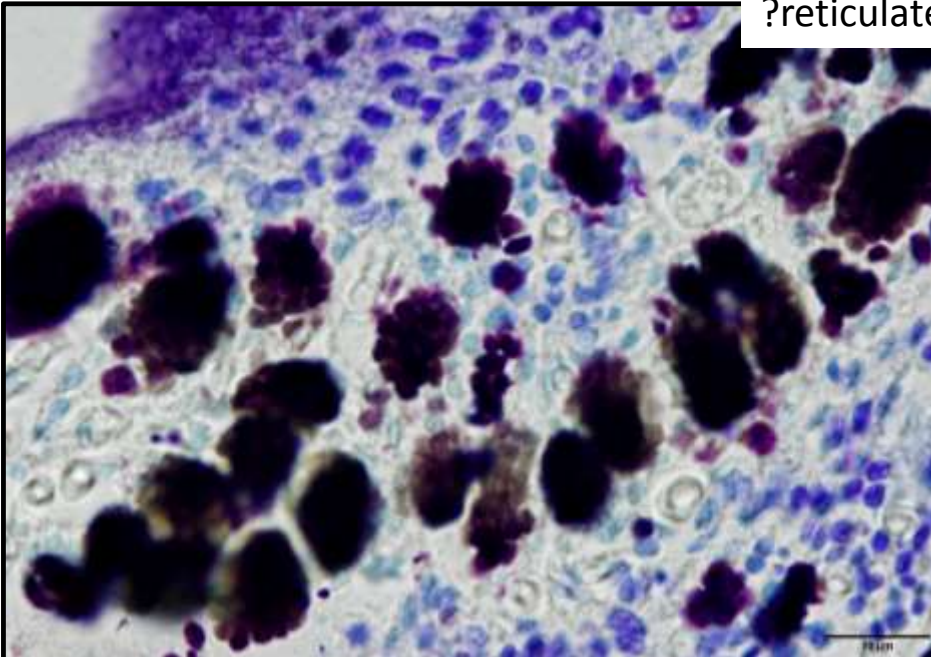
Heavy infection by presumptive RLO reticulate bodies in mucocytes of cnidoglandular band



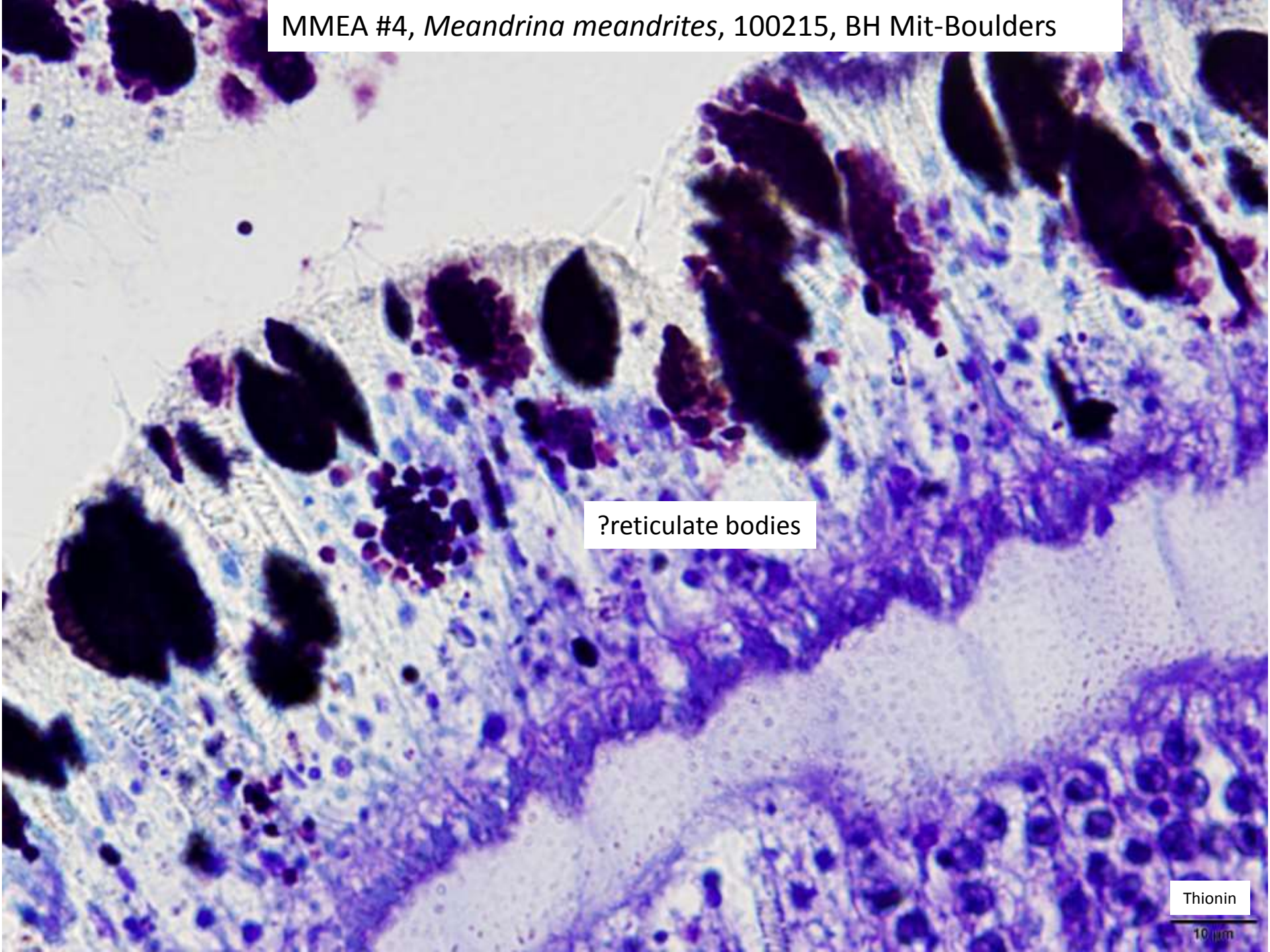
MMEA #4, *Meandrina meandrites*, 100215, BH Mit-Boulders



?reticulate bodies

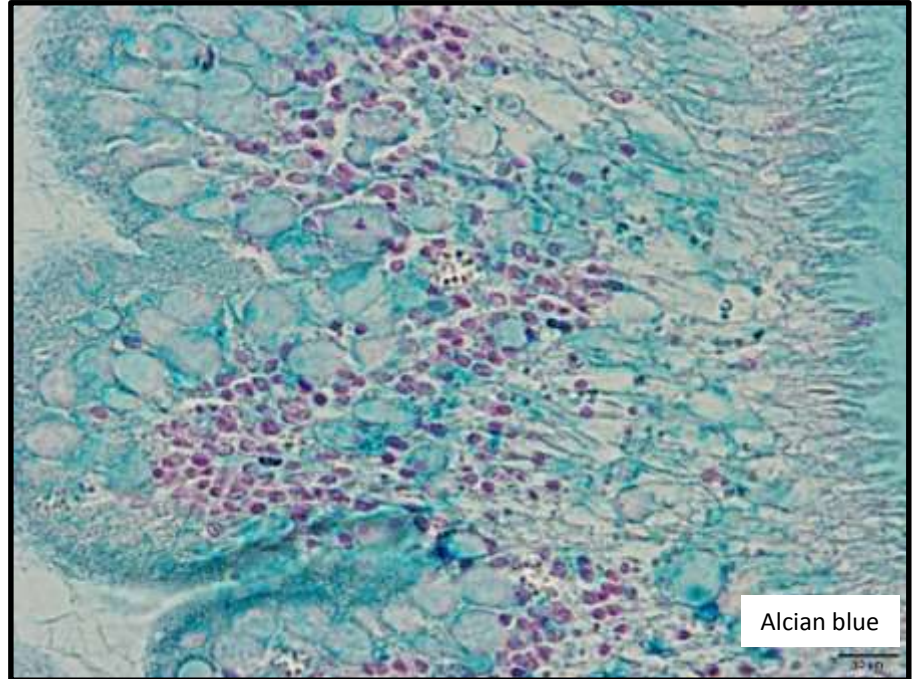
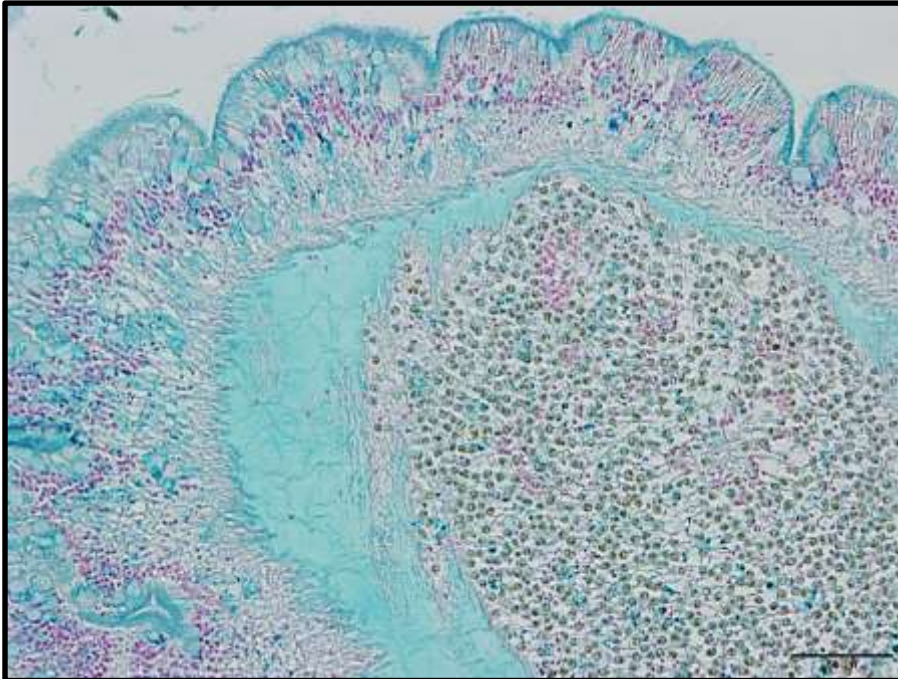


Thionin

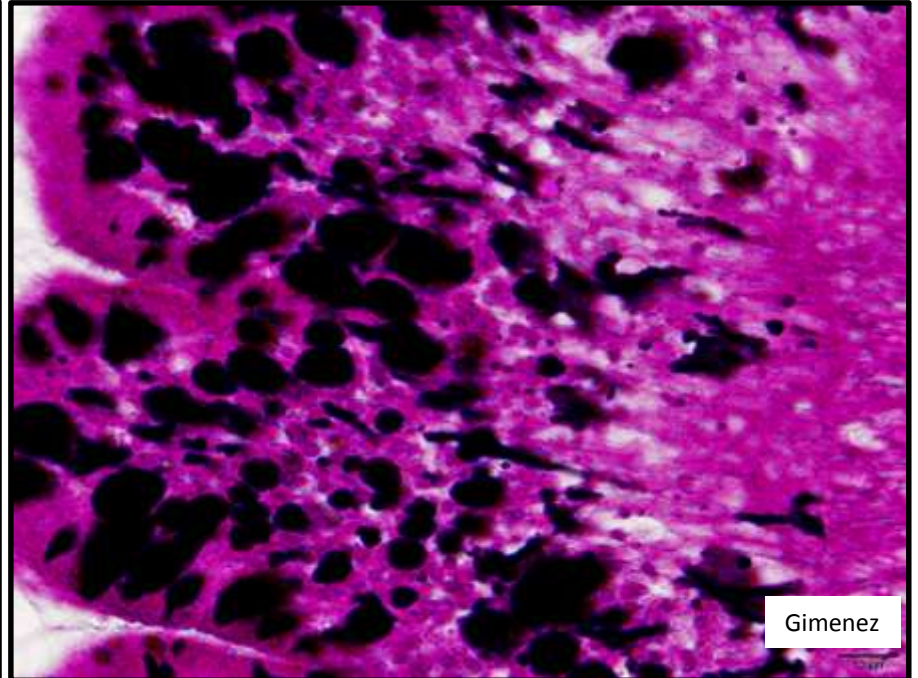
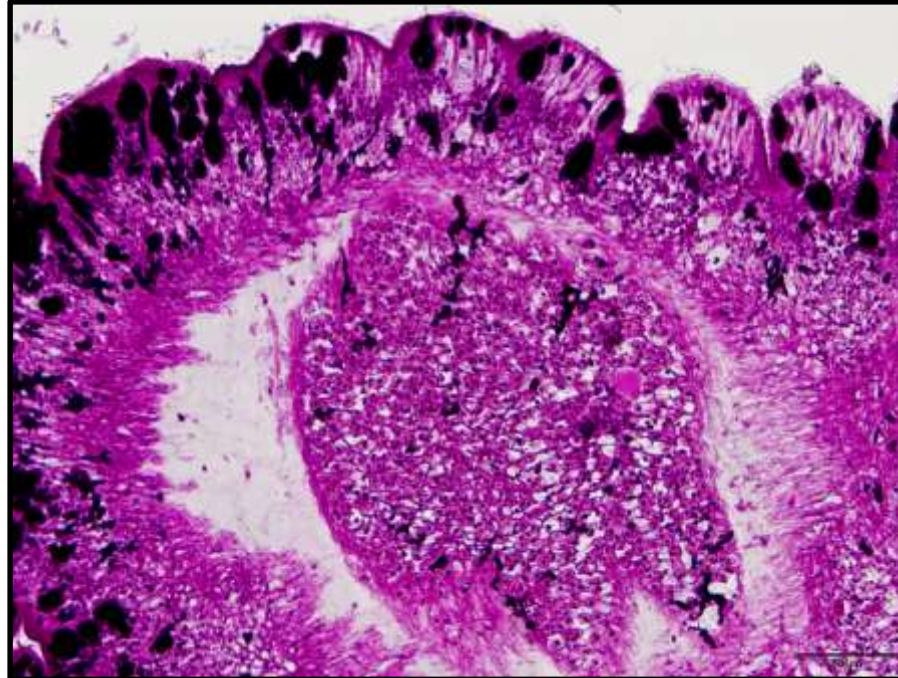


