# To See a World in a Grain of Sand

## Extreme Macrophotography for Microfossils

### *Introduction*

My interest in photographing microfossils began with a YouTube video called "Microsculptures." Developed by an English photographer, Levon Biss, who published a beautiful book of the same name in 2017 (*Microsculpture: Portraits of Insects*),[1] "Microsculptures" presented highly magnified images of insects from every corner of the planet, photographed with striking quality and in impressive detail. Biss's technique was called extreme macrophotography.

How photographic images can be acquired in microscopy or astronomy (from one extreme of the cosmos to another) has fascinated me since I was very young, and my recent discovery of extreme-macro technology pushed me once and for all to put that fascination into practice.

For would-be extreme macrophotographers, there's no need to travel far to find subjects that will afford hours of fulfilling work, research, and image-making. Everything you need to begin exploring the microscopic world can be found in your own home: a seed, a grain of salt or sugar, an insect on the windowsill, or the tiny treasures that hide in sands and sediments, whether they are modern or millions of years old.

Because paleontology is one of my main hobbies, microfossils are, not surprisingly, my favorite photographic subjects. In a marl or sandstone sample, dozens or even hundreds of specimens in diverse and intricate shapes can be found.

Removing specimens from the matrix is probably the most complex (and tedious) phase of preparing them to be photographed. It's essential to eliminate the finest, dust-like particles (dissolved clay, for example) and generally to disaggregate the matrix in which microscopic creatures have been preserved.

Numerous techniques exist for separating microfossils from matrix, and these vary on the basis of the chemistry and composition of the matrix and of the microfossils themselves. Following any sort of blanket approach for all microfossils is dangerous, and it's worth reading and researching before choosing a method for the material you want to process.

In the case of rocky matrix, larger pieces can be crushed into pea-sized fragments and then treated with mild or diluted acetic acid (be sure beforehand that the fossils are not preserved as calcium carbonate, which even weak acetic acid will harm; treatment with acetic acid will generally have no effect on siliceous or phosphatic fossils); with hydrogen peroxide (particularly useful for clayey material); or with surfactants (typically detergents, emulsifiers, or dispersants, and the like; Calgon is sometimes used in the home lab), all of which will aid in separating individual specimens from matrix.[2]

You may want to carry out tests on small subsamples of sediment, repeating them multiple times if necessary, to see what effect any particular technique may have.

[1] Biss's TEDTalk is on YouTube (youtube.com/watch?v=30655tLniko).

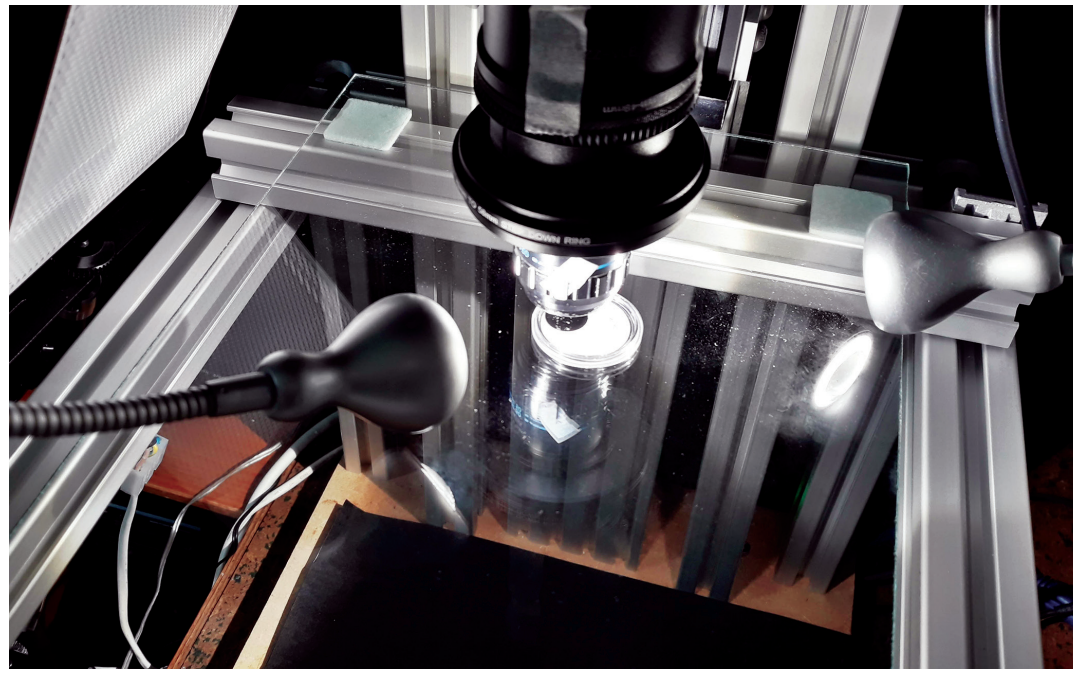
[2] Other, more-or-less complicated techniques include repeated thaw/freezing cycles, boiling, or the use of nitrogen, kerosene, and other materials. Though it is a bit dated, R. L. Hodgkinson's "Microfossil Processing: A Damage Report" (*Micropaleontology*, *37*(3), 1991, 320-326) provides a good review of techniques. Internet searches can also yield in-depth information, and various discussion threads on the Fossil Forum (www.thefossilforum.com) contain practical advice.

*Sediment from the Maastrichtian (Upper Cretaceous) phosphates of Morocco, ready for "picking" under the binocular microscope.*

## Featured Artist

# Enrico Bonino

When you've found a method that works, the time has come to wash and sieve the sample to remove larger pieces. The fossil-bearing sediment is then placed into an ultrasound bath to guarantee the removal of fine-grained materials, making the specimens as clean as possible for photographing.



