Corals and Microscopy.

Laurent Delvoye

Introduction.

The story begins in March 1980. So 40 years ago, as a post-graduate student in Medical Biology, I arrived at the Caribbean Marine Biological Institute – Carmabi for short – in Curacao. I was invited to do histological work on the reproduction of stony corals. They had asked for me, because I had previous experience with that type of research. And probably because I was a little bit older than the average student. For a year I did this with some success, but not without problems. The main problem was, that my mindset as a medical biologist was quite different from that of a field biologist. When I presented my report on the subject in 1982, it got a mixed reception. It described the gametogenesis – development of sperm and egg cells - in a few species of coral in great detail and in it was also noted a correlation between the phases of the Moon and the stages in coral gametogenesis and spawning.

This Moon – gametogenesis correlation was dismissed in the first place, also the detailed description of stages was found interesting but “not necessary”. I eventually got my degree, with specialization in microscopic pathology and I worked in several positions in The Netherlands and Germany. Later on a career switch to education was made, because I wanted a more permanent basis of existence for my family and me. So the last 17 years before retirement I was a biology teacher in high school.

Nowadays, it's all different. From a sideline in the early 1980's, research in coral reproduction has become mainstream. And in every publication the correlation with the Lunar phases is confirmed.

How did this turnaround happen? Well, during my childhood I already lived in Curacao for a couple of years. So I was bitten by the “Caribbean bug” at an early age. And I never gave up the idea of doing coral reef research. So next to my official career I developed a parallel career in coral research. I kept my contacts and in 1988 I got back to Carmabi. With a grant from the Dutch governmental organization Wetenschappelijk Onderzoek in de Tropen (WOTRO), boring algae in coral skeletons were investigated. From then on I was regularly back: For studying coral tissue fluorescence, for a sponge project within a European framework, for studying coral diseases, for coaching and for matters in general concerning the Carmabi institute. At first it was funded with grants, but later I became scientist as a volunteer.

Whenever possible, my wife Annelies accompanied me. Because of her illness and her passing away in 2015 it stopped for some time. But I took it up again in 2018 and for 2020 I was asked to give a course in coral histology for SECORE at Carmabi – nowadays called Carmabi Research Station, as part of the Carmabi Foundation, that has also terrestrial branches. SECORE is an organization devoted to preserve and restore coral reefs. So, despite the pandemic and after my 75th birthday anniversary, I left home in July 2020 to go to Curacao again.

The SECORE Porites Project.

Material and methods.

130 coral samples of *Porites porites* were collected by the SECORE team at five locations along the South coast of Curacao every month from August 2018 until April 2020. Because of technical and organizational problems there are gaps in the collection. They were fixed and stored in the refrigerator in either 4% formalin or 6% glutaraldehyde in seawater. After my arrival, I started decalcification of the samples in 1 N HCl. After rinsing in tap water and neutralizing the acid in 1% sodium bicarbonate overnight, the samples were embedded in paraffin after dehydration through a sequence of graded alcohols and isopropanol. This yielded about 300 paraffin blocks.

The samples were cut in 10 micrometer sections parallel to the surface of the coral with a Leitz rotary microtome using disposable blades. The sections were then mounted with Haupt's adhesive on slides. Staining was done with Krutsay's haematoxylin (7 min) and counter stained in 2% aqueous eosin Y (5 min) and orange-G (10 min) in 96% alcohol. After dehydration through the usual alcohol-isopropanol-xylene series the slides were mounted in Entellan. We shall refer to this procedure as HEO.

Alternatively, also staining protocols with acid fuchsin and alcian blue and Masson's trichrome were used with varying success.

Later, when the need arose for a better fixative, a fixative was formulated with 7.5 grams of zinc nitrate, 100 ml 38% formaldehyde and 900 ml seawater. After fixing for 24 hours, the samples can be post-fixed with 3% potassium bichromate for 3 days in the dark. After thorough rinsing in running tap water the samples are then embedded in the usual way.

The microscopy was done with a Leitz Orthoplan microscope, equipped with NPL Fluotar objectives and a single Zeiss 40x Neofluar. Photography was done with a Canon EOS M10 body on a custom made attachment and a hand-held Samsung Galaxy J3 mobile phone.

Results.

The SECORE team knew in advance that my stay for 99 days at Carmabi was too short to examine all the samples. Therefore, I gave a course in coral histology to two staff members of SECORE and two post graduate students. It was an international class: Canadian, Dutch, French and South-African. I taught them the histological procedures and most important of all: The interpretation of the slides. So they could continue the project after my departure. Because of some technical problem, the majority of the samples were badly preserved when I received them. This posed a problem with the interpretation of the slides, but despite that we succeeded to do so in most cases.