?reticulate bodies

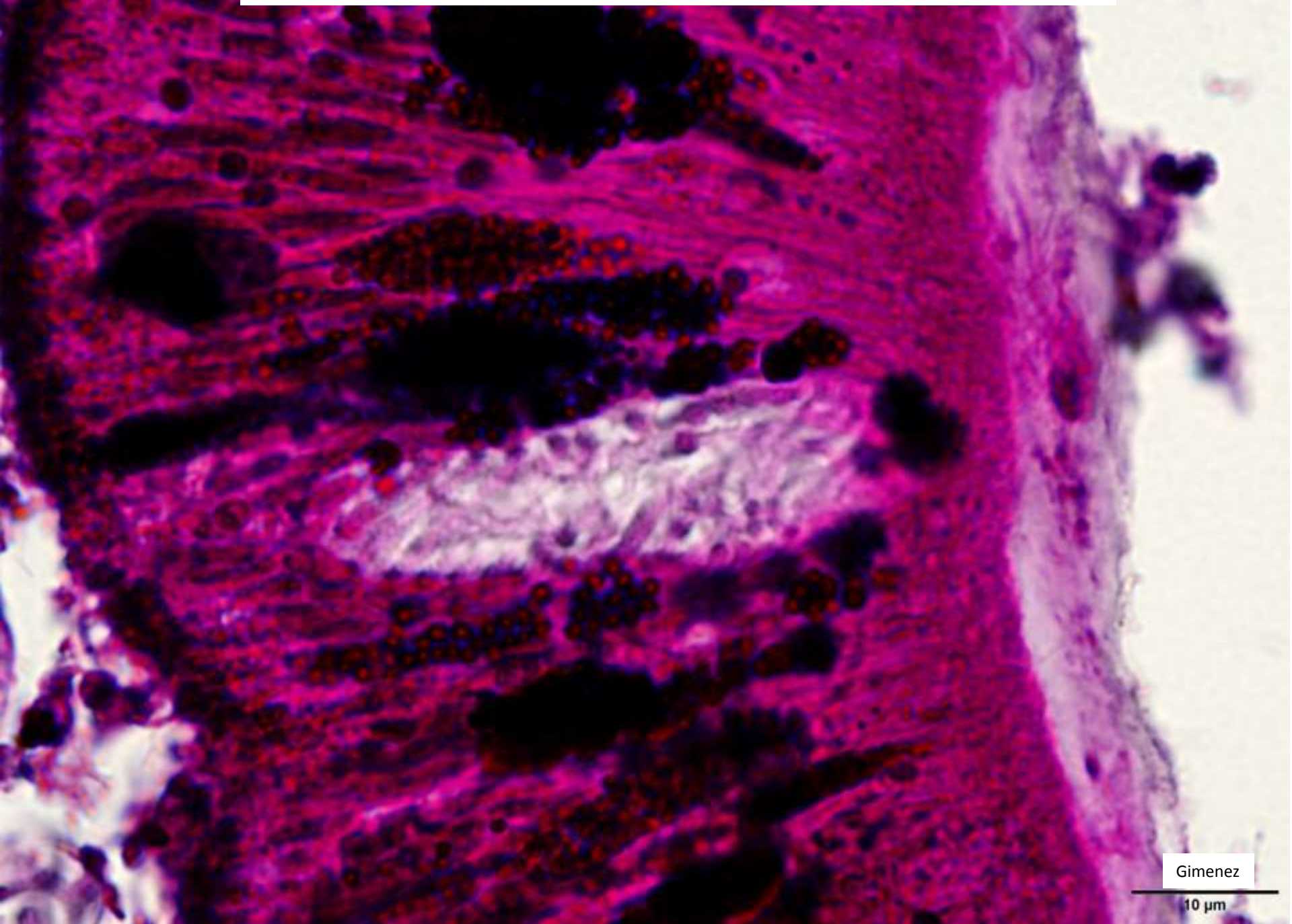
MMEA #4, *Meandrina meandrites*, 100215, BH Mit-Boulders



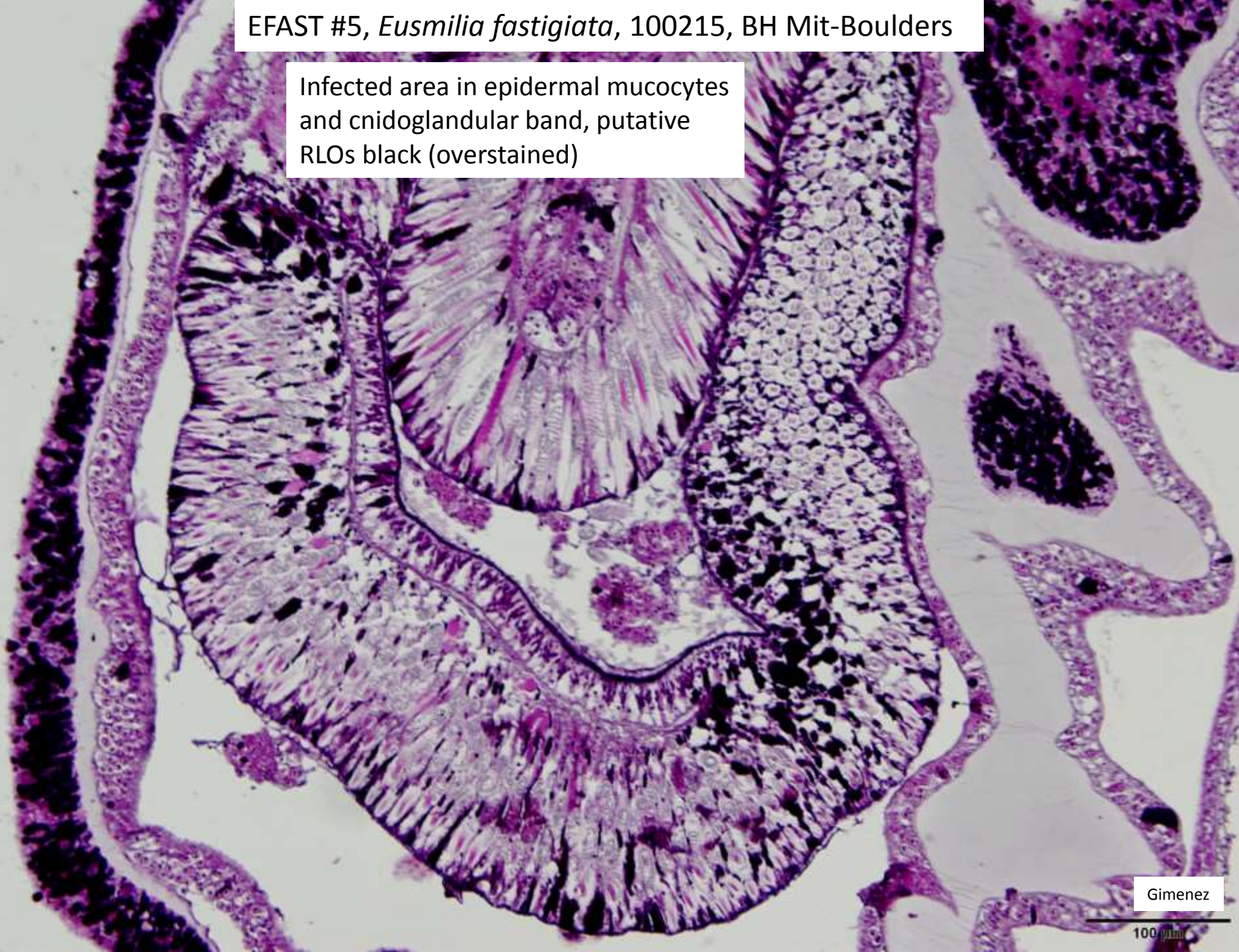
Alcian blue



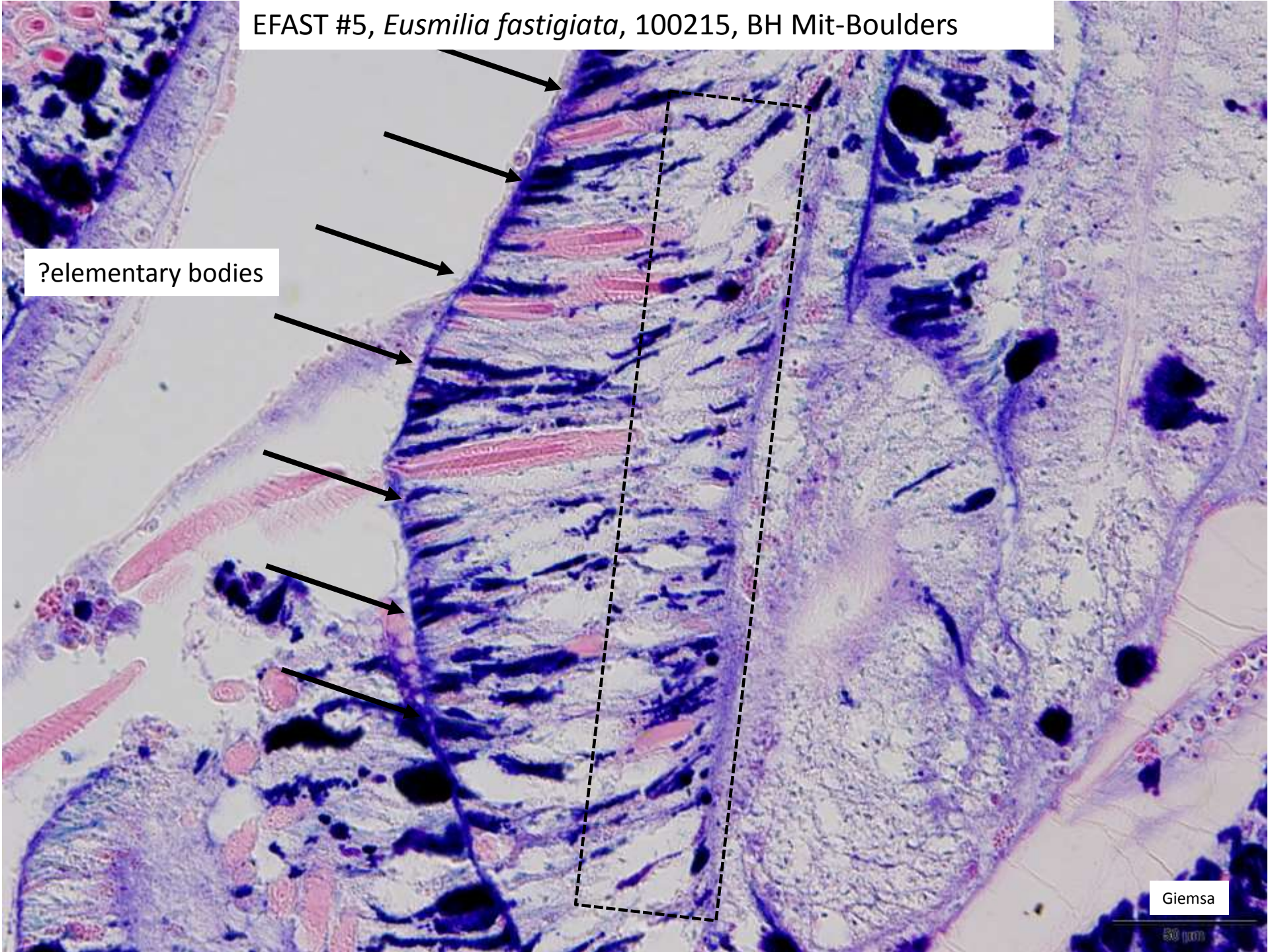
Gimenez



Infected area in epidermal mucocytes and cnidoglandular band, putative RLOs black (overstained)