*Two flexible LED lamps provide lighting for a microfossil subject.*

Suitable subjects can be found in a variety of dimensions—from larger fossils that can be measured in millimeters (small bivalves, gastropods, bryozoans, shark teeth, and corals, e.g.) to those that are submillimetric in size (foraminifers, ostracods, conodonts, scolecodonts, sponge spicules, and others), and on to others that are smaller still, as in the case of diatoms and radiolarians.

### Photographic Techniques

Using a digital reflex camera (I currently have a simple, entry-level Nikon D3300), extension tubes of specific lengths, and microscope objectives, a nearly unimaginable universe of complexity and fascination will begin to reveal itself.

Microscope objectives permit very high magnifications, much higher than those obtainable in classic macrophotography, provided your setup is as invulnerable as possible to both internal and external vibrations (those that come from the camera vs. those that come from the surrounding environment).

The movement of the mirror and shutter in the camera body has a significant impact on the end result. One good piece of advice is to perform image acquisition with "live view" active in order to lock the mirror in a raised position and eliminate the microvibrations that its movement may cause.

New mirrorless technologies help eliminate vibrations because there are no moving mechanical parts. Unfortunately, the price of these cameras is still quite high.

Because vibrations from the environment affect photographic output, anti-vibration feet and a rigid, stable support are essential. When working at high magnifications—for example, when I acquire images of diatoms or radiolarians with a 50x objective—a car passing on the street, a person moving in the room, or even my own breathing can cause vibrations on the screen.

Closely tied to the type of lighting is the sensor's ability to register light. In order to obtain the sharpest images with the least possible amount of background noise, it is best to work with low ISO values, if they are available: 100 is generally the standard. Increasing the sensitivity of the light sensor—i.e., increasing the ISO value—also increases background noise and reduces image quality.

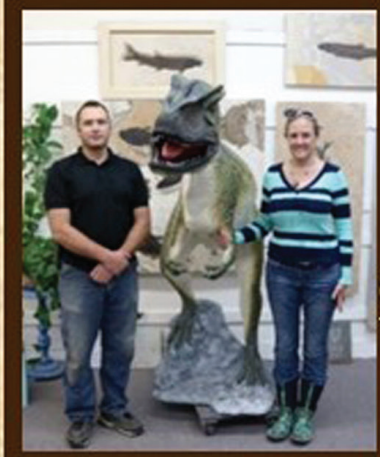
Optimal technical conditions are obtained using the sensor's normal sensitivity—that is, the one for which it was designed—without digital interpolations to increase or decrease values. Nominal sensitivity values can be found in the camera manual.

The use of low ISO values increases exposure time and therefore the movement of current through the sensor. Exposure times are also a function of the optics

# WYOMING FOSSILS

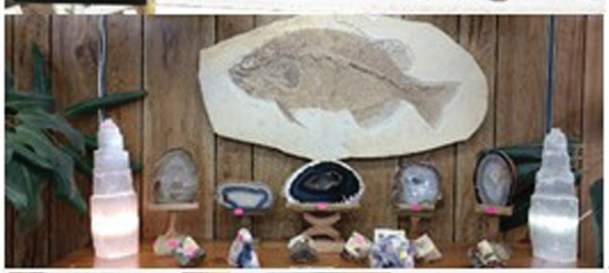
Sawaya's Center
921 Pine Avenue
Kemmerer, Wyoming
(254)223-3204

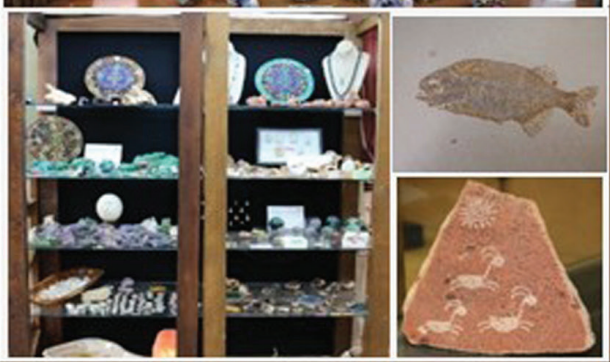
visit our website www.wyomingfossils.com



OPEN DAILY
9:00 a.m.
to 6:00 p.m.

- Fossils
- Minerals
- Crystals
- Unique Items
- Gifts





❖FIND US ON FACEBOOK/INSTAGRAM UNDER WYOMING FOSSILS

Top: *The "18% Grey Card" (also known as "middle grey"), which photographers sometimes use for color balancing, in a set with white and black balance cards.* Middle*: General description of an objective: 1: mounting thread; 2: optical aberration correction; 3: magnification; 4: numerical aperture; 5: image distance; 6: working distance; 7: cover glass thickness; 8: magnification color code.* Bottom*: Set of microscope objectives used with a camera for taking extreme macrophotographs.*

of the objective and the diameter of the lenses. I generally work with exposure times between 1/10th and 1 second, which are more than adequate to acquire a proper image.

Be sure to pay attention to the histogram that reflects the amount of light recorded by the sensor. If you use a black background, you'll observe an asymmetrical curve that will tend toward the left (darker tones—i.e., less light reflected toward the sensor). Being able to modify exposure times is essential because the photographer needs to ensure correctly distributed values (i.e., values that are arranged all along the distribution curve) to avoid over- or underexposure of the subject. Basing the exposure on a homogeneous "gray" area, as close as possible to the Kodak 18% grey card, is definitely helpful.

Acquiring an image on a homogeneous grey surface, under the same lighting conditions, makes post-processing color calibration possible which, in turn, facilitates the preservation of tones and color values.