In *Porites porites* only eggs and larvae have been found so far. For educational purposes I also processed other coral species: *Porites astroides*, *Colpophyllia natans* and *Sideastrea siderea*. A few of them I collected myself by means of surfing and scuba diving in front of Carmabi. Only in *Porites astroides* sperm cells were found.

In all those species some intriguing details were noted, as for example the fluorescent particles in the epidermis of *Colpophyllia* and the yellow brown pigment cells in the epidermis of both the *Porites* species.

Discussion.

From the results so far, it seems that *Porites porites* is indeed parthenogenetic. Comparing the DNA of both parent colony and offspring can confirm this. However, it is interesting to find sperm cells in the related species *Porites astroides*. In both species a yellow brown pigment is found in the epidermis above and between the zooxanthellae (symbiotic algae), shortly known as "zoox". It has interesting properties: A high refractive index and it is chemically stable. It is not noticeably degraded by the histological procedure. Also it absorbs blue and UV-light, as is seen when the normal LED-illumination in the microscope is switched to 455 nm or 395 nm LEDs. All this suggest a role in the spreading of light for the photosynthesis in the zoox and protection against harmful UV.

In contrast we see the fluorescent particles in *Colpophyllia*. They also play a role in photosynthesis and protection. In this case it is accomplished by converting blue and UV in photosynthetic active light by means of fluorescence. Two ways of protecting yourself against UV in one group!

The problem of the bad sample preservation urged me to develop a new fixative for corals, that is both reasonably safe and easy to make for the user and will give a good preservation. The best fixatives contain formalin as well as mercury chloride. The latter substance is ruled out because of its toxicity and its environmental impact. When looking at the periodical table of elements, mercury has two other elements on top of it in its column: Cadmium and zinc. Cadmium is not suitable for the same reasons as mercury. So zinc is an option. I found only a few references in the literature for zinc containing fixatives. Therefore, it was an educated guess when I formulated the fixative: 4 grams of zinc chloride per Liter. But only zinc nitrate was locally available, so it was recalculated at 7.5 grams of zinc nitrate per Liter. After all, it are the zinc ions that do the job! The results were better than expected, as shown in the photographs.

At home as well as in the institute a Leitz Orthoplan microscope is at my disposal, both equipped with a similar set of objectives. This gives continuity in work, since results from both can directly be compared. And since 2017 Canon EOS M and M10 camera bodies are used. First fitted on a Leitz Leica photo attachment, later a custom attachment was made. Making photographs cannot be easier: Just touch the display! The exposures in RAW are "developed" in Canon Digital Professional 4 software and adjusted in brightness and contrast with GIMP software. And by chance I found making photographs with my mobile phone behind the eyepiece an easy way to share results with colleagues and students. The technique involves a steady hand and a few tricks, but the results are impressive.

As always I had a great time, it is always rewarding to help others with my experience and skills and the students Valerie, Kelly, Nina and Matthew were very motivated and critical. They will continue the project for the year to come. As for me, I look forward for the next stay, because there is so much work still to be done!

References.

- www.carmabi.org
- www.secore.org
- www.stainsfile.com
-

Comments to the author Laurent Delvoye welcomed, email - laurentdelvoye24 AT gmail DOT com.

Images.

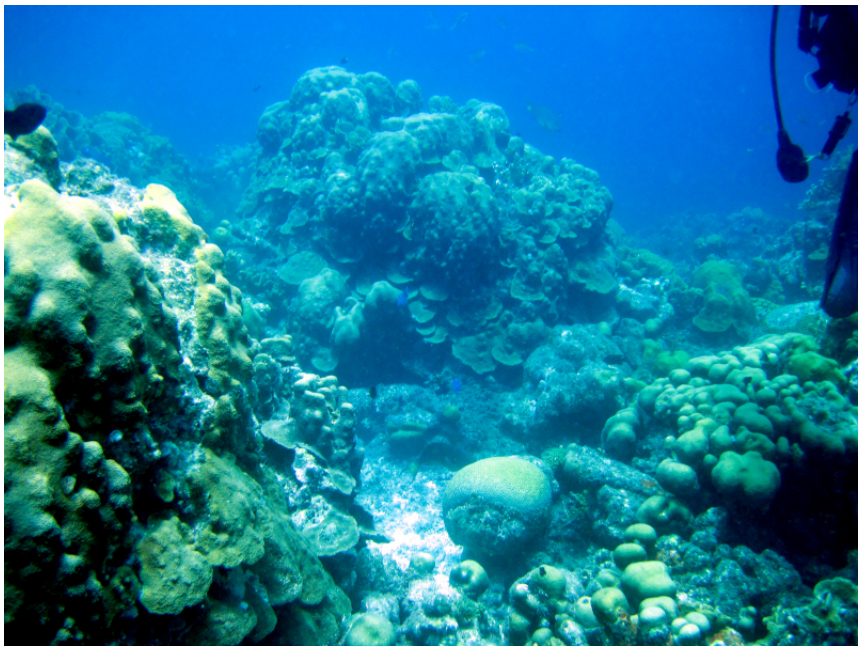

Fig. 1 Typical reef environment in Curacao, between 10 and 25 m depth.

