

Biological Entities Isolated from the Stratosphere

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We have isolated what we believe are biological entities at a height of between 22-27 km in the stratosphere. Sampling of this region was carried out in the UK in July 2013 using a relatively simple low-cost, balloon-borne sampler carrying aseptically clean scanning electron microscope stubs onto which particles were directly captured. The entities varied from a diatom frustule to two unusual biomorphs and a filamentous organism. As far as we can tell, biological entities of this nature have not previously been reported occurring in the stratosphere. We challenge readers with our assertion that these biomorphs came from space.

A number of workers have shown that bacteria and fungi occur in the stratosphere at heights from 20-60km. (Wainwright, 2008). Early studies by members of our team showed that bacterial clumps of size in excess of 10micron can be found in the stratosphere and we have suggested that since these are too big to have been lofted from Earth, they must have come from space (Wainwright, 2008). To date our claim that microorganisms in the stratosphere come from space (Wainwright et al., 2003,2004a,b,c) has not been widely accepted, largely because it has been argued that there must be a means of elevating 10 micron particles from Earth to space. In order to counter such arguments we needed to find much bigger biological entities in the stratosphere, particles which are so large that an Earth origin is unlikely enough to be highly improbable, if not implausible. During a recent balloon-based sampling of the stratosphere we isolated such large particles (Wainwright et al., 2013a, b) the biological nature and presumed origin of which we discuss here. By presenting scanning electron microscope images, some accompanied by EDX elemental analysis data, we allow the reader to judge for themselves whether or not these particles are biological and whether they agree with our conclusion that they must have come from space.

How we sampled the stratosphere

A balloon-launched sampling device was released from Chester, NW England on 31.7.2013. The sampler included a drawer mechanism that could be opened and closed at any desired height using telemetry (Fig.1). The stratosphere sampler carried a video camera by which the opening and closing of the sampling drawer could be viewed, confirmed and recorded.

The sampling apparatus was protected from downfall of particulate matter from the balloon by a cover. Prior to launch, the inside of the draw device (Fig.1) was scrupulously cleaned, air blasted



Fig.1. The airtight drawer sampler which was opened and closed by remote control in the stratosphere, showing scanning electron microscope studs onto which stratospheric particles were directly deposited.

and finally swabbed with alcohol. New scanning electron microscope studs were placed in rows inside the drawer with their top surfaces facing outwards so that when the draw was opened any particulate matter in the stratosphere would attach to them and they could later be removed for examination under the scanning electron microscope. The protective layer on the surface of the stub was peeled off just before launching under a cover to prevent any particulate contamination. After sampling, the apparatus was transported to the laboratory and opened under conditions which avoided exposure of the stubs to contaminating dust and the stubs were similarly transferred under cover to the scanning EM. The stubs were then sputter coated with gold for 30secs at 30mA and then examined using a SEM (JEOL 6500F).

The balloon was launched from an open field near Dunham on the Hill (near Ellesmere Port, Cheshire, England) during daylight hours and traversed to just south of Wakefield in West Yorkshire (England). The sampling drawer was opened for 17 minutes as the balloon rose from 22026m to 27008m. The sampling apparatus was returned to Earth, by parachute, undamaged and completely intact.

A separate control flight was made to the stratosphere prior to the sampling flight, when the draw was not opened, but all other sampling procedures were observed. No particulate matter

was found (using the SEM) on any of the unexposed microscope studs, showing that the draw remained airtight and that none of the stubs was exposed to particles at, or near, ground-level or at any height up to the stratosphere. These results also show that no particles contaminated the stubs during any of the sample processing procedures, thereby demonstrating that the scrupulous procedures used to prevent ground level contamination proved effective and that no such contamination occurred.

A diatom fragment isolated from the stratosphere

A major problem which faces anyone researching the microbiology of the stratosphere is the difficulty of distinguishing between biological entities; particularly bacteria and fungi from particles of cosmic dust which in some cases can mimic the morphology of these entities, notably bacteria (Wainwright et al., 2004a,b,c; Wainwright et al., 2006; Wainwright, 2008). The finding of diatom frustules in stratosphere samples would avoid this problem since these structures can be unequivocally recognised as biological entities.

We have in fact isolated a fragment of a diatom frustule (Fig.2) from a height of 220-27 km which, we assume, is clear enough for experts on diatom taxonomy to identify it (possibly it is a species of *Nitzschia*). The diatom fragment shown in Fig.2

