Tabletop Electron Microscopy at the British Antarctic Survey

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The seas that encircle the continent of Antarctica are known as the Southern Ocean. Occupying the only band of latitudes on the planet where ocean waters encircle the globe, the Southern Ocean connects the Atlantic, the Pacific and the Indian oceans, and is a crucial cog in the Earth's climate system. The Southern Ocean is one of the largest marine ecosystems in the world. Its cold and often ice-covered waters harbour a web of species from the smallest single-celled plants and animals to the largest mammal on Earth, the blue whale. Every new species discovered needs to be properly described and microscopy plays an important role in this process.

Working from the Royal Research Ship James Clark Ross and research stations on South Georgia - Bird Island and King Edward Point – as well as bringing samples back to their Cambridge headquarters, scientists from British Antarctic Survey (BAS) are studying many crucial aspects of the Southern Ocean and the species that depend on it. One of the key challenges for BAS scientists today is to predict how human activity and climate change will affect the Southern Ocean, and how the creatures that depend on it will respond. Parts of Antarctica are among the most rapidly warming areas of the planet. At BAS, scientists are monitoring the effects of this climate change on the Southern Ocean's ecosystem. BAS marine biologists utilise a number of techniques to inspect and characterise species found in the Southern Ocean, and variable pressure electron microscopy is a powerful recent addition to their capabilities.

Variable Pressure Scanning Electron Microscopy

The benefits of variable pressure electron microscopy are well known. Scanning electron microscopy offers much better resolution and depth of focus than optical microscopy and allows high resolution studies of sample morphology. Conventional scanning electron microscopy is a high-vacuum technique, which means that examination of biological material requires special preparation techniques. These range from cooling, freezing or drying to avoid the loss of water vapour from the sample (and subsequent deformation) in the vacuum environment, to coating the sample with a thin carbon or metallic layer to conduct away any electrical charge induced by the electron beam. With variable pressure scanning electron microscopy, however, the pressure in the sample chamber is maintained at a much higher value than in a conventional SEM. This has a two-fold benefit. Firstly, the higher pressure reduces the rate of water evaporation from the sample and secondly, the ionisation of the gas molecules in the chamber by the electron beam results in dissipation of the excessive charge that would otherwise build up on the surface of the sample. The nett effect is that the variable pressure instrument greatly simplifies the examination of biological samples. The emergence of the tabletop variable pressure scanning electron microscope has made this powerful technique much more readily available to hands-on scientists, since a dedicated microscopist or microscopy technician is not needed for operation. Samples can be mounted ready for use and high quality images produced by non-specialists in just a matter of minutes. Such is the compact size and ease of use, that in principle, the microscope could be installed on board the research ship for use during a research cruise!

Tabletop Electron Microscopy at BAS

The tabletop microscope contributes greatly to the morphological studies of many of the diverse species found in the seas around Antarctica and helps enhance existing knowledge about biodiversity. Of particular interest are gastropod and bivalve molluscs and isopods. Marine biologists at BAS use the TM3000 tabletop microscope from Hitachi [8], typically at magnifications between 30x - 5000x to view, measure and record morphological details of the hard components of these fauna, such as shells, opercula, radulae and ctenidia. The morphology of the shell of a gastropod *(e.g. Figure 1)* shows the whorls of the adult shell, which starts forming when the larval gastropod settles and becomes a juvenile. The operculum is an anatomical structure found in some marine gastropods. The operculum is also known as a 'little lid' and is attached to the upper surface of the gastropod's foot. In its most complete state, it serves as a sort of "trapdoor" to close the aperture of the shell when the soft parts of the animal are retracted [1]. An example is shown in *Figure 2*. The radula [2] is a minutely toothed ribbon, which is typically used by gastropods for scraping or cutting food before the food enters the oesophagus (analagous to the mouth parts). The arrangement of teeth on the radula ribbon varies considerably from one group to another.

Whilst examination of these structures is a powerful tool in charactering species, the microscope is also used to compare fauna currently existing in the region with previously discovered examples. For example cores from marine sediment retrieved from the region contain diatoms that can be thousands of years old. Diatoms are one of the largest and ecologically most significant groups of organisms on Earth. Comparisons with current examples and changes in species abundance within a sediment core can be used to infer possible changes in climate over the period, since changing climate could alter the mixing depths and delivery of nutrients to diatoms and their subsequent sizes. Shorter term comparisons can also be made with archived fauna collected since the earliest sample collecting expeditions in the 1940s.