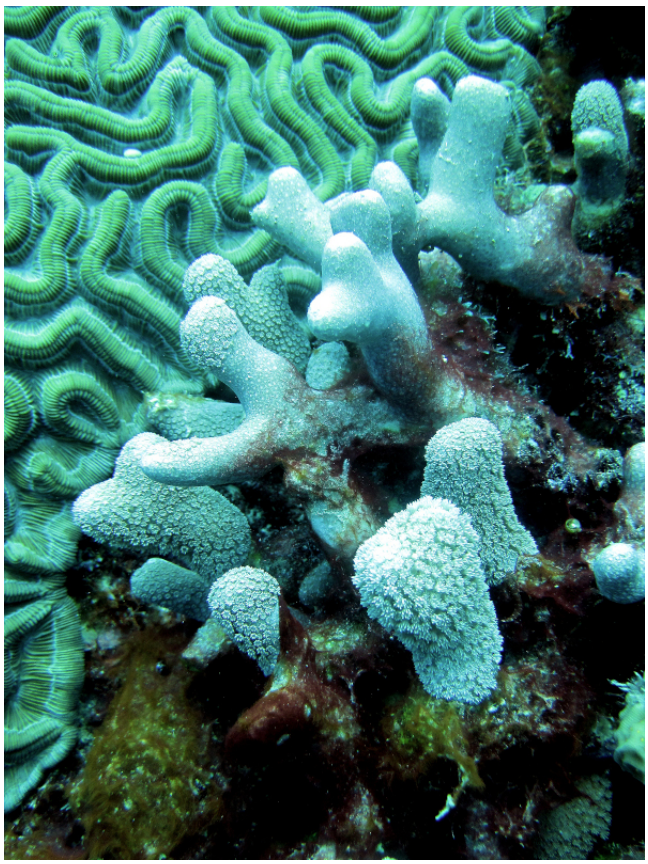

Fig 2. Two coral colonies: Top left *Colpophyllia natans* and *Porites porites*.

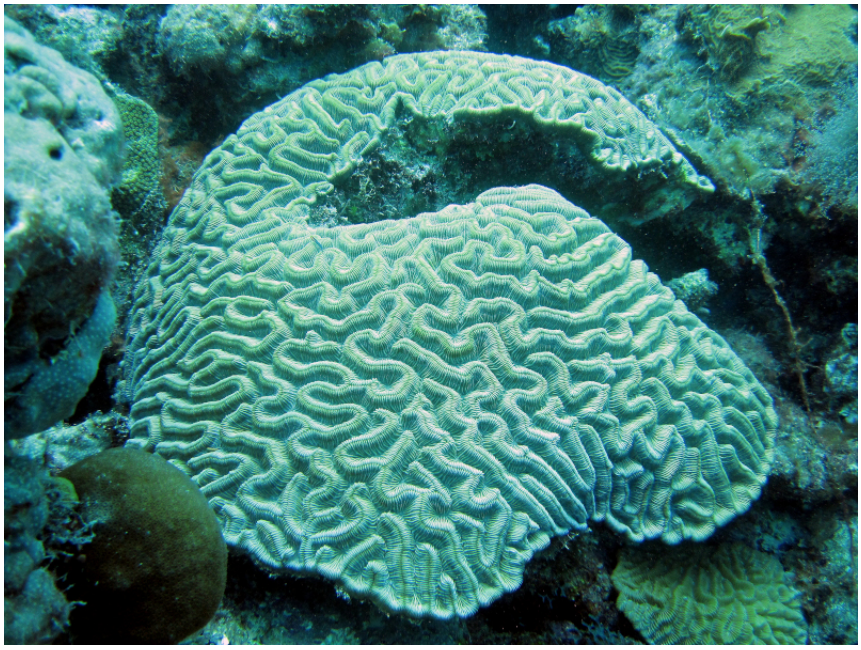

Fig. 3. *Colpophyllia natans*. “Natans” means floating. The dried skeleton is a loose structure, that will float on water because of the air entrapped in the skeleton.