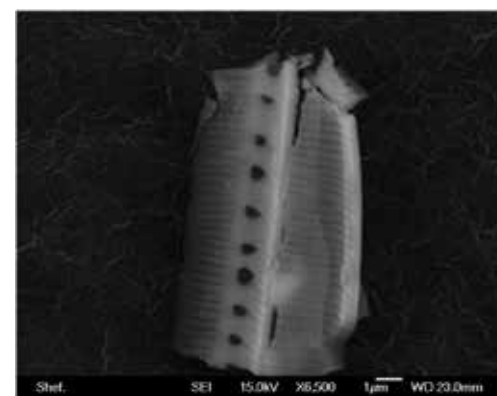


Fig. 2. SEM of a diatom frustule (freshwater *Nitzschia* species?) captured on a stub from a height of 22-27km in the stratosphere

is obviously incomplete and probably does not contain a protoplast, and is therefore part of an empty frustule. We do know if the diatom reached the stratosphere as a fragment, or whether it arrived with a viable protoplast inside an intact frustule. Critics might claim that the bacteria we early isolated from the stratosphere do not come from this region but are contaminants which originate from the balloon itself, or during later processing of the stratosphere-derived samples in the laboratory. This criticism however, emphatically does not apply to the diatom frustule shown in Fig.2, simply because diatoms do not occur on pristine balloons and in laboratory air, nor are they associated with laboratory workers. The methods used to clean the sampling apparatus would also prevent such contamination, as would the methods used to maintain sterility before the EM stubs were exposed to the stratosphere. It is therefore clear that the particle shown in Fig.2 is a fragment of a diatom frustule which was isolated from the stratosphere at a height of between 22 and 27km.

Other biological entities from the stratosphere

The structures shown in Fig. 3A, B are distinct from inorganic cosmic dust and are clearly biological in nature. Figure 3A shows a complex organism which has a segmented neck attached to a flask-shaped body which is ridged and has collapsed under the vacuum of the stratosphere or which has been produced during EM analysis. The top of the neck is fringed with what could be cilia or a fringe which formed the point of attachment of the neck to another biological entity. The complexity of this particle excludes the possibility that is of non-biological in origin. It is clearly too large to be bacterium, but could be an algae or a protozoan; it appears however, too small to be an angiosperm seed and the fact that the main body has a thin wall appears to exclude the possibility of it being a pollen grain, which generally have rigid outer coats

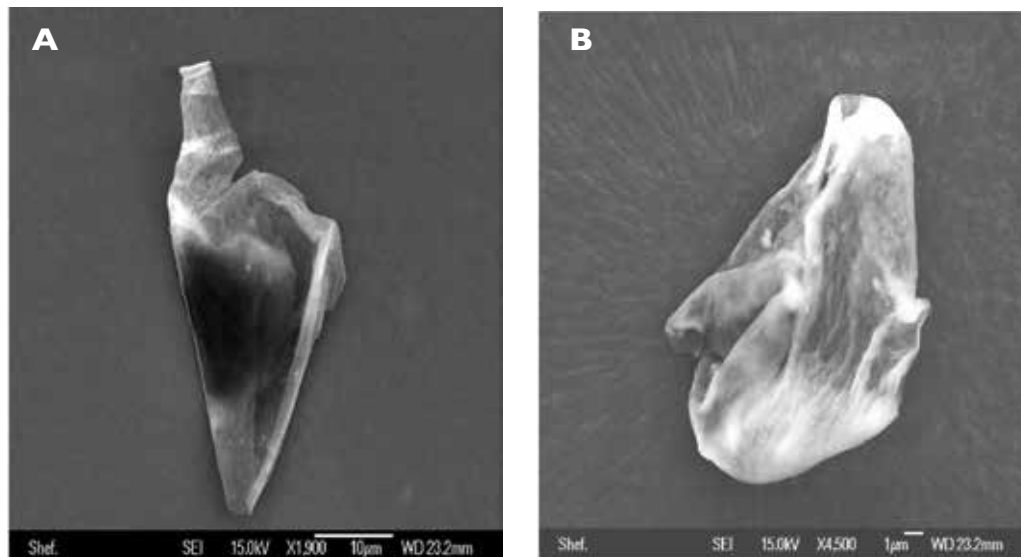


Fig. 3 Unknown biological entities isolated from the stratosphere and A, a complex shaped structure with an ornamented neck; B a collapsed balloon-like biological entity; note "proboscis" to the left, with nose-like openings and the "sphincter" present at the top of the organism. Also note the impact lines radiating from the particle.

that do not generally collapse when exposed to low pressure. The structure shown in Fig.3B is also clearly biological in nature; here we see a somewhat phallic balloon-like structure which has collapsed under low pressure. A "proboscis" is seen emerging from the left of the main cell which has two, nostril-like openings. At the top of the collapsed "balloon" is a sphincter-like opening. Again, this entity is clearly biological in nature, and is not an inorganic particle; although it is clearly not a bacterium it could be an alga or a protozoa. Note also that the particle shown Fig. 3B has disturbed, in a radial fashion, the carbon material comprising the scanning EM stub; suggesting that it impacted at speed, much in the same way as would a micrometeorite impacting

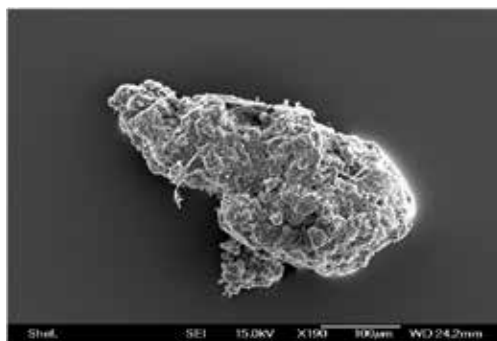


Fig. 4. A stratosphere-isolated particle which exceeds 300 micron in size; it includes biomorphic filaments.

from space. The organisms shown in Fig 3 are again presumably shown clearly enough for experts in the required branches of taxonomy to provide some kind of identification.