EFAST #5, *Eusmilia fastigiata*, 100215, BH Mit-Boulders

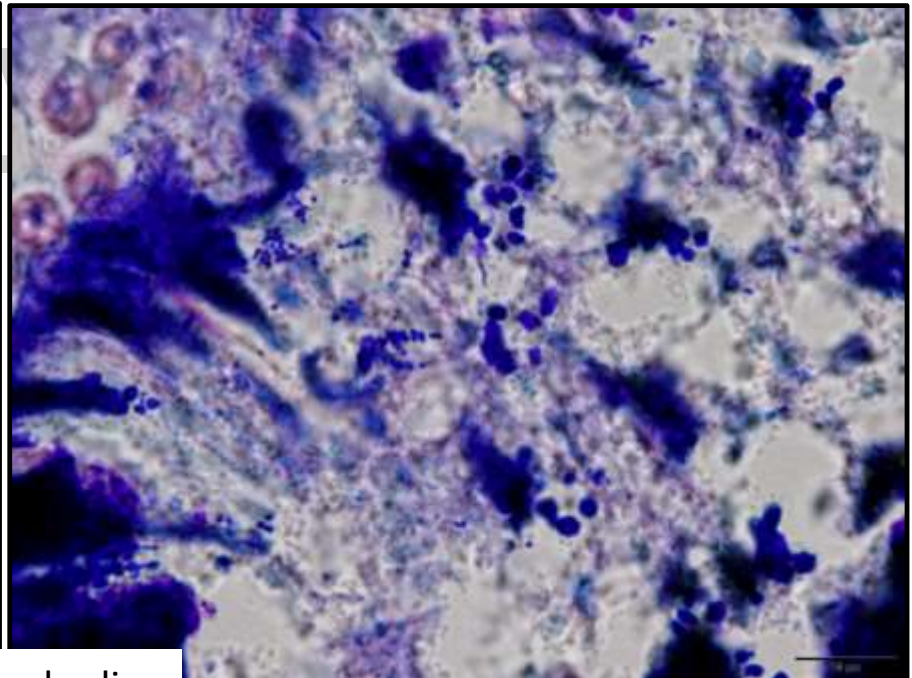
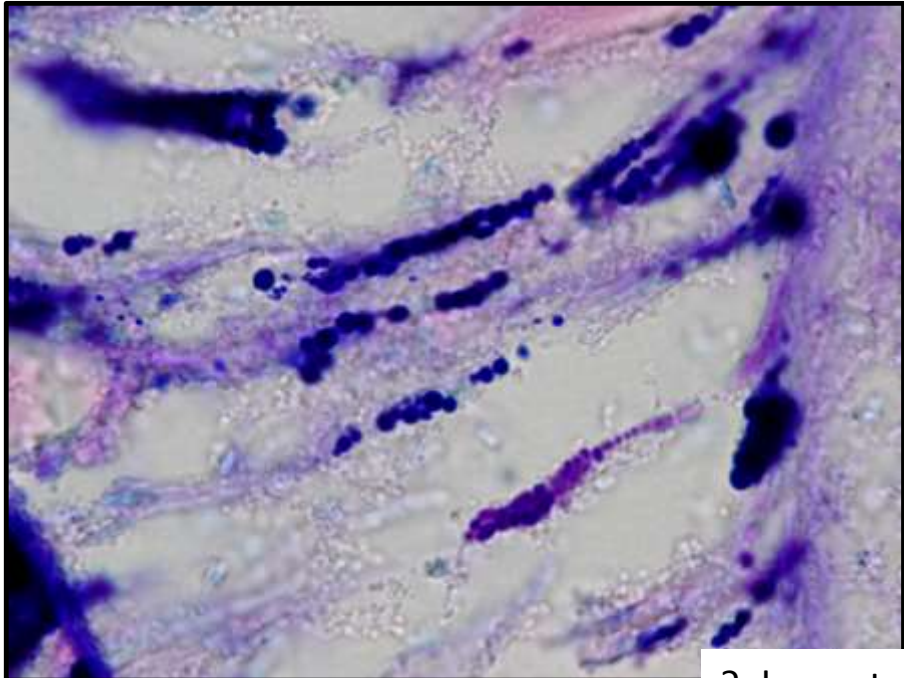


?elementary bodies

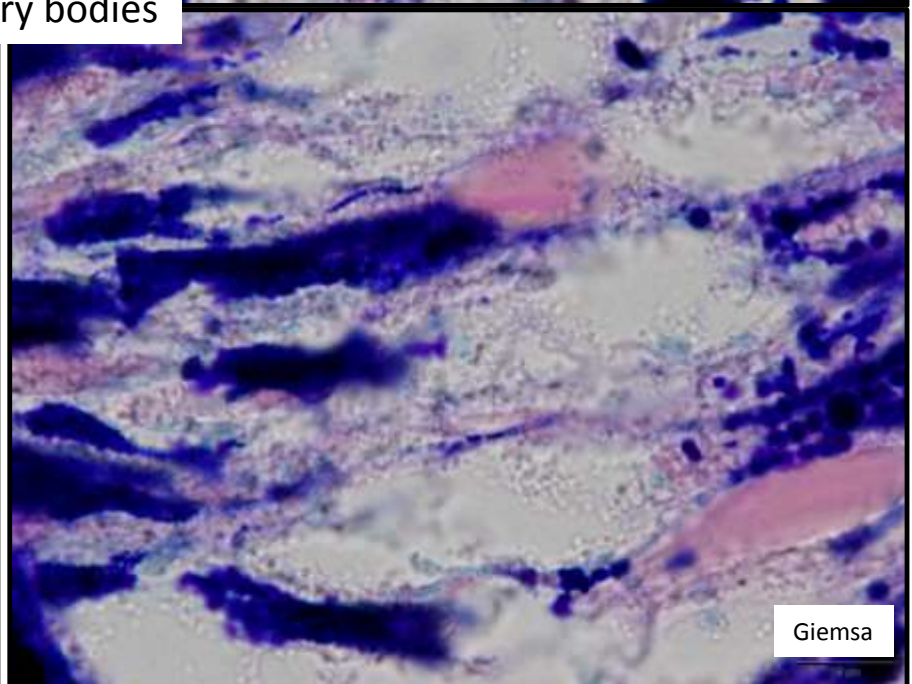
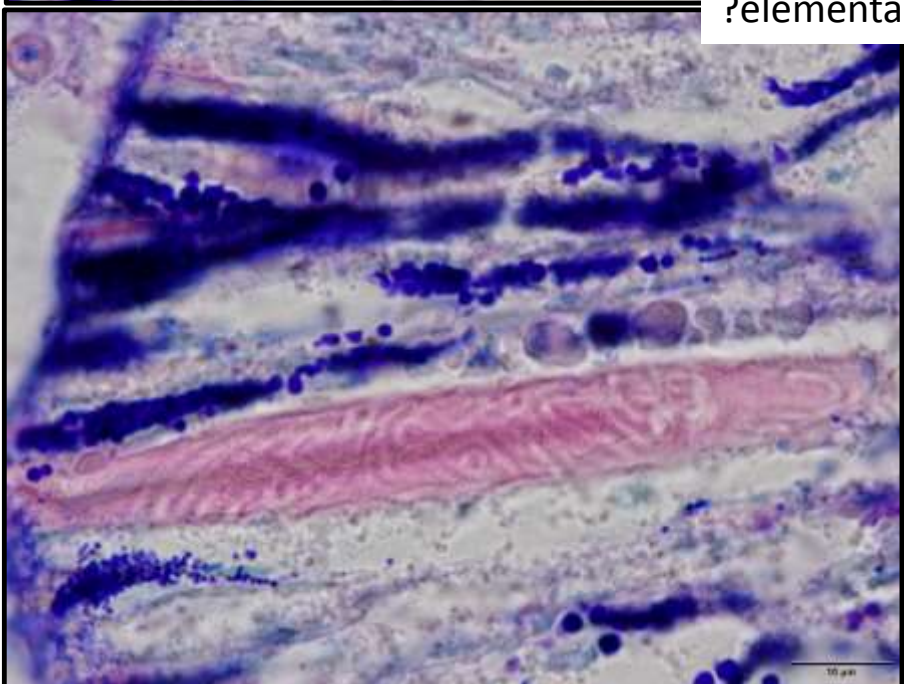
Giemsa

50 µm

EFAST #5, *Eusmilia fastigiata*, 100215, BH Mit-Boulders

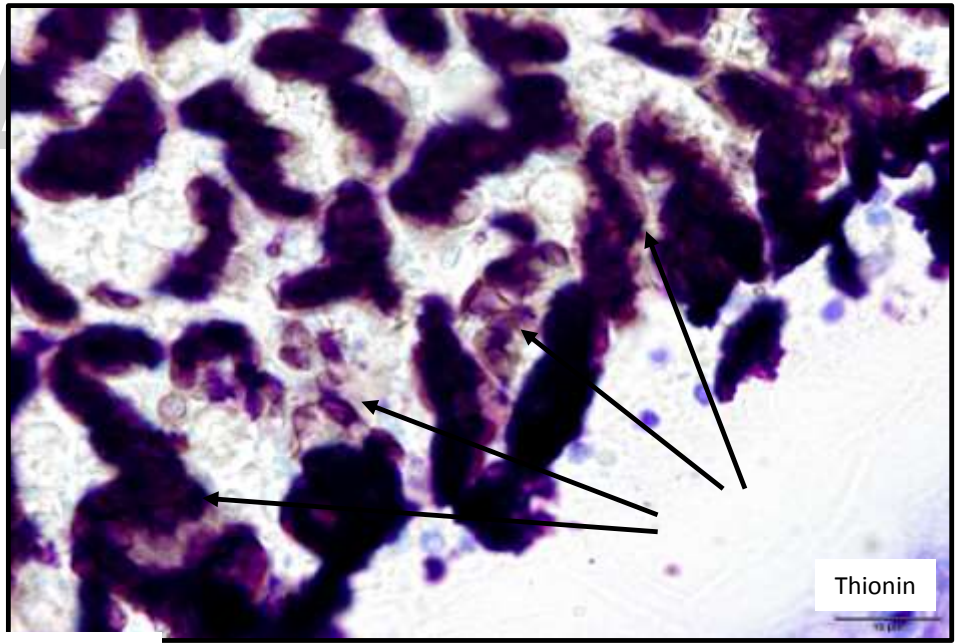
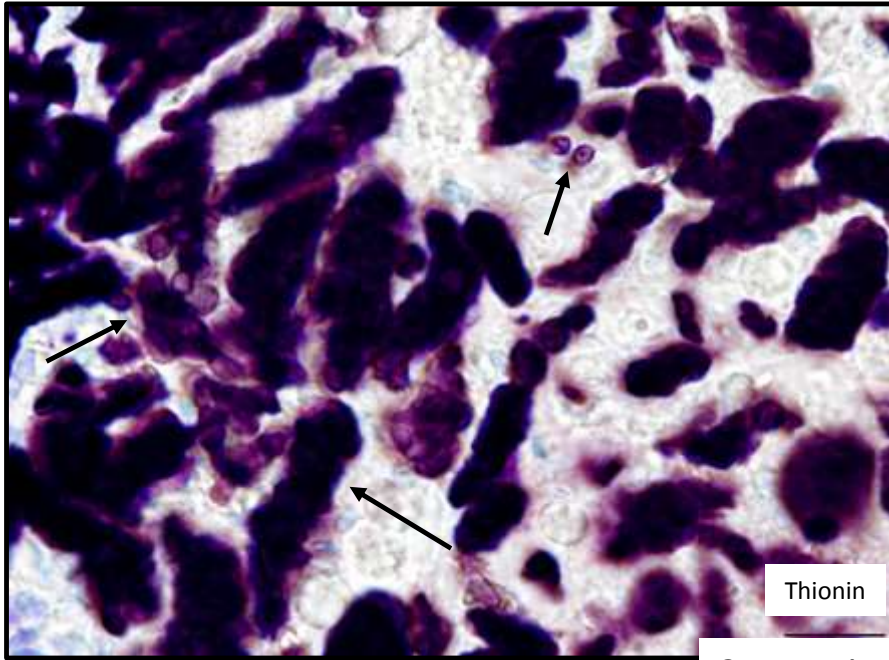