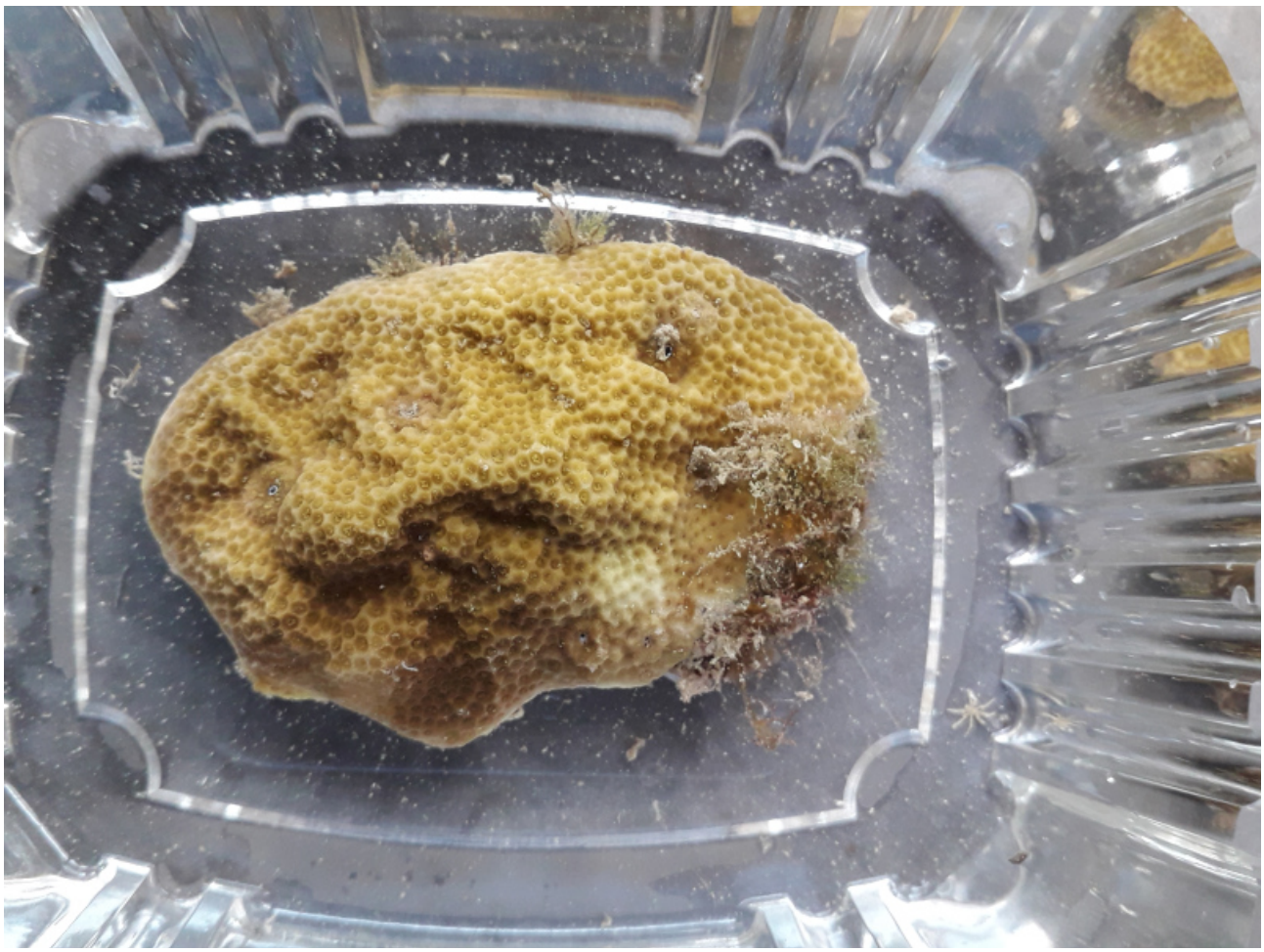

Fig. 4 *Porites astroides*, in the lab after collection. The colony is about 10 cm long. Samsung Galaxy J3 camera.