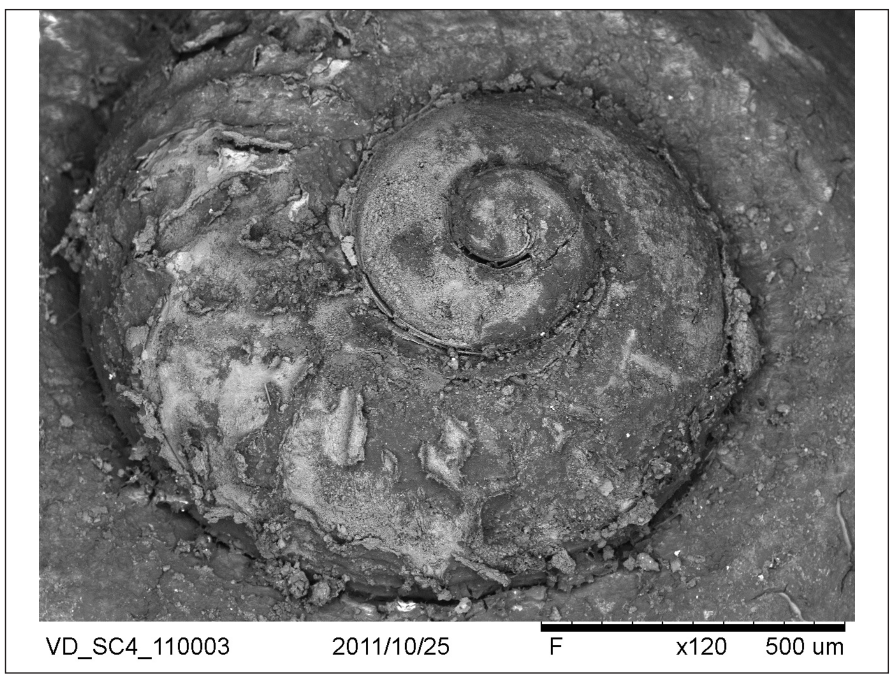

Figure 1. Apical view of Iheyaspira bathycodon shell

Identification of a New Species of Gastropod

While the Antarctic region is naturally the key area of research for BAS scientists, they also collaborate extensively with the environmental science community in the UK and overseas to share best practice and to maximise the scientific impact. One such collaboration has led to the identification of a new gastropod from the Von Damm Vent Field, an active, high-temperature hydrothermal system, on the world's deepest spreading centre, the Mid-Cayman Spreading Centre in the Caribbean, at 2300 m depth [4]. Gastropods are one of the most species-rich macrofaunal taxa in hydrothermal vents.

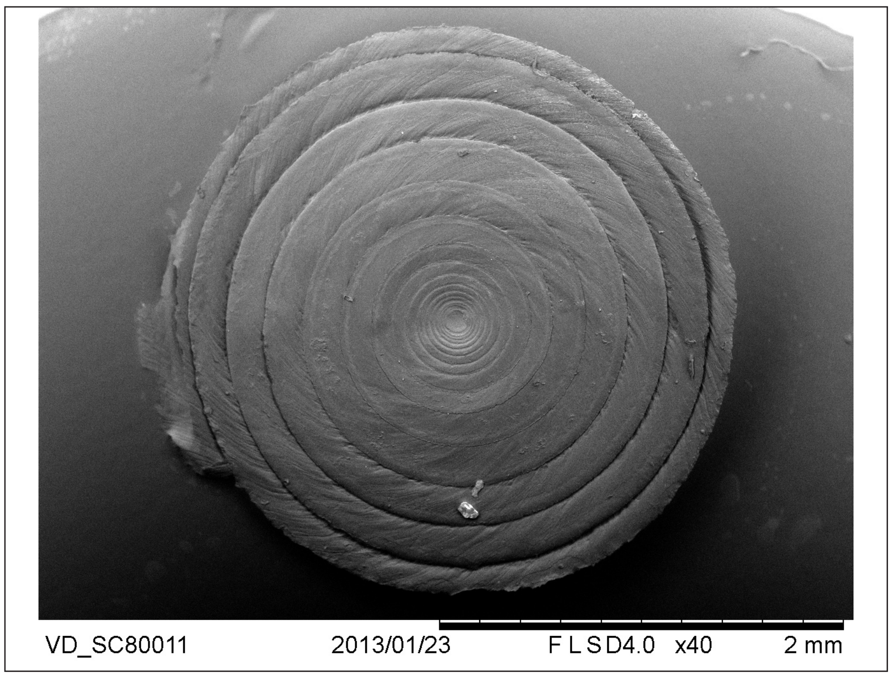

Figure 2. Operculum of Iheyaspira bathycodon

The new species, designated Iheyaspira bathycodon, was evaluated from molecular phylogenetics (the analysis of hereditary molecular differences, mainly in DNA sequences, to gain information on an organism's evolutionary relationships [5]) and morphological differences in the radula and appendage structure of the head-foot compared to the most closely allied vent species. The operculum and radula were mounted uncoated onto an aluminium sample stub and micrographs were taken with the tabletop microscope. The radula, in particular, is conventionally considered as a sample which is highly susceptible to charging and is therefore considered difficult to image. Using the variable pressure tabletop SEM, however, data was acquired quickly and easily without any charging artifact appearing in the image.