?elementary bodies

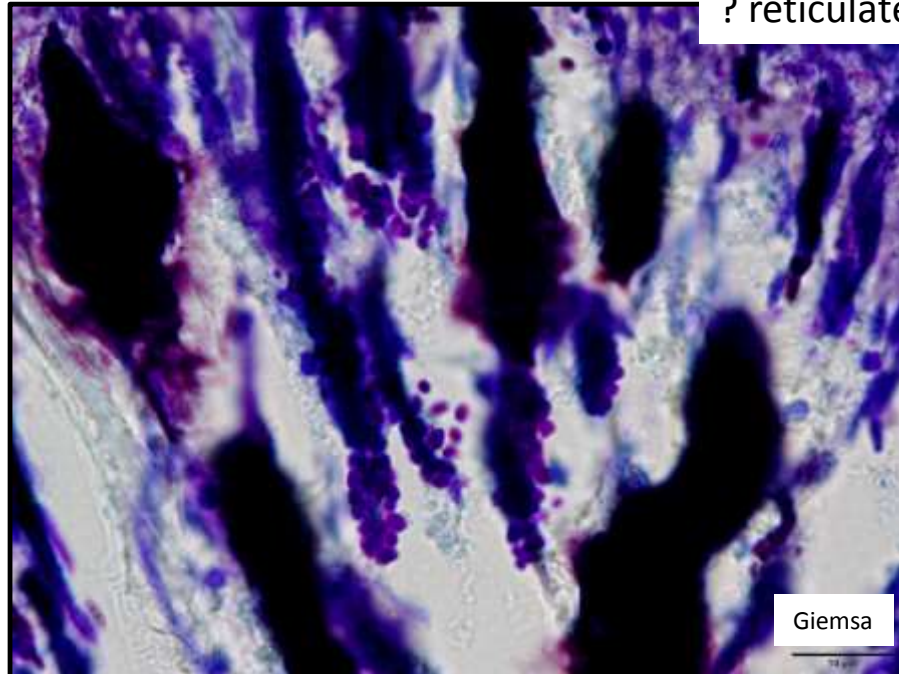


Giemsa

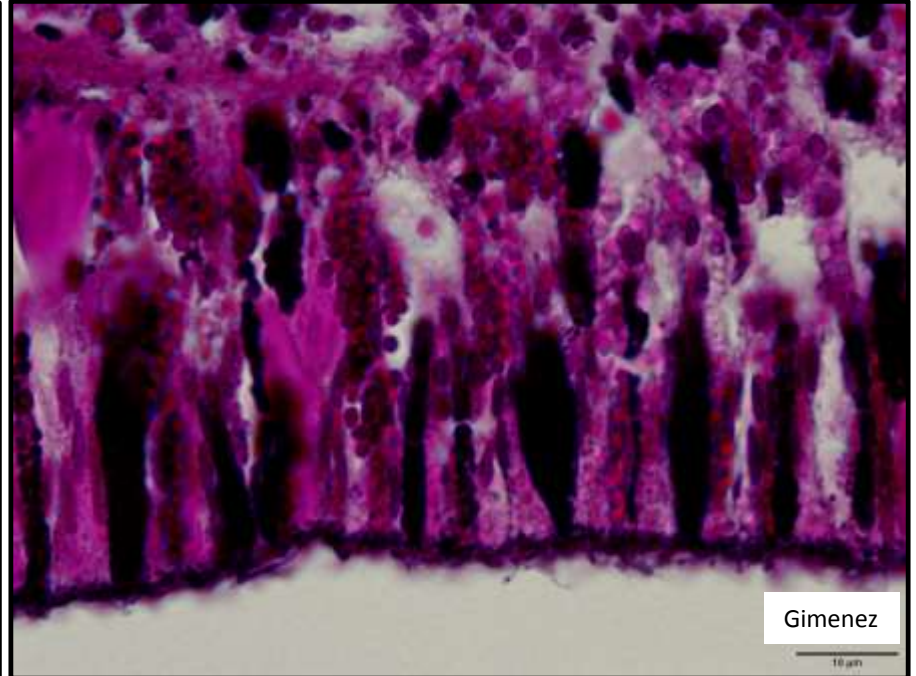
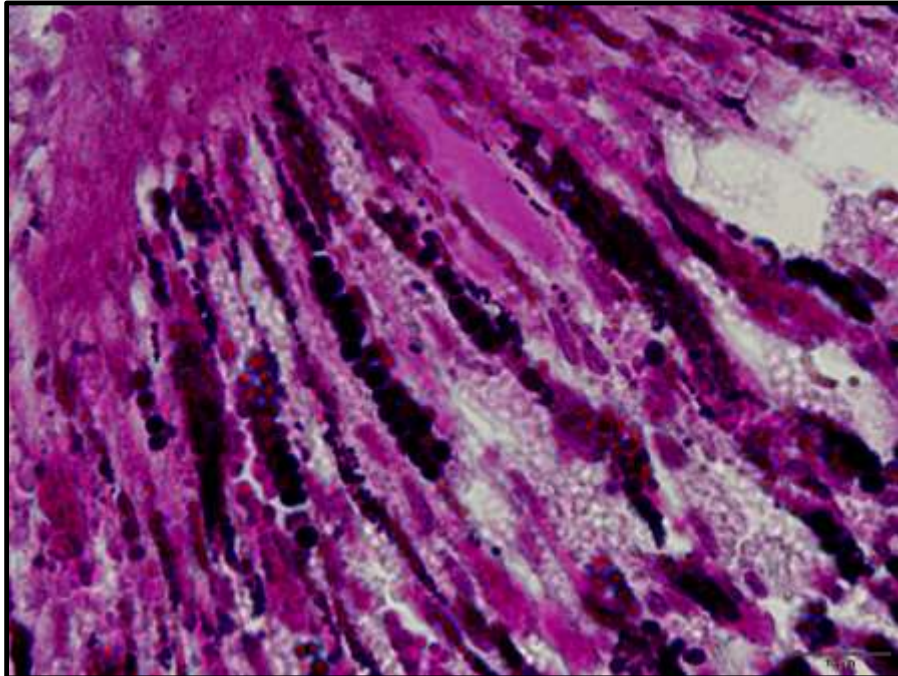
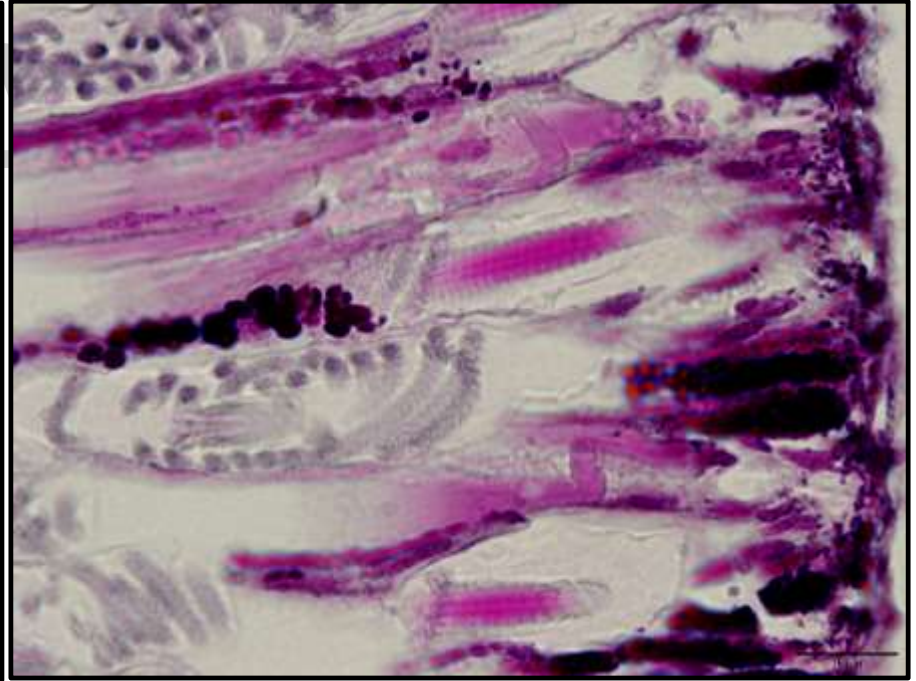
EFAST #5, *Eusmilia fastigiata*, 100215, BH Mit-Boulders