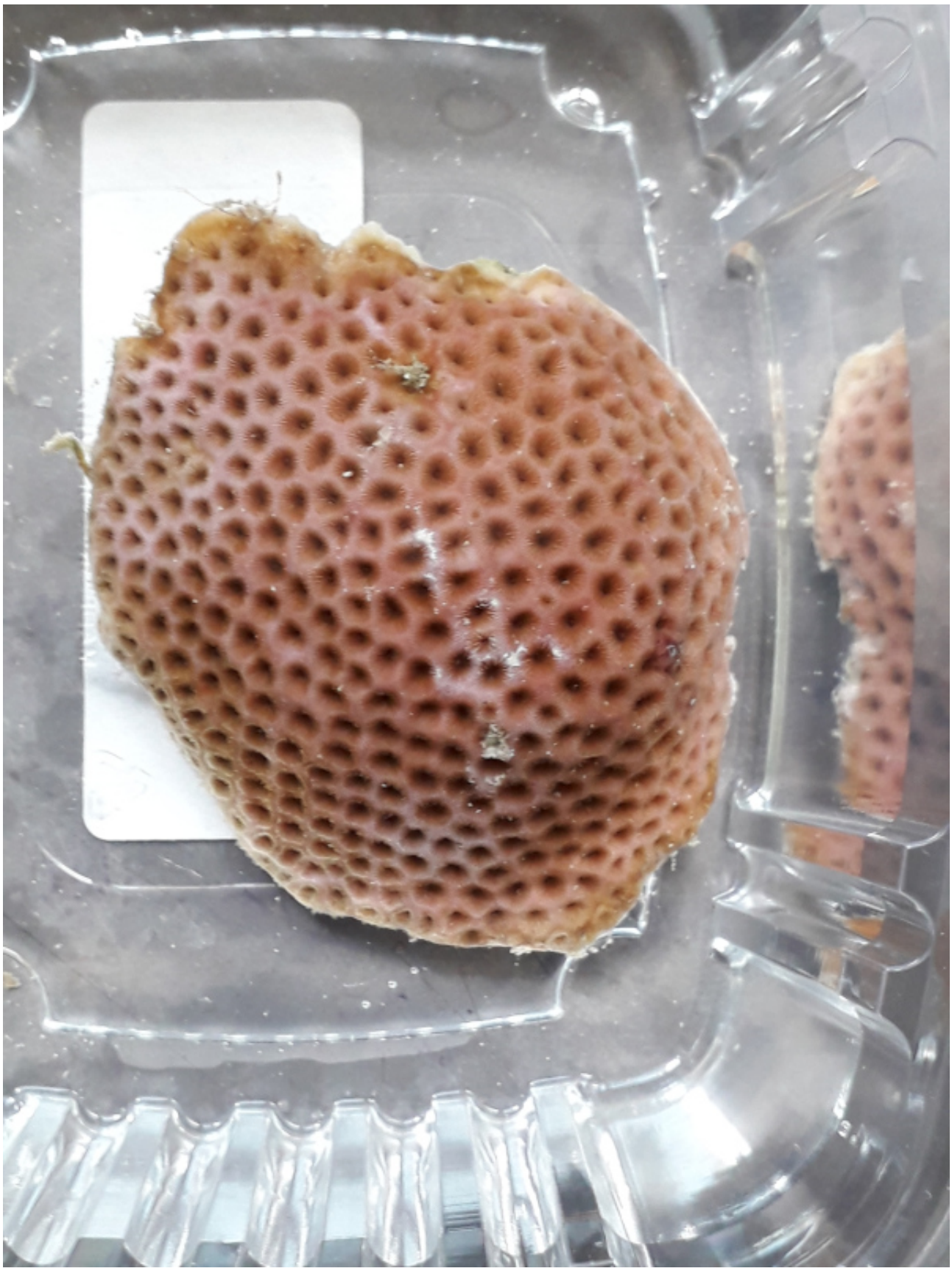

Fig.5 *Sideastrea siderea*. Colony after collection in the lab. Same scale as fig. 4 Samsung Galaxy J3 camera.

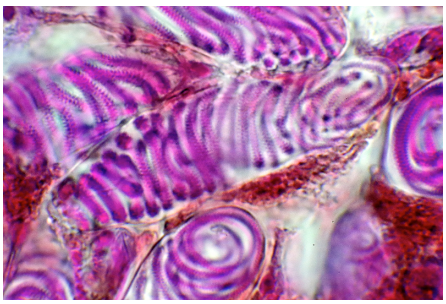

Fig. 6. Nematocysts in the epidermis of *Porites porites*. Planapo 100x oil immersion. Canon EOS M on Leica attachment. These nematocysts are quite large and very distinct. Section parallel to surface of colony.10 micrometers, stained with acid fuchsin and alcian blue.

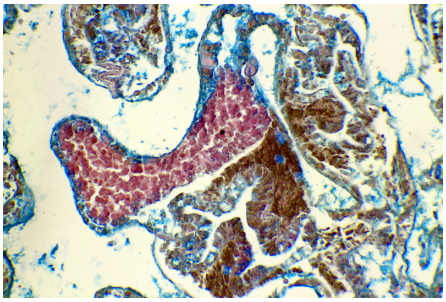

Fig 7. Developing ovum in *Porites porites*, from pilot study. 25 NPL FL objective, section 10 micrometers, acid fuchsin and alcian blue. The yolk particles are stained in red. Same camera setup as in fig. 6. Section parallel to colony surface.

