The Surprising Microscopic Wonders from the Depths of Villefranche-sur-Mer

Les étonnantes merveilles microscopiques des profondeurs de Villefranche-sur-Mer

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ABSTRACT. The Institut de la Mer de Villefranche is located near deep Mediterranean waters, and so is well-placed for investigations of the fauna of the deep sea. Shown here are some rare images of living microscopic organisms that populate the deep sea. Each shows a beautiful and complex morphology, and some images show unexpected features. **RÉSUMÉ.** L'Institut de la Mer de Villefranche est bien placé pour les recherches sur la faune des grands fonds car il est situé à proximité des eaux profondes. Voici des images très rares d'organismes microscopiques vivants qui peuplent les profondeurs marines. Chacun montre une morphologie belle et complexe, et certaines images présentent des caractéristiques inattendues.

KEYWORDS. Deep-Sea, Microscopy, Plankton, Protists. **MOT-CLÉS.** Grands fonds de la mer, microscopie, plancton, protistes.

Introduction

Of all the marine laboratories in Europe, the Institut de la Mer de Villefranche (IMEV), is the only one located near deep waters. This makes the IMEV uniquely well-placed for studies of deep-water fauna because organisms can be collected, and within minutes, brought back to the laboratory for study. A deep-water station, Point C is regularly sampled at the IMEV. The total depth at Point C is over 325 meters; to put this depth in perspective, consider that in 325 meters depth, the Eiffel Tower could be completely submerged. There is almost no light in the depths of Point C, the mesopelagic zone of the sea. It is in the transitional "twilight layer", overlying the abyssal zone of complete darkness. In popular culture, there is a long tradition of the portrayal of the deep sea as populated by strange and frightening creatures, such as giant squids and bizarre toothsome fish. In reality, it is largely populated, just like the surface layer of the sea, by the microscopic organisms of the plankton. The biology of the organisms is poorly known as collecting deep-water specimens and observing them living presents practical difficulties. The organisms are not only difficult to access, but they are also only present in relatively low concentrations. Consequently, observations on living organisms from the deep sea are few and far between. However, during the years 2021 and 2022, samples were obtained at Point C with a "closing plankton net", sampling between 275 and 225 m depth. This allowed samples from large volumes of relatively deep water to be quickly collected and rapidly brought back to the laboratory for observation (Fig. 1.). The images shown here of "microscopic wonders of the deep sea" are all of living microorganisms photographed in the Laboratoire d'Océanographie de Villefranchesur-Mer of the IMEV, and some images show not only a beautiful microorganism, but also a feature that was unexpected!



Fig. 1. The top right photo was taken at the entry of the Bay of Villefranche, about 2 minutes before arriving at the deep-water sampling site, 'Point C', located off the Cap de Nice, approximately 2 km (or 20 minutes travel time), from the laboratory. The bottom right photo shows the plankton net being lowered, on its way to a final depth of 275 m. The fanciful collage on the right shows the IMEV's vessel, the Sagitta 3, at the surface, and the plankton net at about 250 m depth, a distance approximately equal to that separating the tip from the first floor of the Eiffel Tower.

Twelve Microscopic Wonders from the Deep



Fig. 2. A surprising time-series showing the radiolarian Dictyocorne profunda. It was placed in filtered seawater and immediately withdrew all of its fine filament pseudopods, liberating the attached symbionts (12:59). Over the next 2.5 hours, pseudopods were extended and the symbionts were re-attached to the pseudopods via an unknown mechanism. The shell of D. profunda is about 100 µm in longest dimension.



Fig. 3. The acantharian Amphilonche elongata with its typical yellow symbionts. It is about 200 μ m long.



Fig. 4. An acantharian with two types of symbionts. While the yellow spheres are typical symbionts, the small red spheres on the spines appear to be quite unusual. The longest dimension (from tip to tip of the longest spines) is about 200 μm.



Fig. 5. The phaeodarian Euphysetta lucani, when living, forms a large reticulated web of pseudopods to catch prey. The ovoid upper portion of the shell is about 100 µm across.



Fig. 6. The phaeodarian Challengeranium diadon forms pseudopods as long, fine filaments to feed. The shell is about 75 μ m in length.



Fig. 7. The phaeodarian Challengeron willemoesii uses whip-like pseudopods to feed. The shell is about 200 μ m in length.



Fig. 8. A nassularian radiolarian, a Pterocanium sp., extends an array of fine filaments of pseudopods from the central portion of the shell. The shell is about 200 μm in longest dimension.



Fig. 9. Another nassularian radiolarian species, Spirocyrtis cornutella, appears to use pseudopods extending chaotically in all directions.



Fig. 10. The nassularian radiolarian Carpocanistrum cephalum extends pseudopods only out of the base of the shell.



Fig. 11. The tintinnid ciliate Daturella striatura. The ciliate cell lives inside a lorica (shell). In preserved specimens there are often particles attached to outer surface of the lorica. The image shows that particles are also attached to the lorica in living specimens. The lorica is about 150 μm long.



Fig. 12. The tintinnid ciliate Xystonellopsis paradoxa. The species is commonly found in the surface waters. This specimen, contracted in its lorica, contains the remains of ingested prey items (the orange and red spheres in the lower portion of the cell) showing that it was very recently feeding in the deep waters.



Fig. 13. A diatom, likely a species of the genus Pleurosigma. Diatoms generally rely on photosynthesis, but this species was commonly in the deep-water samples in near darkness where photosynthesis is not possible. It may then survive by absorbing dissolved organic matter. A short video showing another specimen, gliding along the bottom of the observation chamber, was recently filmed and is available: https://www.youtube.com/watch?v=ZtlgIWKju-8

Ways, Means, and Details

The plankton net used to collect microorganisms from deep water was a large "Nansen closing net". The mouth of the net is 70 cm in diameter and the total length of the net is 3 m. The mesh (or pore) size is 52 µm. The net was lowered to 275m, and then brought up at a slow speed (0.5 m per second), to 225 m depth at which point a messenger weight was sent down the wire to activate the choke line, closing the net. The closed net was then brought to the surface rapidly (1 m per second), and contents of the collector emptied into a chilled glass bottle for immediate transit to the laboratory. The time elapsed between the closing of the net at 255 m, and the first examination of the sample in the laboratory, was approximately 30 minutes. Thus, the images shown in the preceding pages were all created in the laboratory shortly after sample collection. First, a small portion of the net sample (i.e., 1 ml of the 500 ml net sample) was placed in a 3 ml glass-bottomed chamber, diluted with 2 ml of filtered seawater, and examined using an inverted microscope. Individual microorganisms were located, and using a pipette, removed, and put into another 3 ml chamber filled with filtered seawater for individual observation and imaging. For imaging, an inverted microscope equipped with Differential Interference Contrast optics (Olympus IX71) and a digital camera (Canon Eos 5D Mark II), was used to capture, as rapidly as possible, a series of images, typically 5-12 images, focusing

down through the organism. For most of the images, a 20x microscope objective was used. Usually several series were attempted to obtain one at least in which the organism was not moving! From the focal series of images, a 'stacking program' (Helicon Focus) was used to produce a single composite image. The final composite image was edited using Photoshop. The organisms in the images shown here are all protist taxa, that is, single celled organisms. The actual sizes vary between about 100 and 200 μ m in longest dimension. Most are known to be deep-water species, rarely found in the surface layers of the sea.

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