## Objectives

In microscopy, specific types of objectives are used for various techniques. In this case, I've limited the choices to brightfield observation and reflected-light microscopy, but the important detail is that the instruments are intended for use without a cover glass (look for the symbol "0" for use without a cover glass or "–" for use with or without).

Another feature of these optical systems is the extremely reduced working distance, from a few millimeters to a few tenths of a millimeter for those with sustained magnification. Fortunately, there are longer-distance versions. Through abbreviations may vary by manufacturer, the most common are LW or LWD (long-working distance) and UL or ULWD (ultra-long working distance).

In addition, there are two other distinct categories:

- In a so-called *finite conjugate system*, light from a source is focused down to specific finite position. In microscopy, the image of the object under inspection is magnified and projected onto the eyepiece or onto the sensor if a camera is being used. This requires a tube length that is typically 160mm, though there are many exceptions (170mm, 190mm, 210mm are the most common). Systems of this type can be customized by using a spacer tube between the stop of the objective and the sensor. Some optical systems allow greater or lesser variations, but, in others, aberrations arise at distances other than those indicated.

- In *infinity-corrected (infinite-conjugate) optical systems*, light is focused down to a small point, which is the object under inspection, and infinity points toward the eyepiece or sensor if a camera is used. An additional tube lens placed between the object and the eyepiece directs the light beam in order to produce an image. This system has the advantage of allowing the user to introduce any optical system (such as filters, polarizers, or beamsplitters, e.g.) between the objective and the tube lens without the need to make corrections.

In macrophotography, an objective with the focus set at infinity is used instead of the tube lens. The greater the focal length, the greater the resulting magnification.

I use a Raynox DCR-150 lens (whose position can be reversed for use at higher magnifications). This lens features three or four elements in multi-coated achromatic glass, with a high optical index that allows for extremely sharp images and minimal chromatic aberration.

Because the depth of field (a measurement of the thickness of the plane of focus) at which the subject appears sharp and in focus is greatly reduced with the use of microscope objectives, numerous frames (tens or generally hundreds of images in sequence) must be acquired in order to ensure that the subject is clear across its entire, three-dimensional surface. The individual images in this series may be no more than a few tens of microns apart (sometimes even fractions of microns), and they are acquired through the mechanical movement of a motorized platform connected to the camera and controlled by a microcomputer.

This mechanical process allows the user to define such parameters as the start and end of the shot, the amount of advancement between images, and the length of the pause before or after the image is shot. The photographs in these pages were acquired using the following optics:

- The Schneider-Kreuznach Componon-S 2.8/50 objective (working distance about 60mm), inverted. This is an excellent lens for subjects that can be measured in millimeters (gastropods, bivalves, shark teeth, bryozoans, and echinoderms, for example);

- The Mitutoyo 2.5x QV objective (working distance 33mm). The nearly perfect optical quality allows the user to

work in a range of magnifications up to 6x; and the LOMO 3.7x / 0.11 objective (working distance 27mm), a well-known Russian product known for the excellent quality of its optics and of the images it produces. These two objectives are used for subjects from 1mm to 500µm in size (µ or "*mu*" indicates microns, or thousandths of a millimeter);

- The PLAN 4x / 0.10 infinity objective (working distance 11mm), an entry-level lens, performs well and, like the LOMO3.7x / .011, can be used for subjects of between 1mm and 500µm in size;
- The Nikon CFI Plan 10x / 0.25 objective (working distance 10.5mm), essential for photographing foraminifera, scolecodonts, conodonts, the oogones of green algae, ostracods, and sponge spicules;
- The Olympus LWD MSPlan 20x / 0.40 objective (working distance 11mm) for the smallest foraminifera and organisms with a diameter of less than 150µm;
- The Olympus ULWD Neo SPlan 50x/0.55 objective (working distance 8.1mm) for subjects with dimensions less than 100µm, such as radiolarians, diatoms, and microforaminifers.

The information on the barrel of the objective describes optical characteristics and functions.

## Lighting

Lighting is of enormous importance. The photographer needs the most homogeneous diffusion of light possible without causing undesirable reflections or shadows (except when shadows or reflections are needed to highlight particular structures). Light sources may include LED panels (I have two LED panels of 20W each that produce a white light of 4000 Kelvins and 1600 lumens), LED lights on flexible arms such as the excellent Jansjö lamp by Ikea, or even flash setups.

## "The System" (Photographic Set Up)

I call the setup required for acquiring extreme macrophotographs simply "The System." Shooting microfossils requires placing the subject on a horizontal surface, which means that the camera and the motorized rail are installed vertically and are solidly attached to the aluminum V-slot board. All of this is connected to a standard 30x30 aluminum extrusion mount that rests on a wooden stage fitted with rubber feet to absorb vibrations.

The computer that runs the motorized plate is connected both to the motor and to the camera, and commands are sent via an infrared remote.