? reticulate bodies

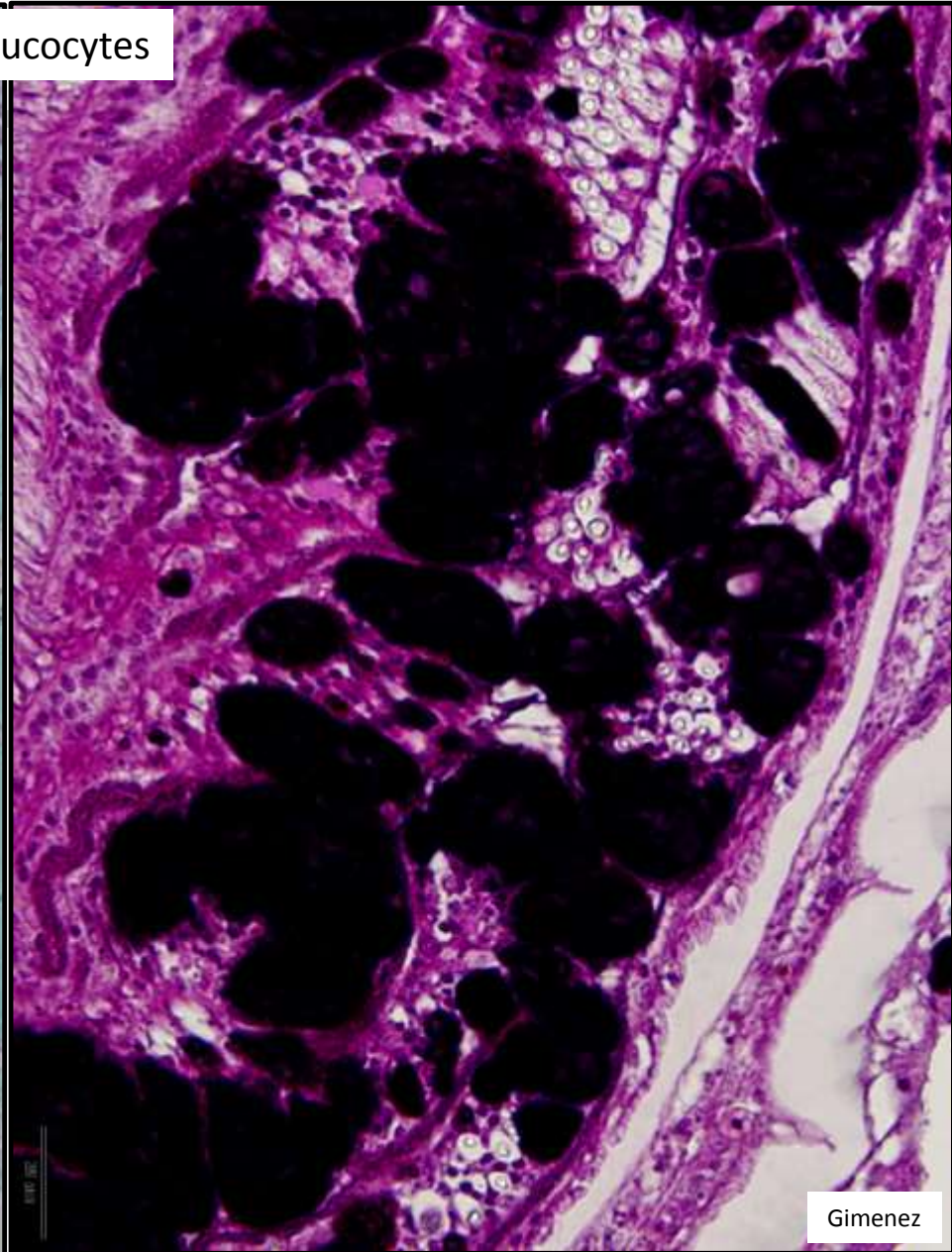
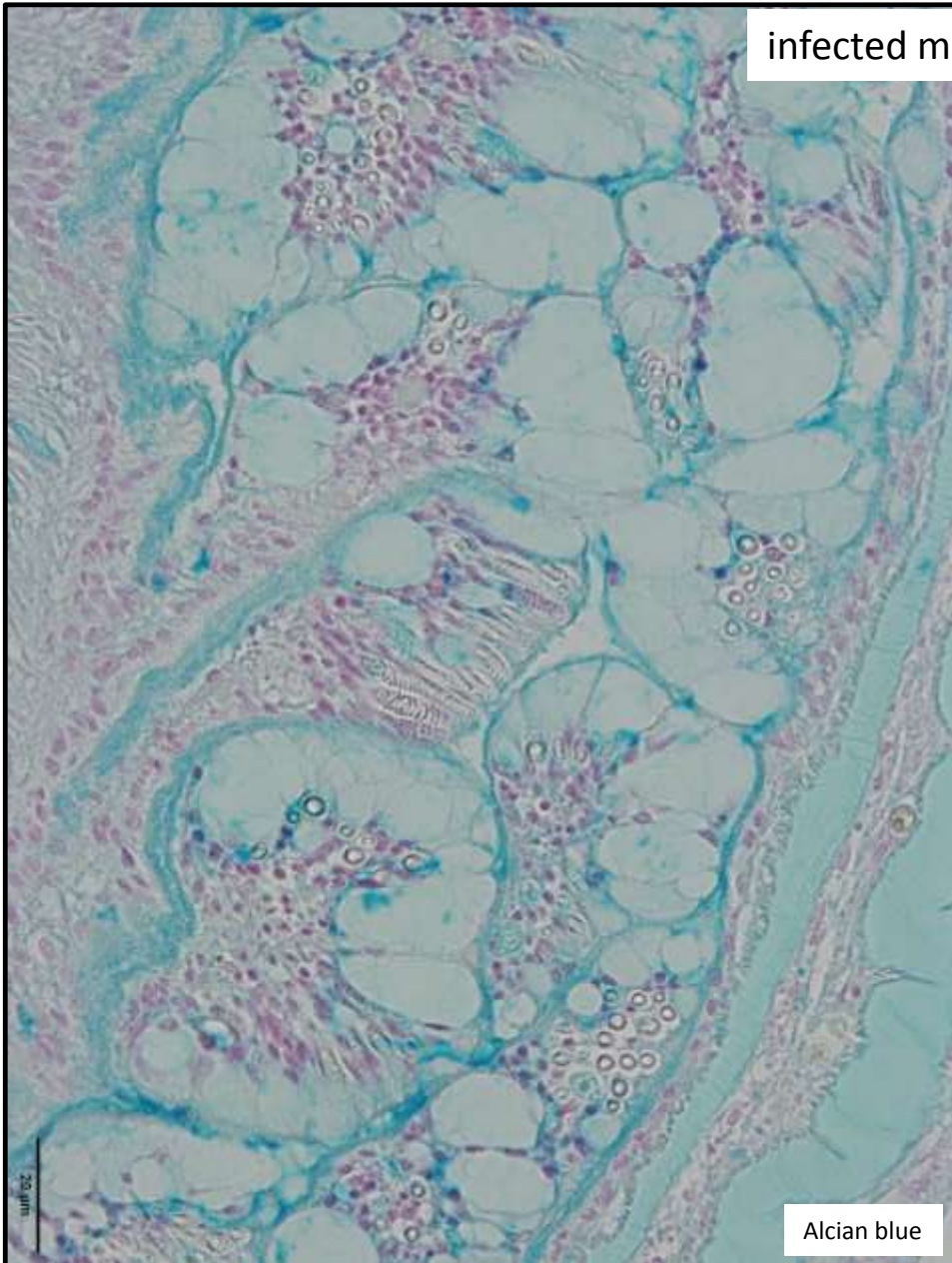