Radula Tooth Structure

Micrographs of the radula produced by the tabletop microscope *(Figures 3, 4 and 5)* show bilateral symmetry with at least 60 transverse rows of teeth along the total length. The central tooth is different in form from the lateral teeth *(Figure 3)*. It is smooth-sided, bell-shaped and wider proximally than distally, with a single incurved central cusp. The lateral teeth *(Figures 3 and 5)* are of similar size to the central tooth, increasing in size outwards; with a long, rounded single central cusp, and an outer apical margin with several flanking denticles; dentition attenuates towards the cusp and is strongest on the outermost lateral. *Figures 3 and 4* show more than twenty marginal teeth on both sides. The cutting plate is concave, terminating in a single short cusp, The apical margins are oblique, each with about 10–14 denticles that are longer and finer than those on the lateral teeth. The outermost marginal teeth in a row are smaller, with weaker dentition and straighter shafts. The marginal rows overlap each other and no jaws are present. This radula pattern appears to be unique in both number of teeth and shape. The new species is similar in morphology to Iheyaspira lequios [6] and Fucaria mystax [7], both of which are known only from hydrothermal vents in the Pacific at water depths less than 1500 m. However, differences in the radula (as well as the appendage structure of the head-foot not discussed here) differentiate it from both these species. The central tooth on the radula of the new species is bell-shaped, not rhombic/arrow-shaped like Iheyaspira lequios and there are only nine (not twelve) pairs of lateral teeth. It is closest in morphology to I. lequios and so the generic name Iheyaspira has been given to the new species to indicate the similarity between the two species. Iheyaspira bathycodon sp. nov. is the second new species to be described from the Von Damm Vent Field, and the tenth turbinid gastropod to be described from a hydrothermal vent environment to date.