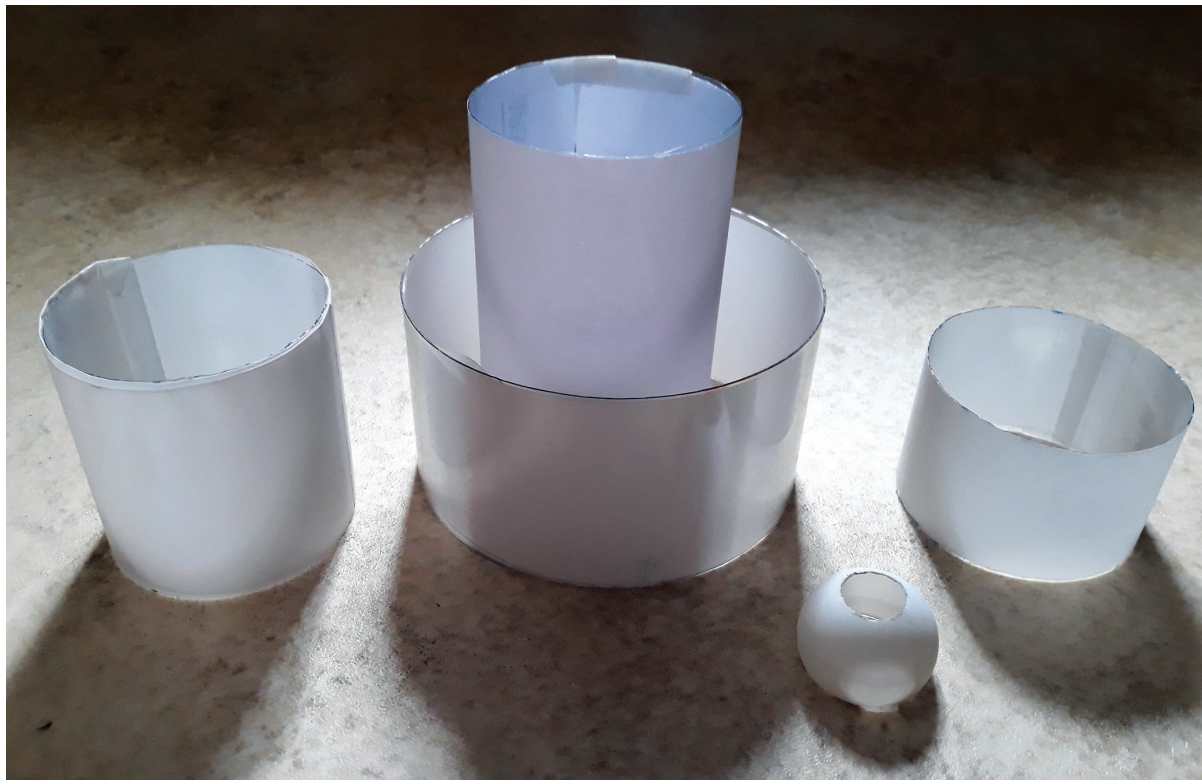
A wide variety of motorized systems are available on the market that allow the acquisition of very high-resolution images with extreme macrophotography. I use

the QOOL Model sold by MJKZZ Europe (www.mjkzz.de), which comes with a 400-step motor for precise movements without vibrations.

LED panels and flexible LED lights are attached to a separate external mount so as not to transmit vibrations to the column that supports the camera. Finally, a seven-inch screen is connected to the camera with an HDMI cable. The screen is especially useful for determining the start and end point as a subject is brought into complete focus as well as for visualizing shutter speed and the color-distribution histogram.

The specimen to be photographed is identified under a binocular microscope and then chosen or "picked" with a "000" brush or a slightly moistened toothpick and placed on a microscope slide.

The slide rests on a fixed glass surface (about 20cm above the base) that is covered with a black velvet cloth. The subject is generally approximately 1cm from the bottom of the objective (the list of objectives above indicates relative working distances), and around it, resting on the glass surface, is a cylindrical diffuser of variable diameter (8-14cm). This allows for the most homogeneous diffusion of light.



*DIY light diffusers can be fashioned from a variety of materials and then covered with white paper.*

Many different kinds of diffusers exit, especially homemade ones, all developed with the needs of different photographic subjects in mind. Personally, I've used yogurt containers, ping-pong balls, drinking glasses, and plastic cylinders of various sizes wrapped in drawing or tracing paper. The paper, by the way, must be white in order not to introduce unwanted chromatic variations and should be relatively thin to avoid altering the colors of the subject during acquisition. The paper used to make photocopies is fine.

### Software

Images are recorded in native RAW format (.NEF is Nikon's proprietary format) at sixteen-bits or with the greatest number of bits that can be obtained. They are then imported to a computer and digitally manipulated with software intended for that purpose.

Quite a few programs allow the user to make compilations of images in an extremely fast and effective way. Some of these are open-source while others are commercial products. Among the latter, the Zerene Stacker (https://zerenesystems.com) and Helicon Software (https://www.heliconsoft.com) are widely used.

These two are similar in function and are equipped with complex algorithms that can process hundreds of images in an extremely short time, resulting in a perfect image of the whole subject. This technique of overlapping, combining, and geometric correction of images is called focus stacking. Adobe Photoshop also allows images to be compiled with excellent results, but the calculation times are extremely long compared to the two software packages I've mentioned. Note that the Zerene Stacker, unlike Helicon, allows

the user to import .JPGs or .TIFFs only and not native RAW formats.

To preserve all the metadata associated with an image, which allow the user to modify color dynamics, geometric correction, and other parameters in post-processing, the composited image is saved in .DNG format (digital negative, an open-source format developed by Adobe) or .TIFF. Exporting images in .JPG format is not recommended because, although this format takes up considerably less space and calculation times are much shorter, it does not allow for precision color calibration.

The file is imported into Adobe Camera in .RAW format, and the image is balanced with color, sharpness, contrast, and all the other parameters that allow the optimal display of detail and shading.

Any defects (such as dust on the sample or on the camera sensor) are removed using the tools available in Adobe Photoshop.

If details require sharpening, the filters in the Sharpen AI extension (https://topazlabs.com/sharpen-ai) can be applied. They allow the level of sharpness and contrast of the image to be increased through deep-learning technology. Because we are dealing with micro-subjects, be sure to insert a scale to indicate the size of the specimen. For each lens configuration or tube length, a scale engraved in microns on special calibration slides is photographed and recorded. Calibration is done in Adobe Photoshop, and the measurement appears as a bar in microns.