EFAST #5, *Eusmilia fastigiata*, 100215, BH Mit-Boulders



EFAST #5, *Eusmilia fastigiata*, 100215, BH Mit-Boulders

infected mucocytes

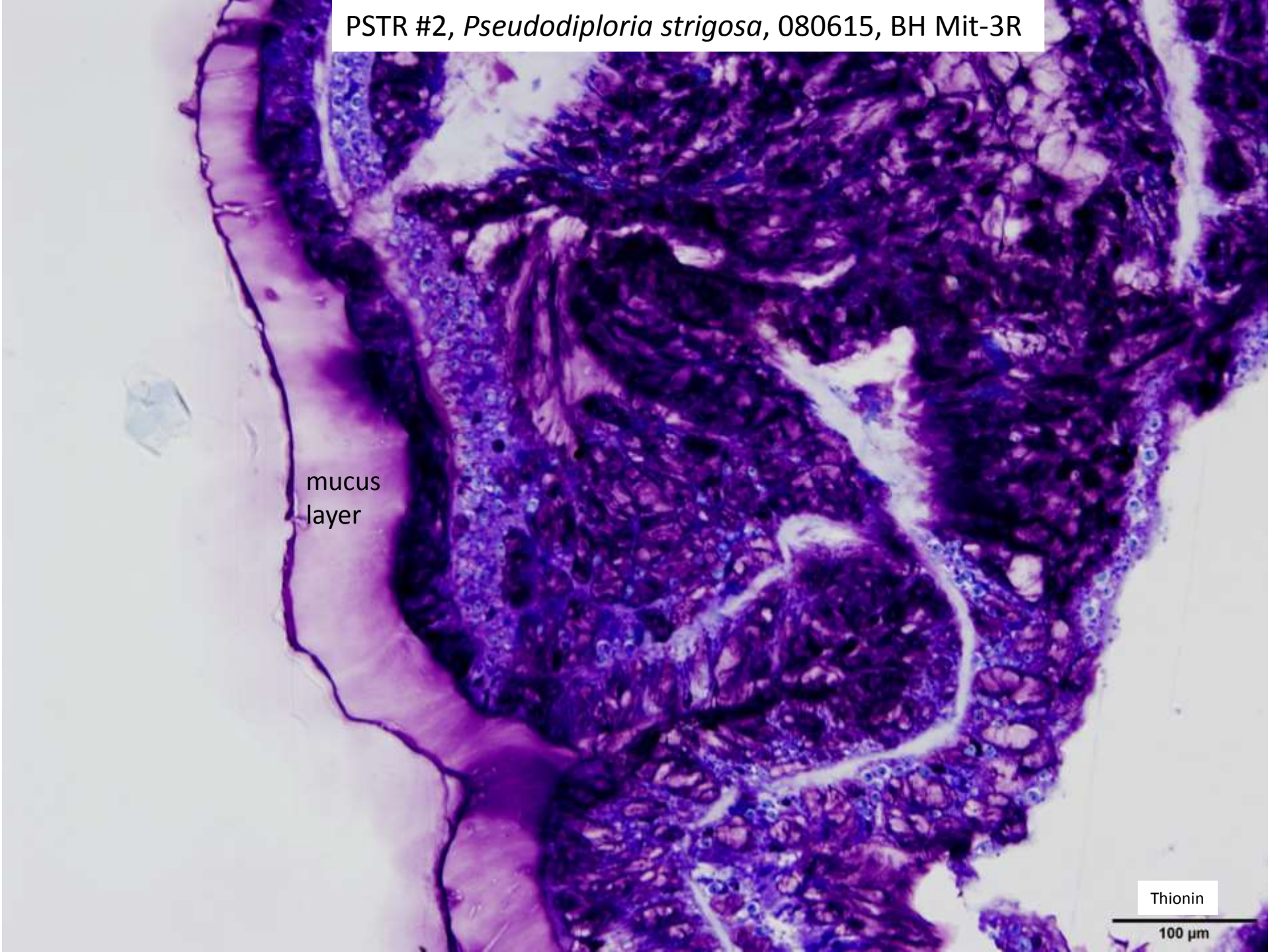


PSTR #2, *Pseudodiploria strigosa*, 080615, BH Mit-3R

mucus
layer

Thionin

100 μ m



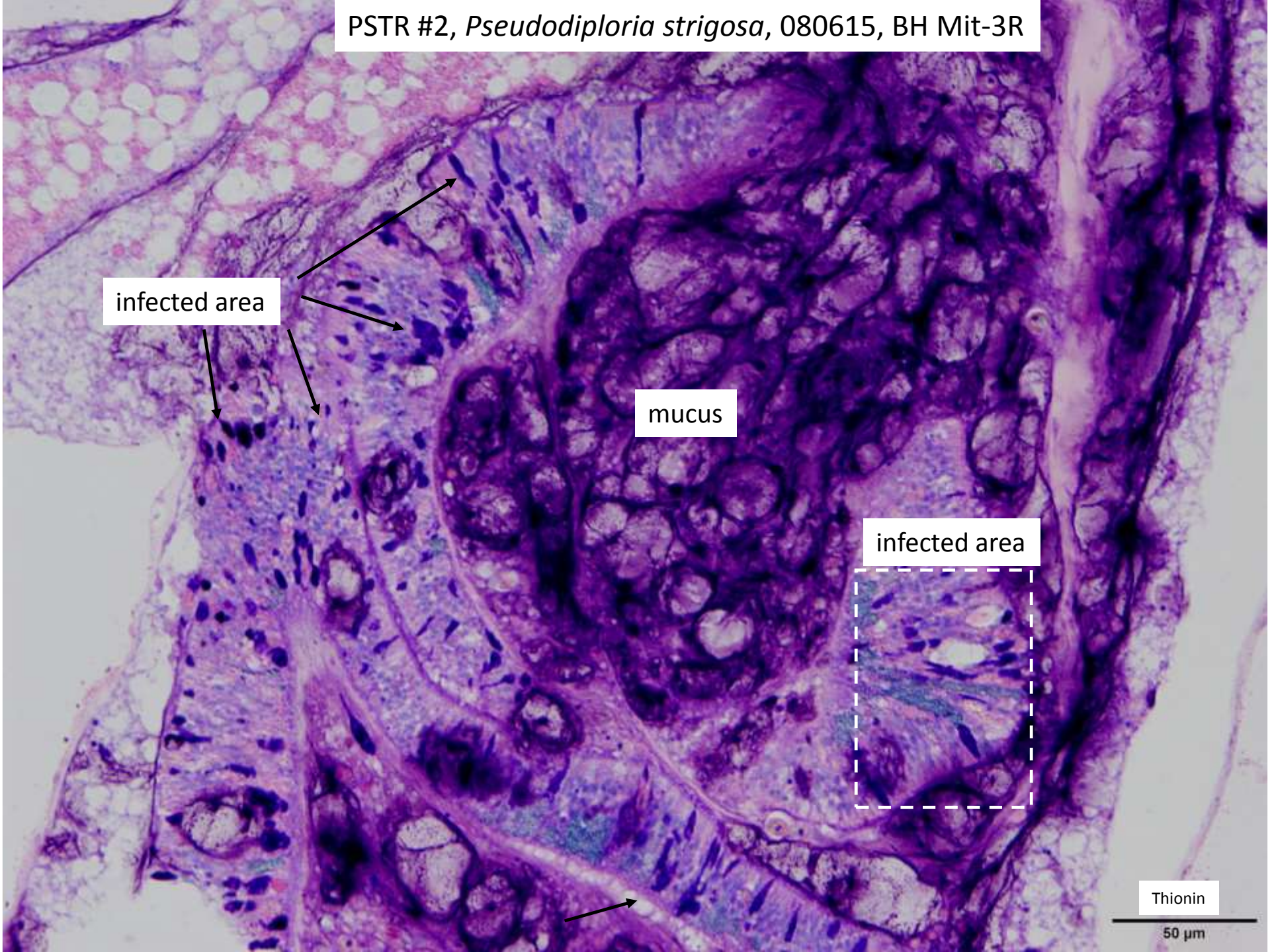
infected area

mucus

infected area

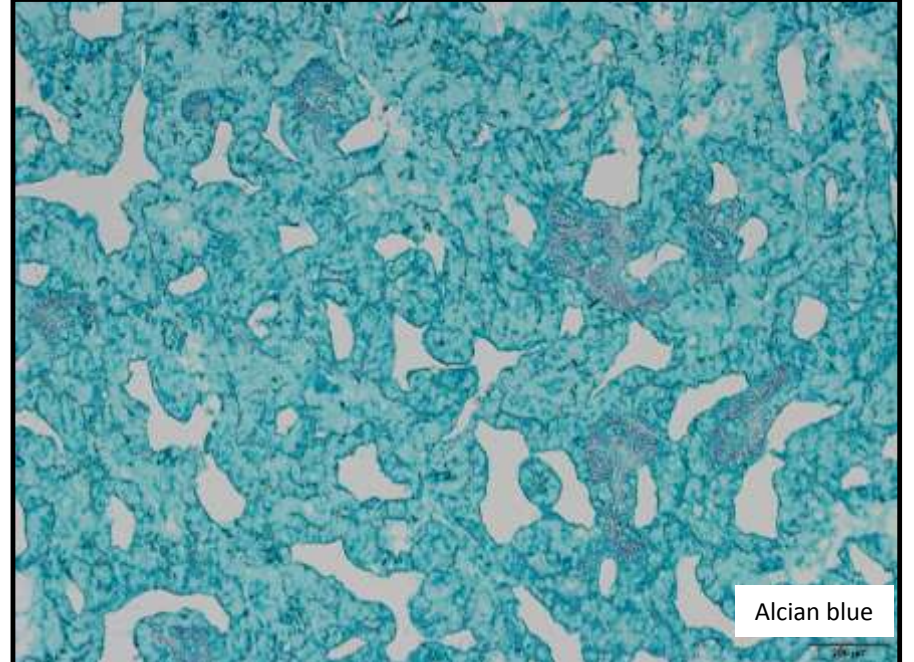
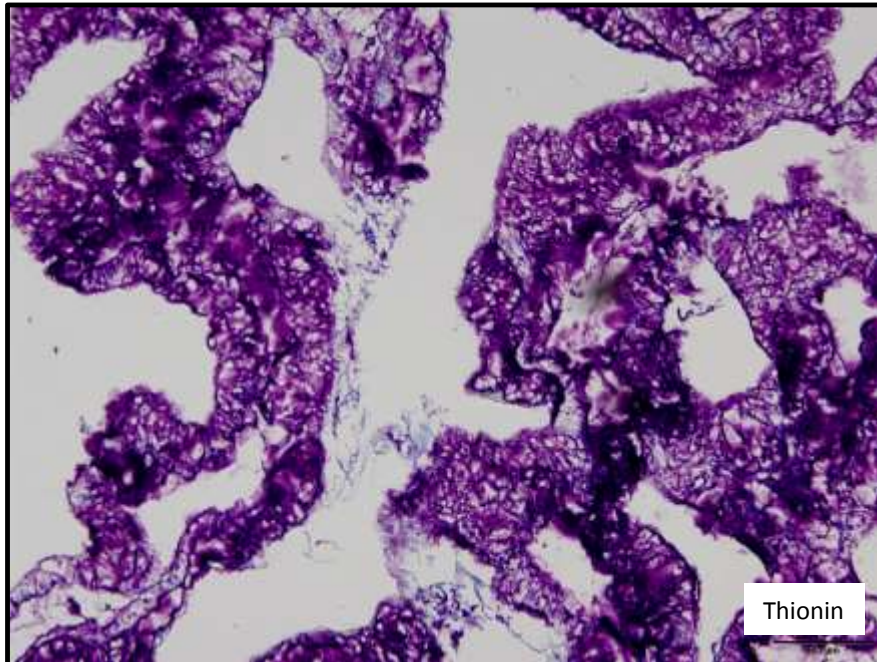
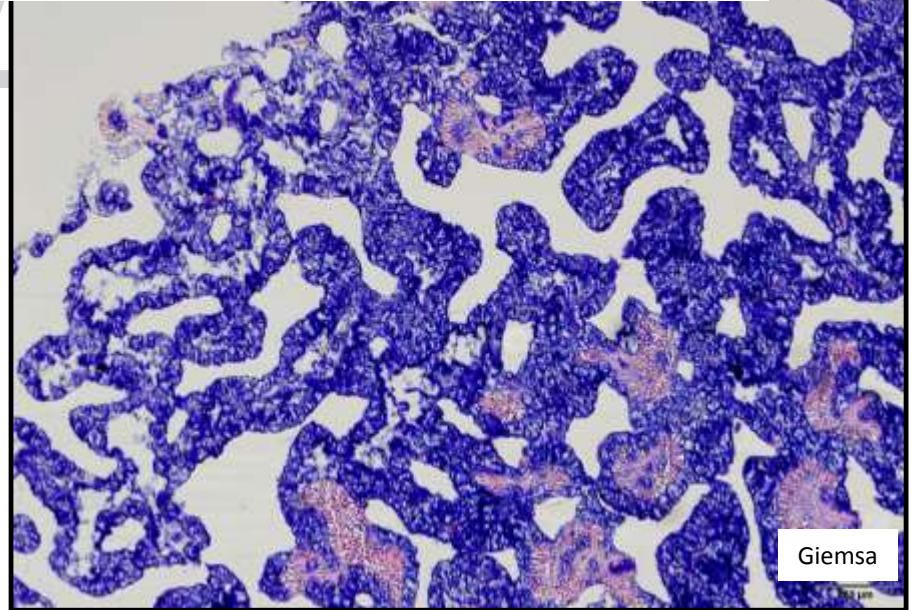
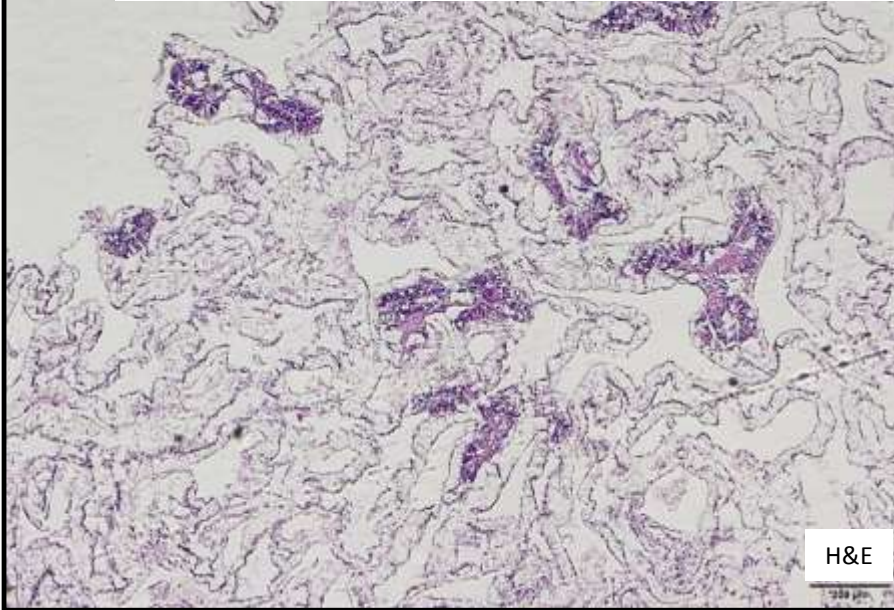
Thionin

50 μ m



PAST#3, *Porites astreoides*, 091115, BH Mit-3R

Necrosis, masses of mucus, less common to rare RLOs with background level in cnidoglandular band



Summary

DRAFT

Field obs.	Species	Pathology	?RLOs*	Date	Location	Collector	Org
BBD	MCAV	active infection (AI)	+++	090415	FTL 5	K. Bohnsack	FDEP
WPL	MCAV	necrosis, +mucus, AI	+++	080615	BH Mit-3R	S. Thanner	MDC
WPL	MMEA	AI	+++	090415	FTL 5	K. Bohnsack	FDEP
WPL	MMEA	AI	+++	100215	BH Mit-Boulders	S. Thanner	MDC
WPL	EFAST	older infection (OI?)	+++	100215	BH Mit-Boulders	S. Thanner	MDC
WPL	PSTR	++mucus, OI?	++	080615	BH Mit-3R	S. Thanner	MDC
other	PAST	++++mucus, necrosis	rare (CGB)	091115	BH Mit-3R	S. Thanner	MDC

* RLO = Rickettsia-like organism. Not quantified, subjective observation only.
Located more commonly in cnidoglandular band (CGB) epithelium and in the epidermis

- Note other pathologies, parasites, pathogens not reported
- 2016 samples in process for histology, special stains, EM, IHC, molecular

A Rickettsiales-like (?) Bacterium is Responsible For The Tissue Loss Diseases Of Caribbean Acroporid Corals



2014

Esther C. Peters, Ph.D.



Department of Environmental Science & Policy, George Mason University, Fairfax, Virginia 22030-4444

The Problem

Staghorn (*Acropora cervicornis*), elkhorn (*A. palmata*), and their hybrid (*A. prolifera*) coral populations in the Caribbean Sea and tropical western Atlantic Ocean have declined greatly since the early 1970s when tissue loss due to white-band disease was first reported (Gladfelter 1982, Weil & Rogers 2011). Only a few studies have used histopathological and microbiological techniques to study the affected corals, despite the importance of doing so to confirm the links between suspected microbial pathogens and tissue damage (e.g., Bythell et al. 2002, Work et al. 2008, Williams et al. 2011).

This report presents progress on a histopathology study based on recent sample collections and archived tissues and histologies to try to improve our understanding of the tissue loss disease(s).

Four categories of acute to subacute tissue loss (focal, multifocal, or diffuse), not associated with predation by corallivores, have been identified on Caribbean acroporids, based on rate and pattern of tissue loss:

- White-band disease type I (WBD-I) with sloughing or straight tissue loss margin (Fig. 1A), reviewed in Bythell et al. (2008).
- White-band disease type II (WBD-II) with bleaching at tissue loss margin (Fig. 1B), reviewed in Ritchie and Smith (1998).
- White pox or white patch disease (WPD) (Fig. 1C), reviewed in Patterson-Sutherland & Ritchie (2004), and
- Rapid tissue loss (RTL) (Fig. 1D), Williams & Miller (2005).

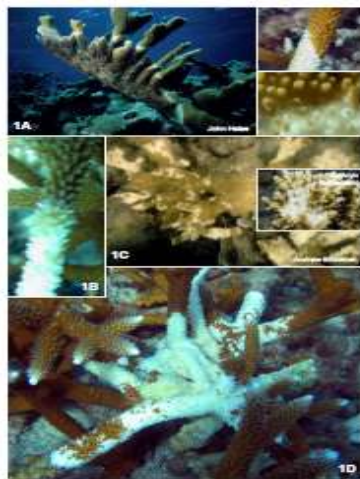


Fig. 1. A, WBD-I on *A. palmata*, Grecian Rocks, Florida Keys, upper right straight margin on *A. cervicornis*, Broward County, Florida, bottom right sloughing tissue on *A. palmata*, Tague Bay, St. Croix, US Virgin Islands. B, WBD-II on *A. cervicornis*, Broward County, Florida. C, WPD on *A. palmata*, Puerto Rico, Inad, WFO on *A. palmata*, Loose Key, Florida. D, RTL on *A. cervicornis*, Broward County, Florida.

Identifying the Pathogen

To restore lost acroporid populations, coral nurseries are culturing branch fragments and replanting them on reefs. For this process to be successful, we need to know what causes the tissue loss and whether and how it can be controlled. These studies have reported finding a microorganism that might be the pathogen causing the tissue loss:

- WBD-I bacterial aggregates by histopathology (Peters et al. 1982, not seen in Bythell et al. 2002), identified by Pufson (2007) by molecular analysis as Gram-negative *Pseudomonas* spp. Found in apparently healthy samples and not always found in diseased samples.
- WPD: Gram-negative *Serratia marcescens* by microbiology. Koch's postulates satisfied (Sutherland et al. 2011), only found in *A. palmata*.
- WBD-II: Gram-negative *Vibrio cholerae/harveyi* by microbiology (Ritchie & Smith 1998). Koch's postulates almost all satisfied, incomplete (Gil-Adgado et al. 2006).
- WBD-I: Gram-negative *Rickettsiales*-like bacterium by molecular analysis (90% similarity to unclassified Rickettsia) with BLASTn based on cloning and sequencing of bacterial 16S rDNA (Cassas et al. 2004). Also in apparently healthy staghorn corals and in other coral species, conclusion: not the pathogen of WBD-I.