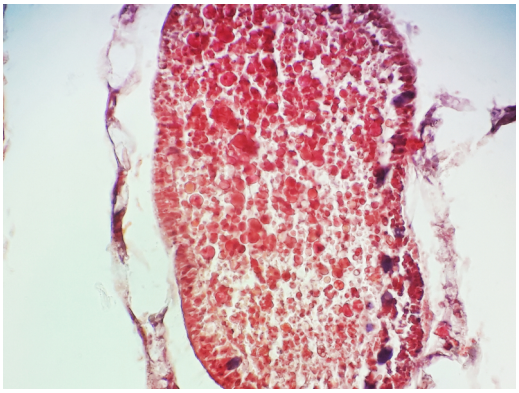

Fig. 8. Young developing larva in *Porites porites*. An epithelium is forming, these are the columnar cells on the outside. A lot of yolk is still present. 25x NPL FL objective, Galaxy J3 camera. 10 micrometer section, HEO stained. Section parallel to colony surface.

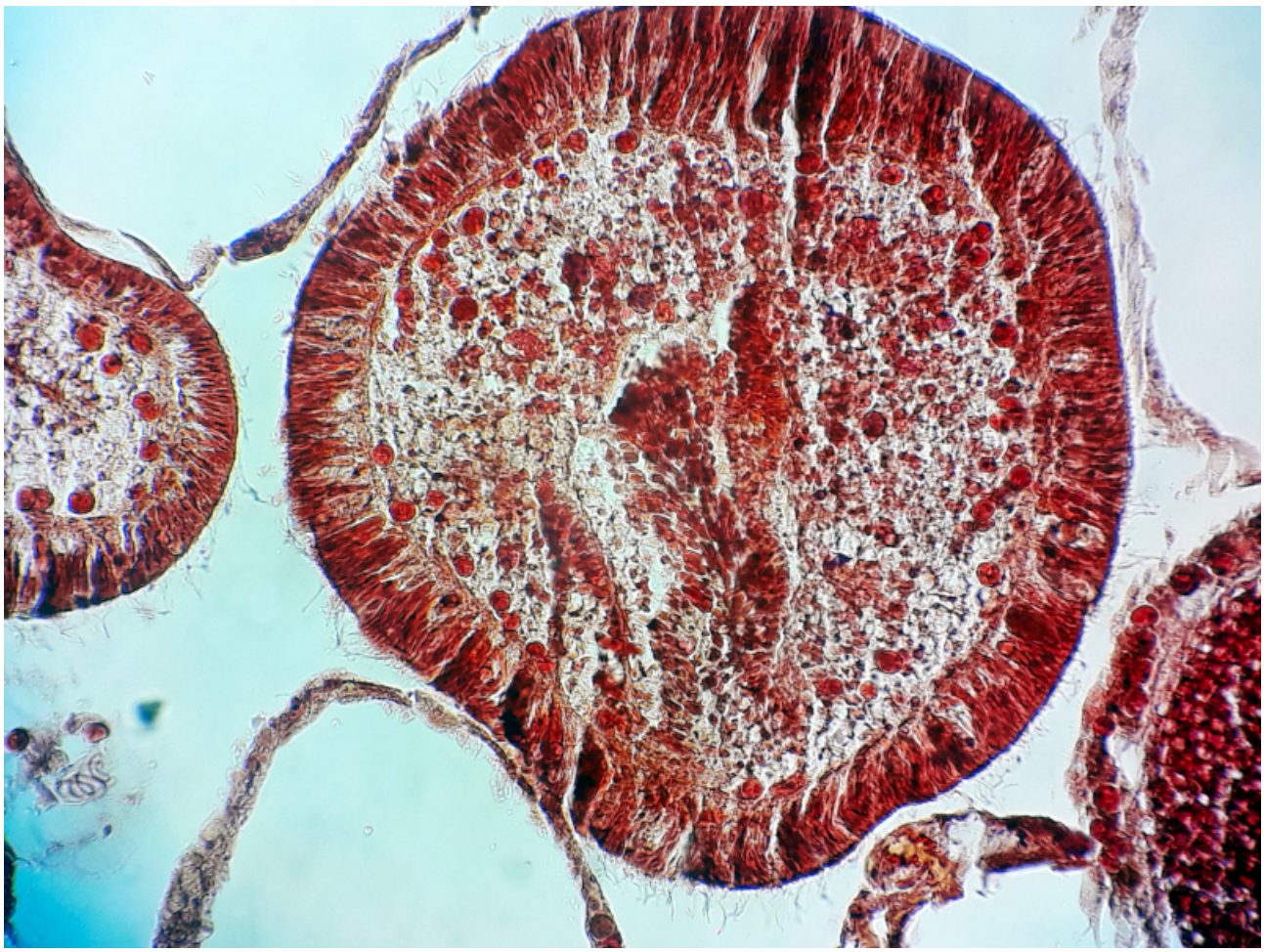

Fig 9. Larva in *Porites porites*. The long epithelial cells and their cilia on the outside are clearly visible. Just beyond that small round cells can be found: The zooxanthellae. They are about 5 micrometers