To calculate the magnification factor, measure the width of the photographic field with the millimeter or micron scale and divide this value by the width of the camera's sensor. The width of the Nikon D3300 sensor is 23.5mm, but see the instruction manuals for other models or search the web.

The level of detail that can be achieved with this technology is impressive. Using the configuration I've described and an Olympus 50x objective, pores of about 1µm on a radiolarian can be seen and photographed.

No kind of microfossil is better or worse for extreme macrophotography. Each organism is interesting in its own way. The most complex part of the process is orienting the light source properly to bring out the subject's morphology, including protuberances, spines, and surface texture.

The entire creative process—starting with the positioning of the subject and ending with the final image, generally takes an hour or less, depending on the size of the microfossil and the time needed for post-processing. Once all these operations have been completed, the image is ready to be published.


## Acknowledgments

*I'd like to thank Samir Bandak, Attilio Dalmasso, Rami Djeffal, Alex Liu, and Gianpaolo di Silvestro for providing me with the fossiliferous sediment samples from which most of the examples photographed in this article were taken. I'm also grateful to Walter Biggi, Guido Gherlenda, and Fabio Lena for their comments on the text and for their unparalleled technical support as my involvement with extreme macrophotography deepened.*


— English Translation by Wendell Ricketts

# Conodonts

The organisms I have photographed cover the entire dimensional spectrum from about one millimeter to just over 50µm. They represent geological ages from as early as the Neoproterozoic Era and as late as the Recent, starting with fossilized cells from the Upper Ediacaran of the Doushantuo Formation (China) and moving on through Ordovician conodonts and scolecodonts, oogones of Permian freshwater algae, foraminifera, ostracods, the teeth of sharks and bony fish, gastropods, bivalves, and Recent radiolarians and diatoms. 