Should the Rickettsia-like organism (RLO) be dismissed? RLOs are obligate intracellular parasites and known pathogens of many marine organisms. Could they be harmful to corals without causing gross disease signs?

Materials and Methods

Freshly collected and archived preserved specimens of *A. cervicornis*, *A. palmata*, and *A. prolifera* obtained from sites throughout the region. All specimens immersed in a formaldehyde-based fixative, decalcified, paraffin-embedded, and sectioned (Peters et al. 2007). Tissues stained with hematoxylin and eosin (H&E) for examination by light microscopy to detect pathological changes in the tissues. Additional sections stained with a Giemsa method, which is used to detect rickettsia and other Gram-negative bacteria.

Conclusions

Typical western Atlantic acroporid corals have been infected since the 1970s with an obligate intracellular bacterium that has affinities to the primitive bacterial groups *Rickettsia* (molecular) or *Chlamydia* (morphology) (e.g., Fryer & Lammann 1994). The microorganism infects polyp mucocytes, proliferates, and kills them. Additional molecular and ultrastructural studies to determine its identity are in progress.

Koch's Postulates require modifications for determining the pathogenicity of nonculturable microorganisms and other stressors (USEPA 2000). Experimental exposures to *Serratia marcescens* (Patterson et al. 2004) and *Vibrio cholerae* (Gil-Adgado et al. 2006) resulted in tissue loss, but coral condition was not determined by histology. The suspect RLO/CLO is the primary pathogen, continuously killing cells. If the coral cannot obtain adequate nutrition to replace these cells and counter vital metabolic functions, it becomes highly susceptible to other agents. Probable secondary pathogens include: other microorganisms, temperature, turbidity, sedimentation stress, or toxicant exposure. Different patterns of tissue loss are probably due to the intensity and duration of infections, and the identity of the secondary stressor(s) that trigger them.

The weakened condition of present populations of acroporids explains the intermittent outbreaks, which may occur during transient exposure to additional stressors but then disappear, and inconsistent transmission of tissue loss. Studies are underway to determine whether larvae are infected, when settled planulae become infected, or if any acroporid genotypes resist infection, and to further explore this challenging enigma.

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Results

Carolyn Friedman (University of Washington) identified these structures (Fig. 2) as the suspect RLOs (WBD-affected *A. cervicornis* collected by David Kline, Bocas del Toro, Panama). More than 30 histological sets prepared since the mid-1970s have been located and many obtained (from International Registry of Coral Pathology, Registry of Tissues in Lower Animals, or collectors). Results of observations to date are in Table 1.



Fig. 2. Suspect RLO in mucocyte.

Table 1. Results of histopathological examinations of acroporid samples from the tropical western Atlantic.	Location Year	Collector	Species	Condition*	RLOs**
Loose Key, Florida	1975	W. Jeep	<i>A. cervicornis</i>	AH	20/22†
Florida Keys (1975)			<i>A. palmata</i>	AH	(91%)
Broward National Park (1975-1981)		W. Jeep	<i>A. palmata</i>	AH	13/13†
Puerto Rico (1982-1995)		A. Szwed	<i>A. palmata</i>	AH	Present
Loose Key, Florida	1982	K. K.	<i>A. cervicornis</i>	AH, WBD	9/9
Loose Key, Florida	1987	B. Peterson	<i>A. palmata</i>	AH, WPD	Present
Broward County (2001-2011)		Angel et al.	<i>A. cervicornis</i>	AH	Present
Florida Reef Tract, Key Largo, Florida	2000	M. Miller	<i>A. palmata</i>	AH, WBD, RTL	Present
Key Largo, Florida	2000	D. Kline	<i>A. cervicornis</i>	AH, WBD	24/24†
Key Largo, FL Keys (2002-07)		N. Fogarty	<i>A. prolifera</i>	AH	41/41
US Virgin Islands	2000	C. Woodley	<i>A. palmata</i>	AH	Present
Guantanamo Bay, Cuba (2009-2011)		C. Woodley	<i>A. palmata</i>	AH	71/71
Key Largo, FL Keys (2011-12)		M. Miller	<i>A. cervicornis</i>	AH, WBD, RTL	45/45†
Belize (2012)		L. Carna	<i>A. cervicornis</i>	AH, WBD	5/8
Puerto Rico (2012)		S. Griffin	<i>A. cervicornis</i>	AH	24/24

* AH = apparently healthy; WBD = white-band disease; WPD = white patch disease; RTL = rapid tissue loss.

** Percentages of suspect RLOs in specimens collected in each sample set. Present = suspect RLOs seen in some of the samples but not all of the samples have been inspected yet. † = 100% when slides in sets to be inspected.

Results, Continued

Tissue sections from acroporid coral samples collected in 1981 from Tague Bay, St. Croix, US Virgin Islands, site of the first WBD-I outbreak were re-examined and the results are presented in Table 2. When first studied, coral histology knowledge was limited and these microorganisms do not always stain well with H&E, possibly also due to variations in fixation and acid decalcification. Giemsa staining is necessary to clearly detect them (Fig. 2), the cells then stain dark reddish-purple.

Table 2. Results of histopathological examinations of most of the samples collected in 1981 from Tague Bay, St. Croix.

Species and Condition	<i>A. cervicornis</i> WBD n = 15†	<i>A. cervicornis</i> Healthy n = 4*	<i>A. palmata</i> WBD n = 53*	<i>A. palmata</i> Healthy n = 14
Bacterial aggregates	1.0	75	100	100
Bacterial aggregates 30x	0.7	1.7	2.0	2.9
Epithelial RLOs	100	100	100	100
Epithelial RLOs 30x	3.0	2.0	4.2	4.7
Mucosal RLOs	100	100	100	100
Mucosal RLOs 30x	2.4	3.0	3.7	4.5

* See Peters et al. (2007) for details of methods and results. † See Peters et al. (2007) for details of methods and results. ‡ See Peters et al. (2007) for details of methods and results. § See Peters et al. (2007) for details of methods and results. ¶ See Peters et al. (2007) for details of methods and results.



Fig. 2. A, H&E-stained section, poor quality, difficult to see RLO-infected cells. B, H&E-stained section, better differentiation of suspect RLOs from mucus. C, Giemsa-stained section, RLOs are dark purple, mucus pale and frothy. All sections from same sample, *A. cervicornis* with RTL (11-174), Conch Shallow, Key Largo, FL, 08/23/2011, collected by E. Peters.

Apical polyps have fewer infected mucocytes compared with lateral polyps. Mucocytes of the oral disc and tentacle epidermis usually have the larger bacterial cells within their cytoplasm. Mucocytes of the lateral actinopharynx epithelium and endodermal bands of the mesenterial filaments have smaller cells, mucocytes of the basal body wall gastrodermis lining gastrovascular canals can also be infected. These cells all stain with Giemsa, but differ in size and shape, suggesting they are more likely members of the *Chlamydiales* forming inclusion bodies and elementary bodies, and not *Rickettsiales* (Fig. 3).

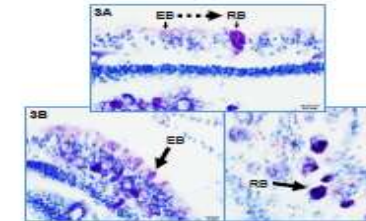


Fig. 3. A, Small ovoid cells similar to *Chlamydia*-like organisms (CLOs), the infective stage elementary bodies (EB), that develop into larger reticulate bodies (RB), as seen in this section of endodermal band epithelium. B, Section of endodermal band epithelium showing more of the small-cell and large-cell bacteria. The pale-pink cells are actinopharyngeal gland cells that secrete enzymes to digest food. C, Section of tentacle epidermis with large bacterial cells, the pale pink structures here are apicoles. All sections from same sample, apparently healthy *A. cervicornis* (11-167), Coral Restoration Foundation nursery, Key Largo, FL, 08/23/2011, collected by E. Peters.

Course offered

"Understanding Corals from the Inside Out: Comparative Histopathology" at Nova Southeastern University Oceanographic Center, May 12-August 1, 2014, 3 graduate credit hours or may be audited, online and one lab week at NSUOC (July 14-18).

Acknowledgements

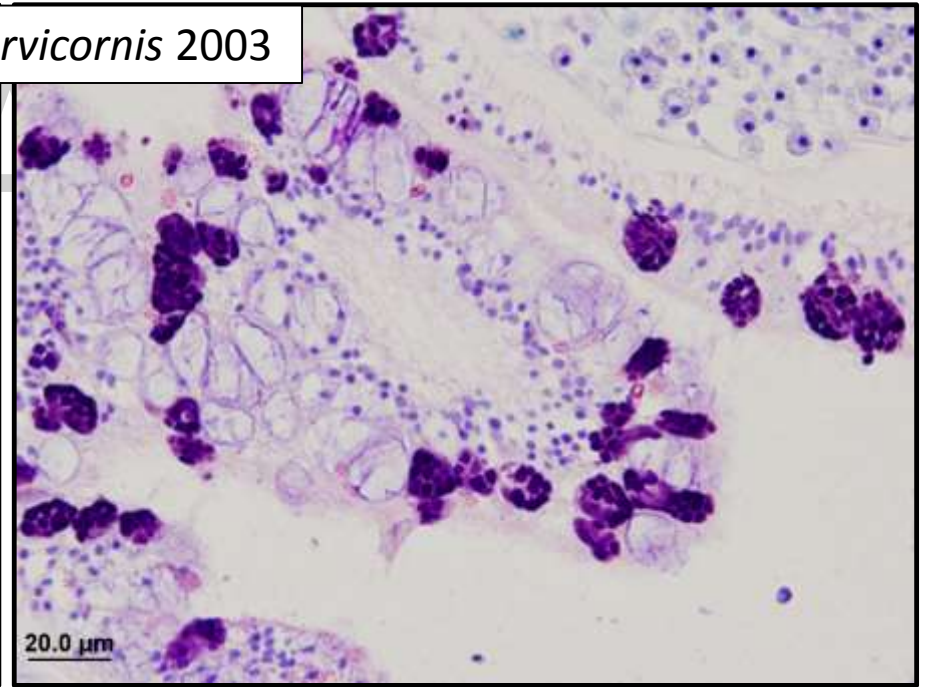
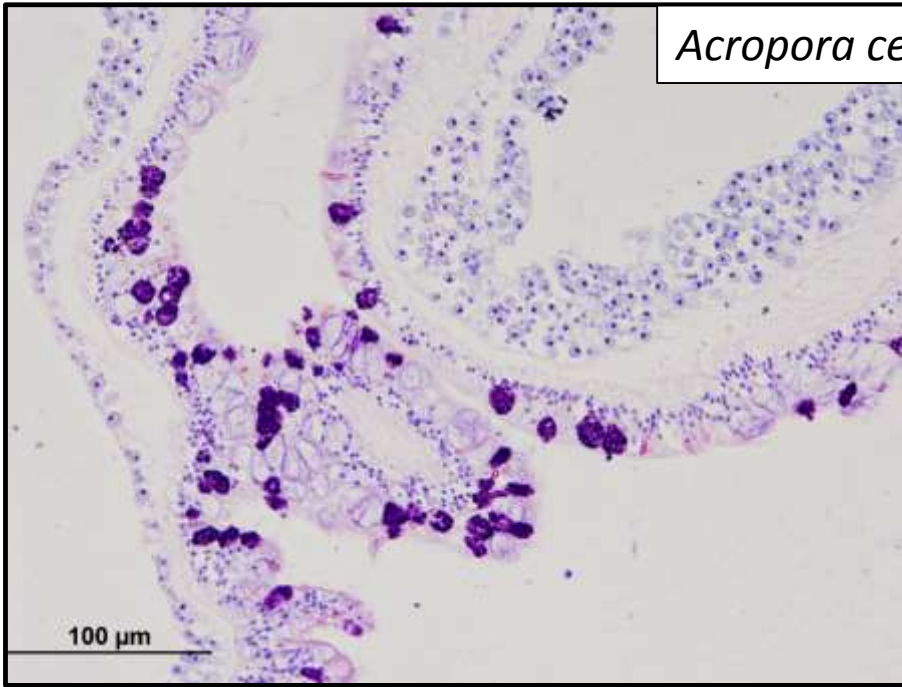
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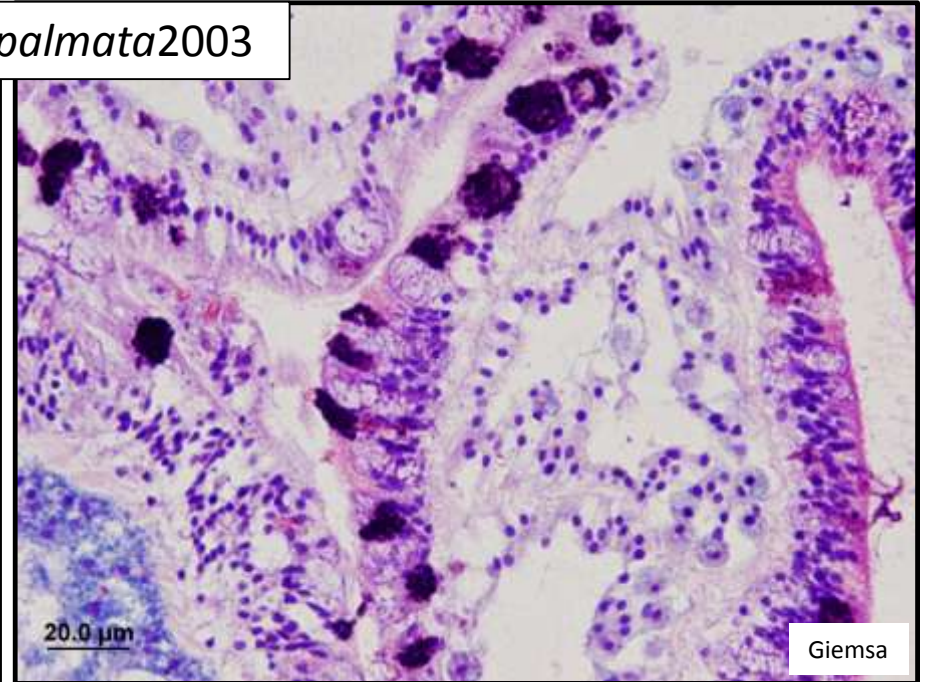
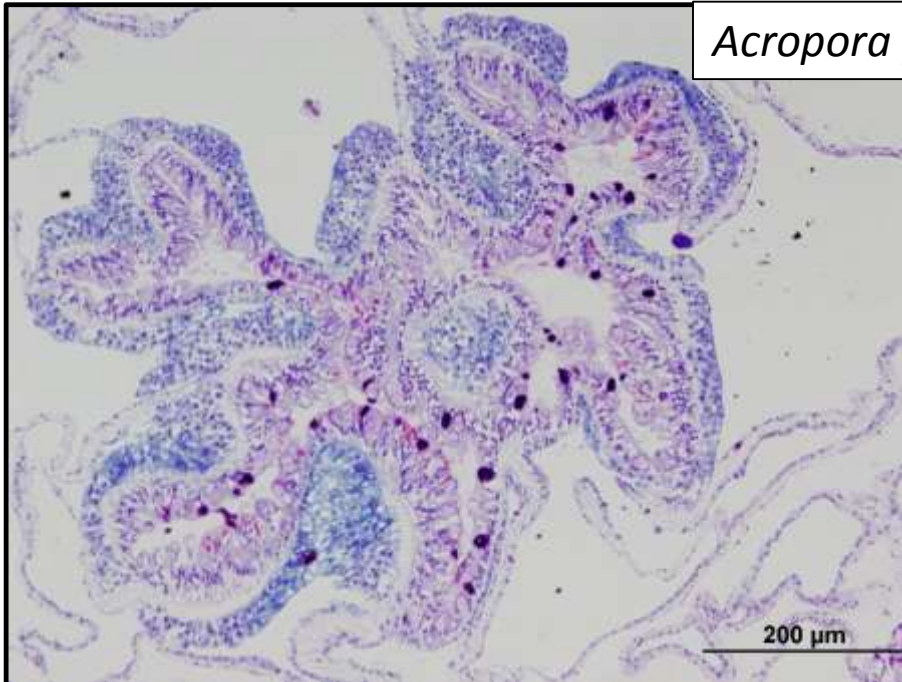
For further information