in diameter. Also the first signs of internal organisation can be seen in the form of septs or “mesenteries”as they are wrongly called. To the right part of an ovum is seen, at left another larva. 16x NPL FL objective, Galaxy J3 camera. Section 10 micrometer parallel to colony surface.

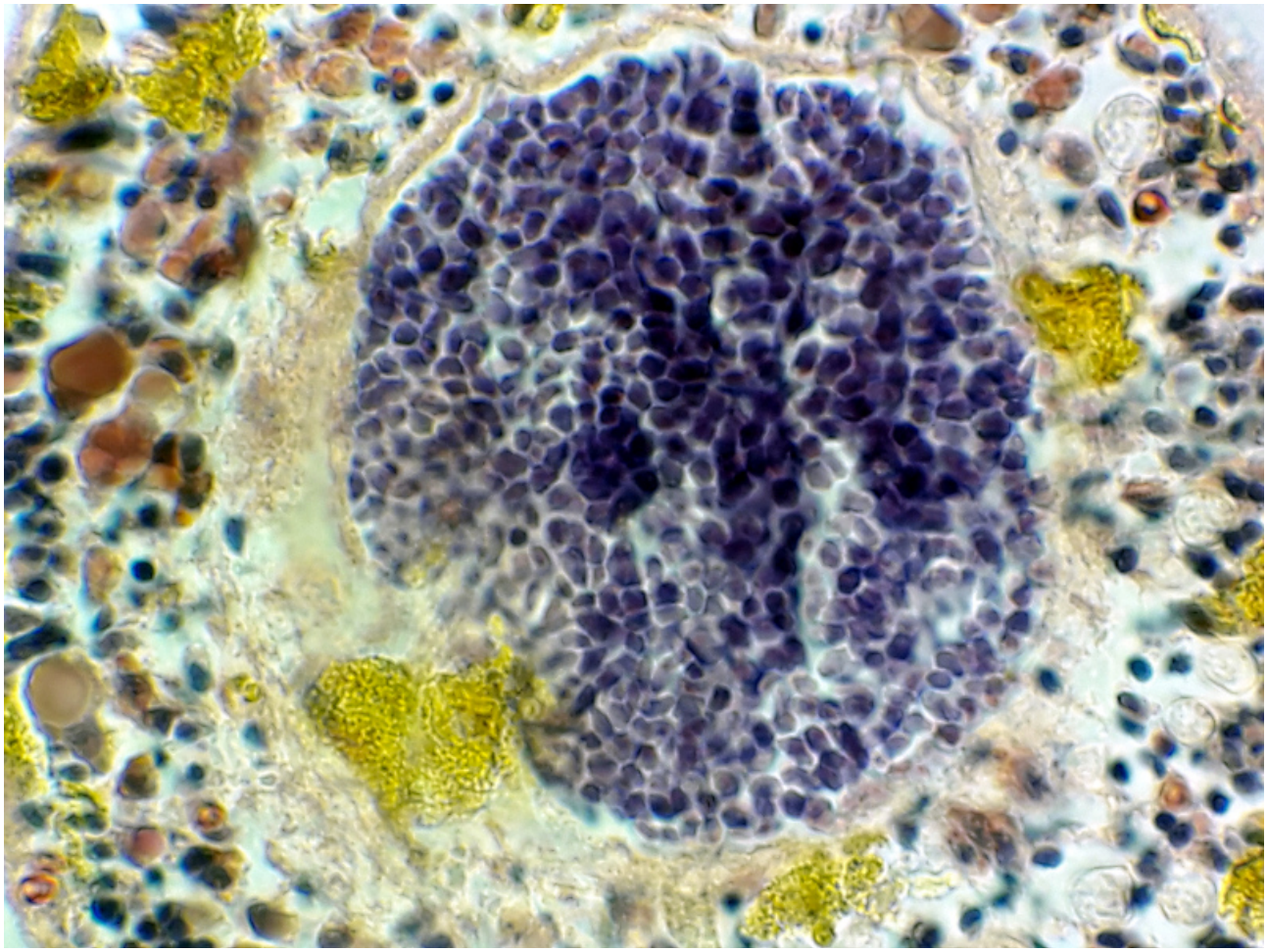

Fig 10. Developing sperm cells in *Porites astroides*. Zinc-formol-seawater fixative. In this stage, no tails have been developed yet. Zeiss Neofluar 40x, Galaxy J3 camera. 5 micrometer section parallel to colony surface, HEO stained.