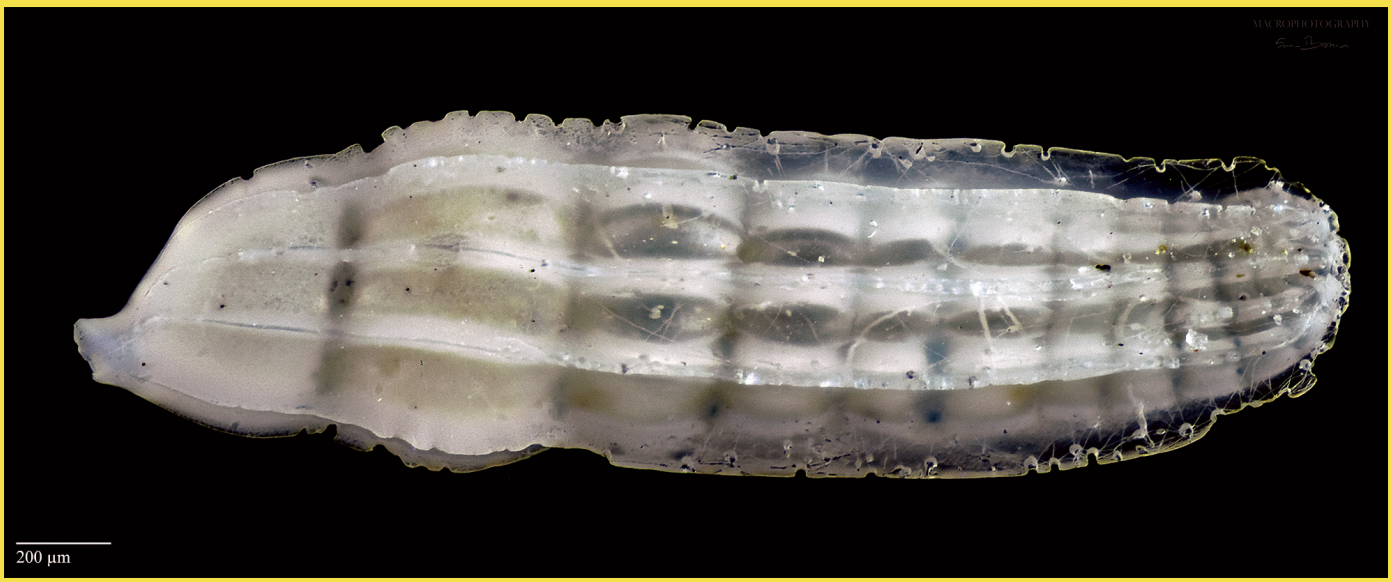


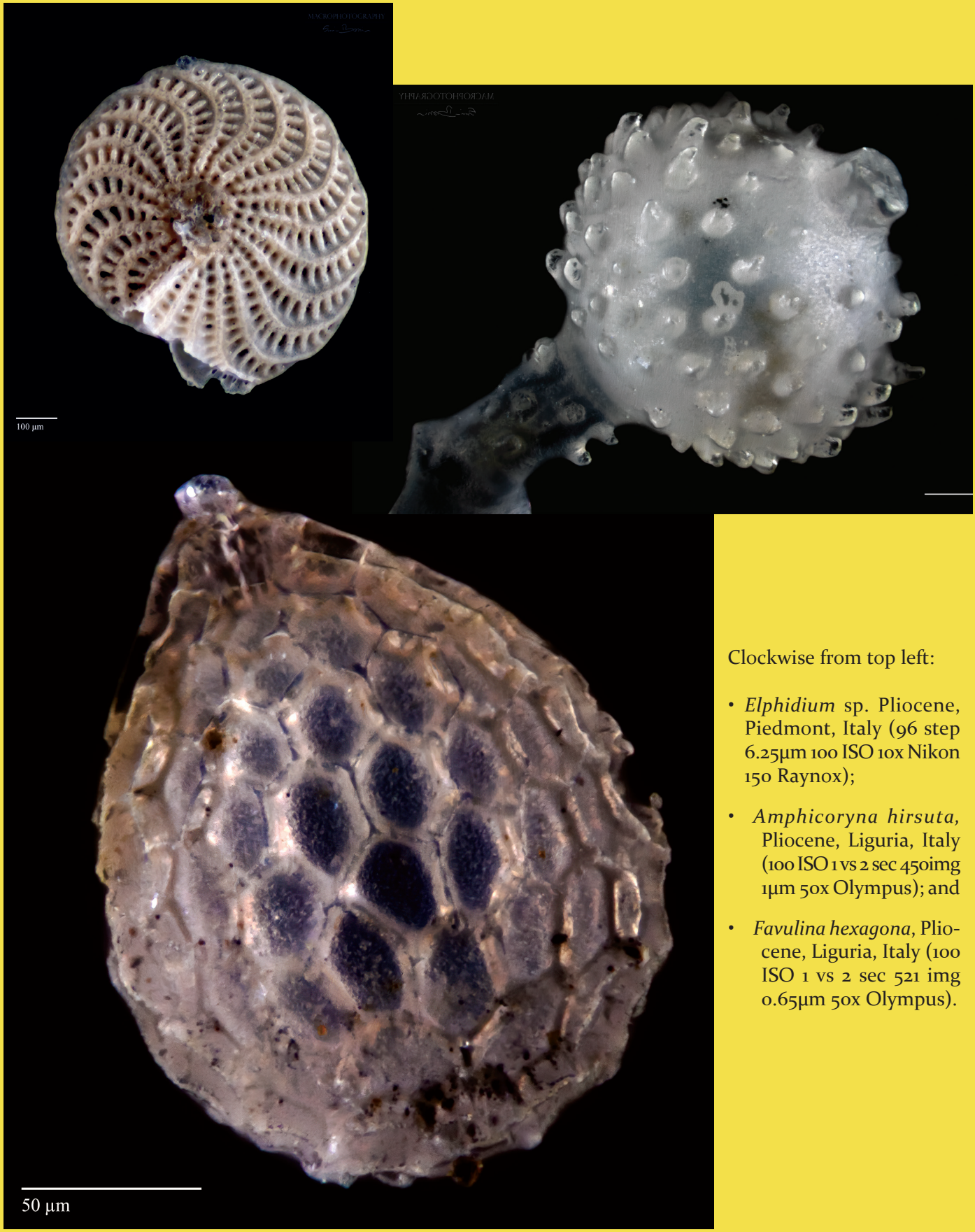
Conodonts are tooth-like microfossils known from the Cambrian to the very end of the Triassic. Though their classification was long in limbo, and knowledge about their soft tissues remains limited, they are believed to be elements from extinct jawless chordates that resembled eels. Conodonts are important index fossils. *Above*: Unidentified conodonts, U. Ordovician, Cincinnatian, Waynesville Fm., Milan, IN. (102 img, 1 vs 3 sec, 6.25µm, 10x Nikon tube lens). Below: *Ozarkodina* sp. Beechwood Limestone Member, Upper Middle Devonian, Speed, IN. (7.5µm, 1 vs 3 sec, 100 ISO, 88 steps, 10x Nikon Raynox 150).

# Foraminifera


Foraminifera are the external shells (or "tests") of single-celled protists (that is, organisms that are classified as neither animal, plant, or fungus). Most foraminifera are marine, and the majority of these species live on or within seafloor sediment. In their forms and shapes, they are among the most diverse of microscopic creatures. As fossils, they are valued as indicators of paleoclimate and as index fossils. At left: *Uvigerina* sp. Pliocene, Liguria, Italy (100 ISO 0.5 sec 255 img 2.5µm 50x Olympus 150 Raynox). Facing page, top: *Lagena* sp. cf. *L. striata*. Pliocene, Liguria, Italy (100 ISO 1.3sec 400 img 2.5µm 50x Olympus 150 Raynox). Bottom; *Marginulina costata* Pliocene, Liguria, Italy (100 ISO, 1vs 8sec, Mosaic 2 stack, 37 img 5µm, 10x Nikon 150 Raynox).


50 µm


200 µm



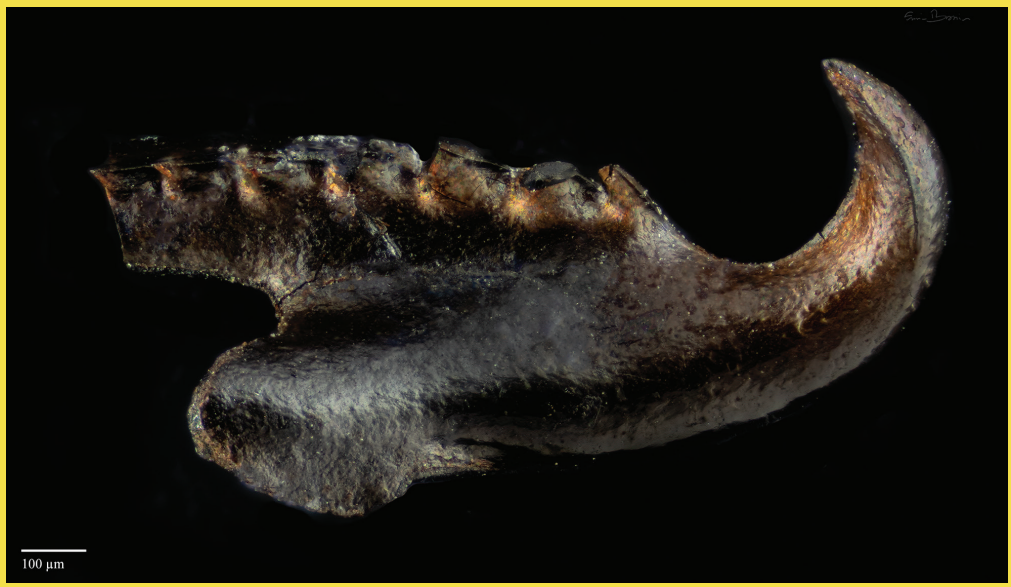

Clockwise from top left:

- *Elphidium* sp. Pliocene, Piedmont, Italy (96 step 6.25µm 100 ISO 10x Nikon 150 Raynox);
- *Amphicoryna hirsuta,* Pliocene, Liguria, Italy (100 ISO 1 vs 2 sec 450img 1µm 50x Olympus); and
- *Favulina hexagona*, Pliocene, Liguria, Italy (100 ISO 1 vs 2 sec 521 img 0.65µm 50x Olympus).

# Scolecodonts

Scolecodonts are the jaws of polychaete annelids (segmented bristle worms). Fossil scolecodonts are particularly common in Ordovician, Silurian, and Devonian marine deposits, but the organisms they come from have existed from the Cambrian to the Present. The vast majority of species are marine. Though scolecodonts are generally tiny, one of the largest scolecodonts ever found—*Websteroprion armstrongi* from the Devonian of Canada (see *FN* Summer/Fall 2020)—was more than a centimeter in length and is estimated to have belonged to a giant worm that may have grown as long as a meter. Top and middle: ?*Hadoprion* sp., U. Ordovician, Waynesville Fm., Milan, IN (1 vs 3 sec, 6.25µm, 10x Nikon tube lens). Bottom: *Atraktoprion* sp. from the same location (0.6 sec, 7.25µm, 140 img 10x Nikon tube lens).





# Oogonia

Charophyta is a group of specialized freshwater algae that includes the closest relatives of the embryophyte plants (that is, most of what we think of as vegetation: liverworts, mosses, ferns, vascular plants, gymnosperms, and flowering plants). Oogonia are female reproductive structures in charophytes and appear as rounded cells or sacs containing one or more fertilizable gametes (sex cells).

Clockwise from Top left: *Stomatochara moreyi*, Florena Shale Member, Beattie Limestone, Permian, Grand Summit, Cowley County, KS (139 img, 6.825µm, 1.6 sec, Nikon 10x; *Sphaerochara andersoni*, Lower Weald Clay, Lower Cretaceous, West Sussex, UK (Nikon D3300, QOOL 250 rail, Nikon + tube lens & Raynox 150, Jansjö LED lamps + cylinder of tracing paper as diffuser, stack of 82 images @ 6.825µm); *Harrisichara tuberculata*, Bembridge Limestones, Upper Eocene, Shalcombe, Isle of Wight (Nikon D3300, QOOL 250 rail, Nikon + tube lens & Raynox 150, Jansjö led lamps + cylinder of tracing paper as diffuser, stack of 231 images @ 6.825µm; *Grambastichara tornata*, Colwell Bay Member, Headon Hill Formation, Upper Eocene, Totland, Isle of Wight (152 img, 6.825µm, 2sec, Nikon 10x).

# American Fossil

*The Only Quarry that lets you keep every fossil you find! **

Full Day Dig (8 Hours) - $120
4 Hour Dig - $85
1 Hour Dig - $35
Children 7-14 Half Price with Paying Adult
Children under 7 Free with Paying Adult
Special pricing for groups over 5
Call or email for info 801-836-7269
Prices Subject to Change

Quarry Lease Opportunities Available
7 Days, 10 Days, 30 Days or Full Season

American Fossil is providing you the adventure of a lifetime. We have the only quarry in the world where you have the opportunity to dig fossils and keep every fossil you find. Our quarry is located in the world famous Green River Formation of Wyoming and is the most exciting destination in the world for everyone who loves fossils. Think of it as your own fossil expedition.

Ours is a world renowned quarry, well known for its fossil fish, and one of only two locations in the world where a complete 3-Toed Horse has been found. At the quarry you can also find stingrays, shrimp, Gar, Paddlefish, Amia, Pike, Dioplomystus, Knightia, and even turtles, crocodiles, bats, birds, ancient rhinos and camels, primates, and much more.

*At our quarry you can keep every fossil you find up to $100,000 in individual value. This means keep all of the turtles, bats, birds, stingrays and fish you find. No other quarry lets you do this. Come share our love of fossil collecting and contact us today!

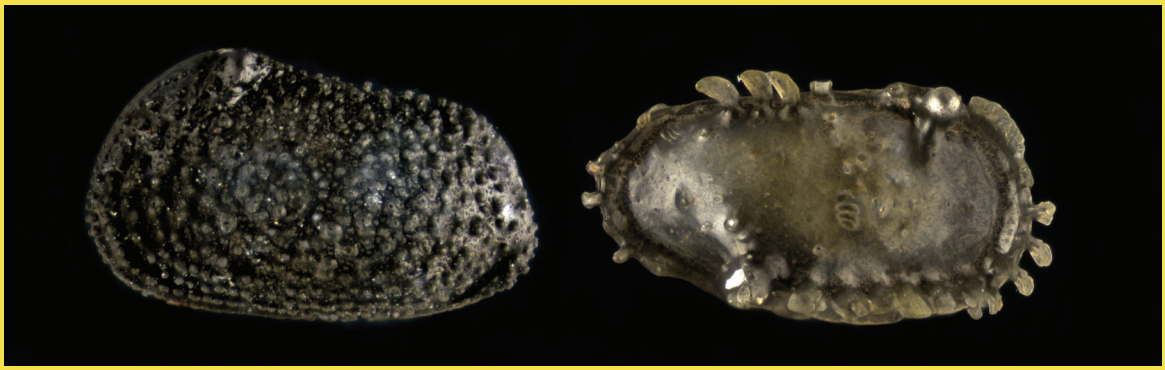
www.AmericanFossil.com or www.FishDig.com
801-836-7269