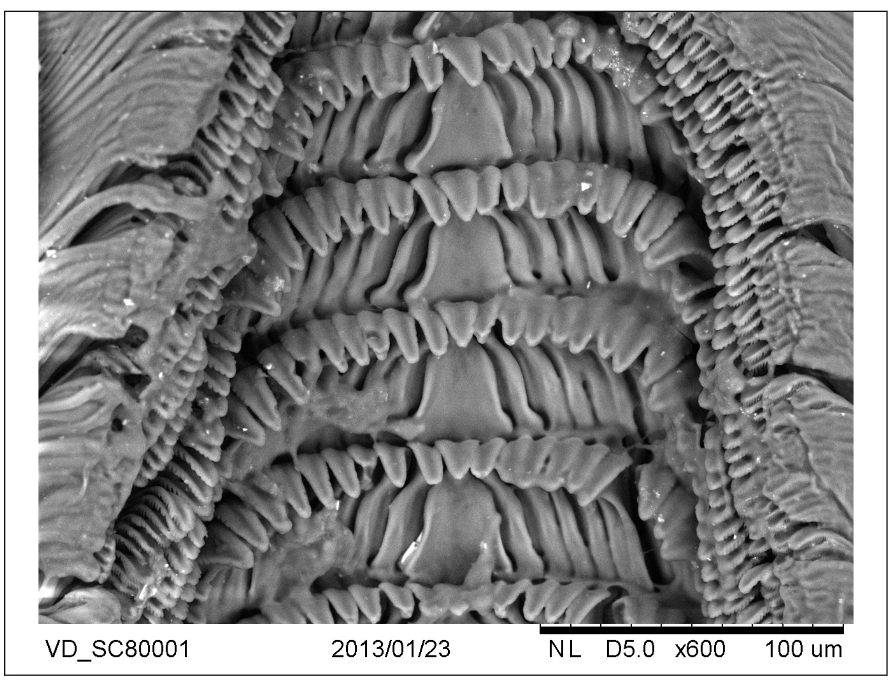

Figure 3. Iheyaspira bathycodon radula

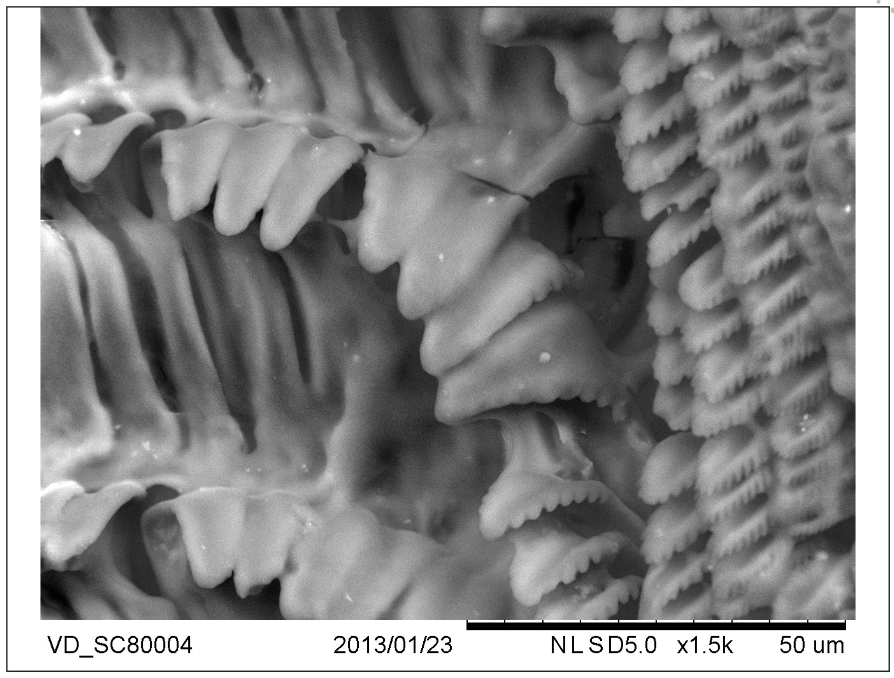

Figure 4. Iheyaspira bathycodon radula marginal teeth

Additional Capabilities

This article has concentrated on the importance of morphological information in marine biological research, and the ease with which high quality images can be produced using the tabletop electron microscope. However there are two other important capabilities offered by the tabletop microscope; atomic number contrast and chemical element analysis. Images in the tabletop microscope are produced by collecting high energy electrons backscattered from the sample. Backscattered electrons contain information related to the average atomic number of the materials in the sample which can result in enhanced contrast in the image if materials of different atomic number are present. In addition to generating backscattered electrons to form the image, the electron beam interacts with the sample to generate X-rays, which are characteristic of the chemical elements present. The addition of an energy dispersive X-ray analysis system to the tabletop microscope allows these X-rays to be collected and analysed to give semi-quantitative or fully quantitative analysis of the elemental composition. These two features have been of particular interest to geologists at BAS elsewhere, and some of their applications will be discussed in a future article.

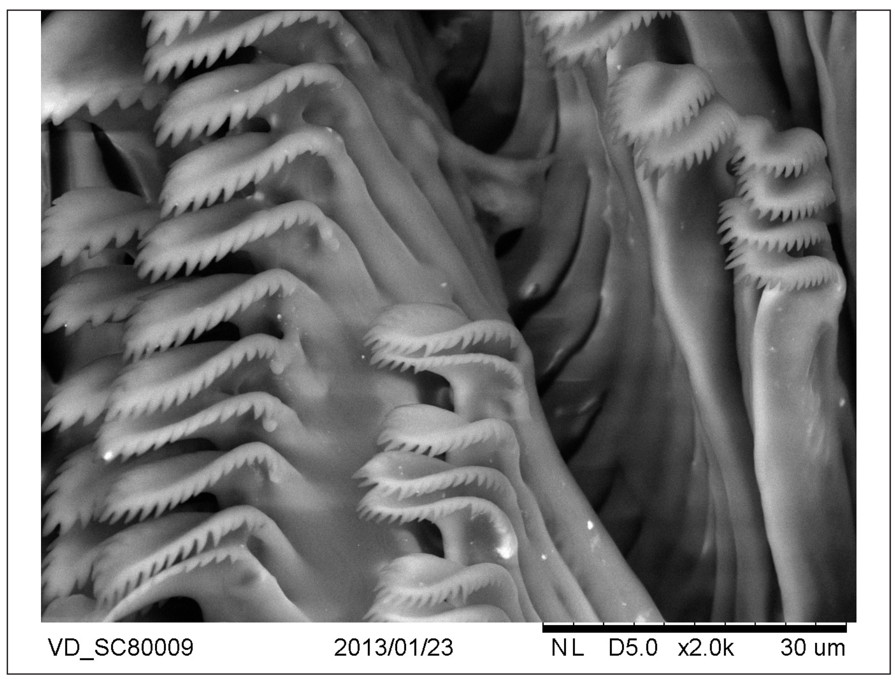

Figure 5. Iheyaspira bathycodon radula lateral teeth

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