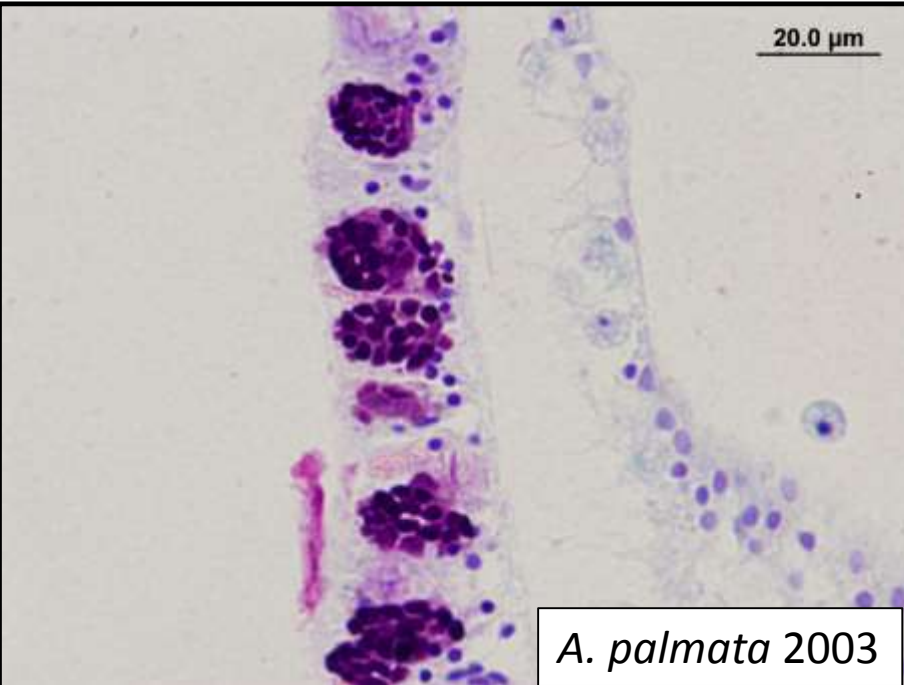
Please contact epeters2@gmsu.edu for more information on this and related projects. Do you know of other collections of formaldehyde-preserved Caribbean acroporid samples? Please let me know!

Acropora cervicornis 2003

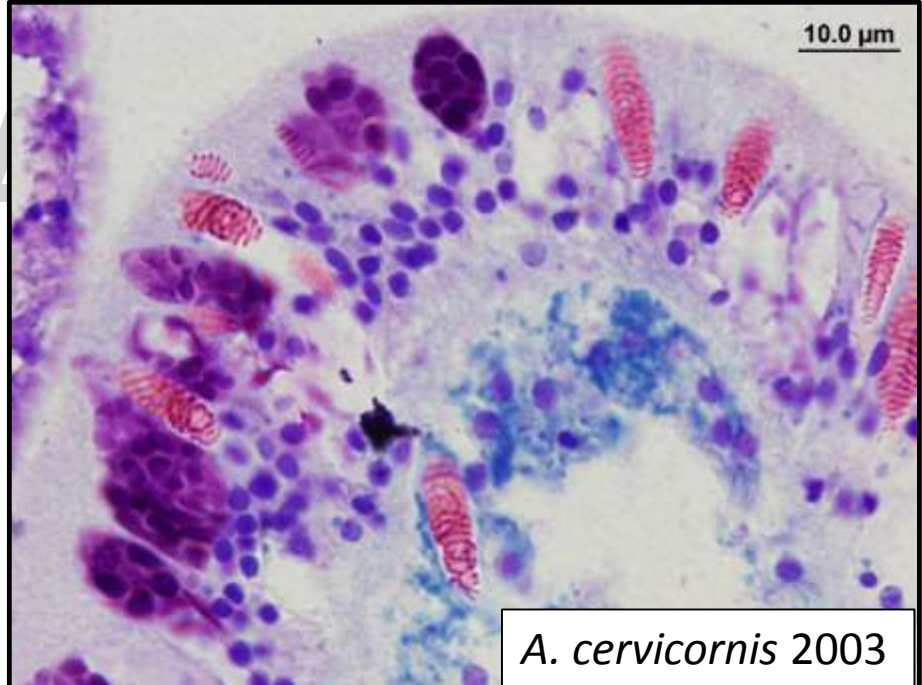


Acropora palmata 2003





A. palmata 2003



A. cervicornis 2003



A. cervicornis 2003

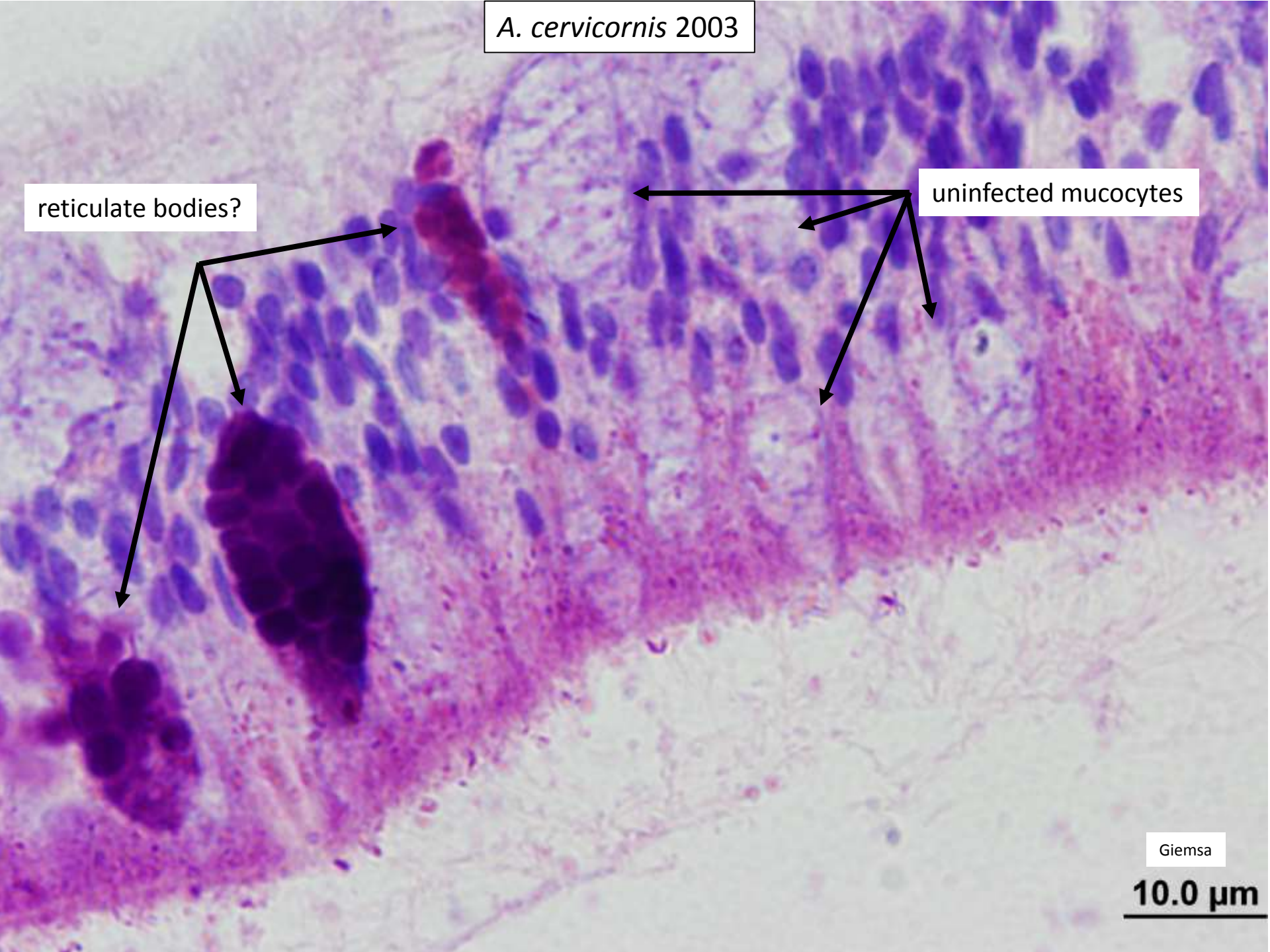


Giemsa

A. cervicornis 2003

reticulate bodies?

uninfected mucocytes



Giemsa

10.0 μ m