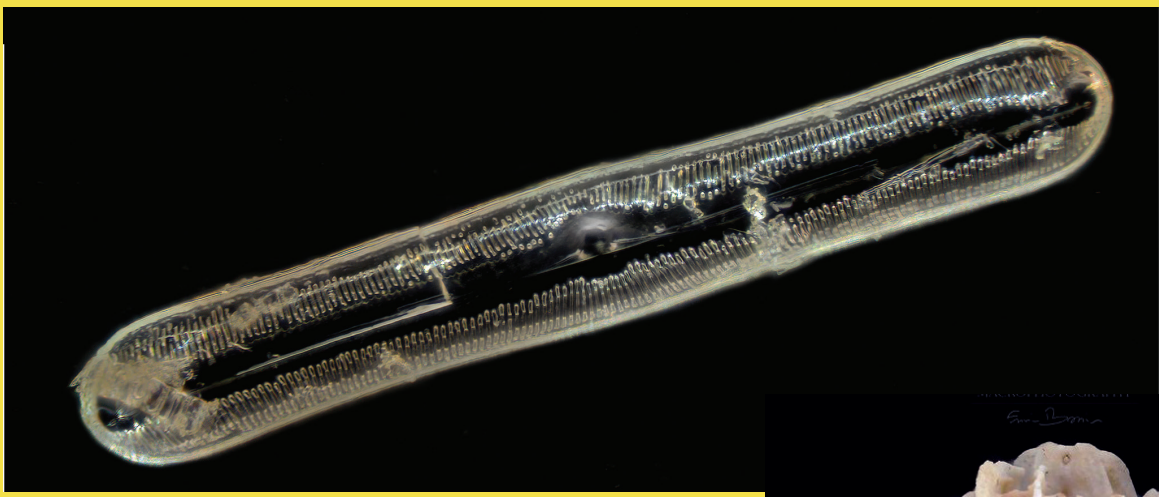
# Hyoliths & Salterellids

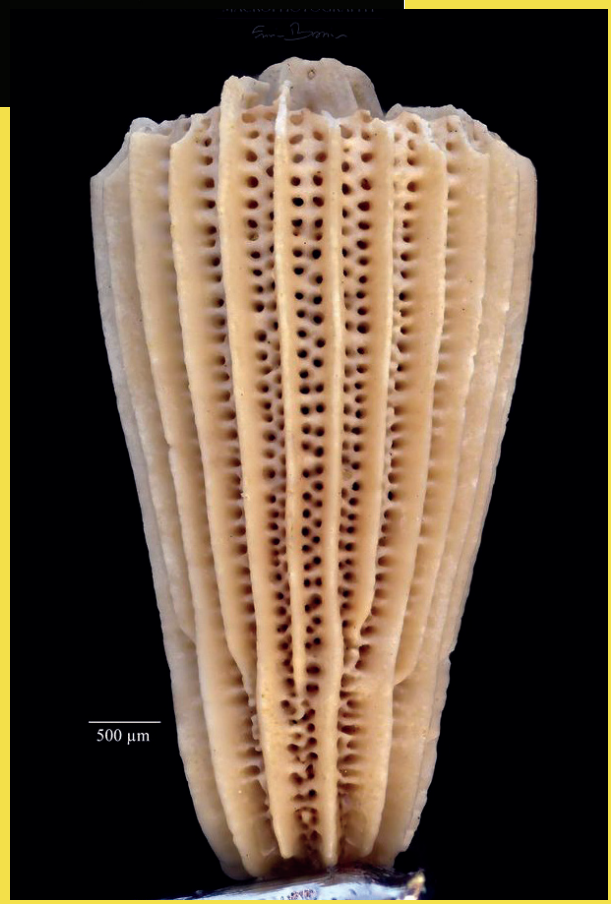

Below is *Conotheca subcurvata* from the Zhujiaqing Formation, Lower Cambrian, Yunnan Province, China. *Conotheca* is a genus of Cambrian hyoliths, which are classed with the lophophorates, or lophophor-bearing animals, as are brachiopods and bryozoa. In addition to a lophophore (a feeding organ that appears as a ring or coil of ciliated tentacles surrounding the mouth), it has a "lid" or operculum, like some gastropods. (72 img, 1 vs 8 sec, 45µm, Lomo3.7x, 160mm)


Above is *Salterella maccullochi* from the Pioche Shale, Lower Cambrian, Miller Mountain, NV. Salterella belongs to a group of fossils that have, so far, resisted definitive identification. They built their shells in part by gluing grains of sediment together and may have been complex multicellular animals or single-celled like the foraminifera. They are likely to have lived on or perhaps embedded in tidal mudflats. (72 img 45µm, 1 vs 8 sec, Lomo3.7x, 160mm)

# Diatoms, Coral & Ostracods








*Top*: Unidentified Pliocene ostracods, Liguria, Italy (100 ISO, 1 vs 3 sec, 663images, 5.25µm, 10x Nikon 150Raynox); *Middle*: Pleistocene diatom, Mount Amiata, Tuscany, Italy. (121 steps, 0.625µm, 100 ISO, 1 sec, Olympus 50x, Raynox 150, ping-pong ball light diffuser; *Bottom*: *Turbinolia* cf. *T. sulcata,* a solitary scleractinian (or "stony") coral from the Stone City Formation, Middle Eocene, Burleson County, TX.