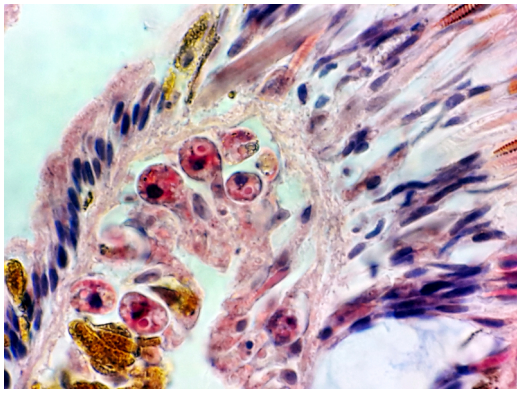

Fig. 11. *Porites astroides*, fixed in zinc-formol-seawater mixture. HEO Staining.Very fine detail is preserved. See for example the zoox just above the centre of the image. They are contained within host cells, as can be clearly seen. Note the long nuclei of the epithelium and the yellow brown pigment. Oil immersion 100x NPL FL , Galaxy J3 camera. 5 micrometer section parallel to colony surface.

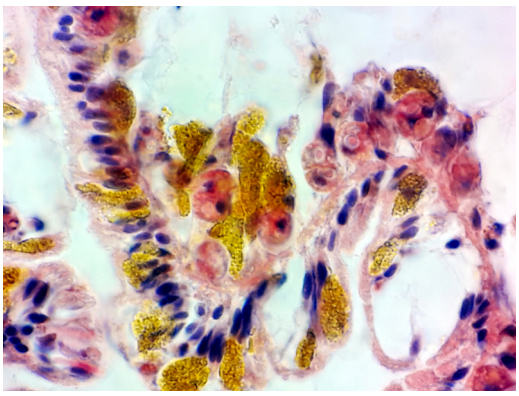

Fig 12. *Porites astroides*, same as in fig 12, but here emphasis on the relationship between the yellow brown pigment and the zooxanthellae. See text for explanation.

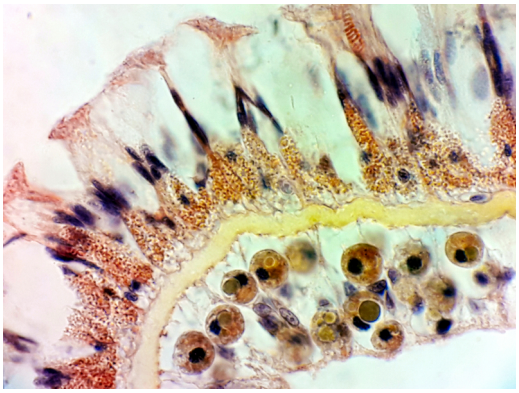

Fig 13. *Colpophyllia natans*. 5 Micrometer transversal section, fixed in Bouin's. HEO staining. Left epidermis, with the high cells and elongated nuclei. On top mucus, near the bottom the particles that cause fluorescence in the living colony. Below that the yellow connective tissue, also called mesogloea. The the layer of zooxanthellae, with the conspicuous nuclei of their host cells.
Oil immersion 100x NPL FL. Galaxy J3 camera.

Fig 14, from same sample as in fig. 14, but now stained with Masson's trichrome.

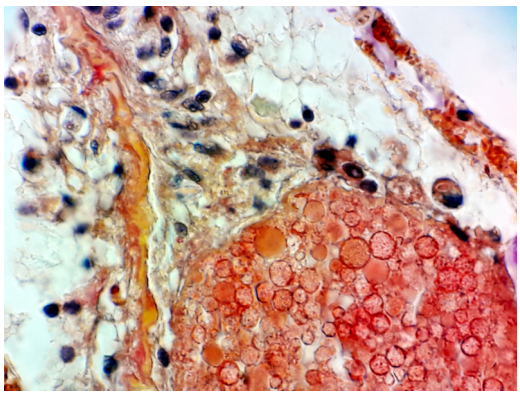

Fig 15. *Sideastrea siderea*. Developing ovum. 5 micrometer transversal section, HEO staining. 100x oil immersion, NPL Fl objective, Galaxy J3 camera.

Published in the January 2021 issue of *Micscape* magazine.

www.micscape.org