A very large particle (exceeding 300 microns) is shown in Fig.4. It is made up of inorganic cosmic dust interspersed with filaments which look like filamentous fungi. A close up image of a filament from a similar large stratospheric particle which was isolated on the same sampling trip is shown in Fig.5. The filament appears biological and could be fungal or a filamentous bacterium such as *Nocardia*. Note that the filament is flattened, either by the low pressure of the stratosphere, or as the result of imaging with the scanning electron microscope. Such flattening is commonly seen when fungal hyphae are examined under the EM and shows that the filament seen here is a tube (i.e. typical of biology) and not a solid rod (i.e., typically inorganic). An EDX analysis of one of the filaments (Fig.5), shows that it made up largely of carbon and oxygen and is therefore an organic (biological) filament and not an inorganic structure, such as a silicon whiskers; we conclude then that the large particle, shown in Fig.4, which was isolated from the stratosphere, contains biological filaments.

From where do these biological entities originate: space of Earth?

How did these biological entities reach the stratosphere? Let us first consider the diatom fragment shown in Fig.2. From the data given by Kasten (1968) we can calculate that a diatom of radius in the range of 3-10 micron would fall at an average of 1cm s⁻¹ at a height of 20km, and that the residence time of any particle lofted to this height is about 6 hours. The conclusion is that even if a major volcanic eruption occurred a few days before the sampling event, no particles of the size of the diatom fragment would have been retained in the stratosphere at the point of sampling. There is no record that any such eruption took place. In fact, the most recent major volcanic activity occurring close to the UK was the Eyjafjallajökull volcano, which erupted on Iceland in early 2010 and caused considerable problems to commercial flights.

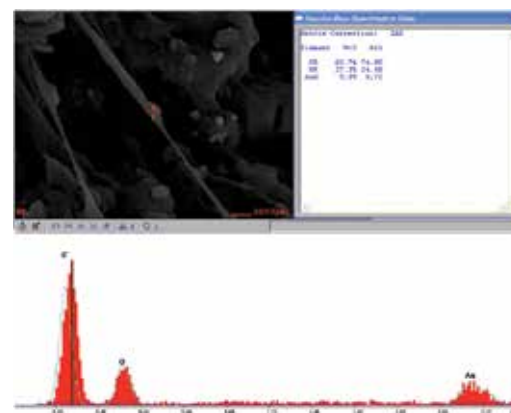


Fig. 5. EDX analysis of a biological filament showing it is made up of carbon and oxygen and is not inorganic.

There is certainly no way, as is often casually suggested by unthinking critics, that a particle, like the one seen in Fig. 2 could simply float into the stratosphere or be carried by winds up to heights well above the tropopause. Other possible mechanisms by which the particle could theoretically be carried into the stratosphere include gravito-electrophoresis and the involvement of blue lightning (Wainwright

et al., 2008), but even these would probably only theoretically elevate particles of radii greater than 1 micron to the stratosphere. Rosen (1969) concluded that particles larger than 5 micron in size do not reach the stratosphere from Earth, so while it is possible that a single bacteria (of size around 1 micron) could be elevated from the Earth's surface to the stratosphere, the biological entities shown here or, the inorganic particle masses which they form a part are too big to make this journey.

The biological entities shown in Fig. 3-5 are particles of relatively large size and mass and by our current understanding of the means by which such particles can be transferred from Earth; such reasoning particularly applies to the 300 micron-plus particle (which contain biological filaments) shown in Fig.. Of course the standard rebuttal to a fragment of space origin is to assert that Occam's razor informs us that there *must* be a mechanism for lofting particles of this size from Earth to the stratosphere and that our findings are proof of the existence of such an unknown mechanism - the search for which can now begin.

While an Earth origin for these biological entities may be invoked in order to meet criteria of parsimony and conservatism, we argue that since no major violent volcanic event or other atmospheric event occurred close to the time and place of sampling, the biological entities discussed here most plausibly came from space and it is worth noting that the sampling trip which resulted in the recovery of this fragment was carried out in mid-late July 2013 during the period of the annual Perseid meteor shower. It is also noteworthy that the biological entities shown here are clean and free of cosmic dust or soil particles, thereby suggesting they have an aquatic origin. Water spout from the Earth's oceans are unlikely to ever reach the lower stratosphere to act as a carrier of marine organism to this region. Comets on the other hand provide a vast cosmic source of water from where such biological entities could have arisen. It is likely that, because of the very low temperatures and high

exposure to UV radiation, such biological entities would not be alive in the stratosphere, although bacteria existing within clumps of cosmic dust would be protected from such radiation and could arrive to Earth in a viable state. Even if these entities were not alive when they arrived to the stratosphere and then Earth, they might still carry DNA and RNA, which could possibly integrate with the genomes of Earth's life forms or make up part of the vast pool of environmental DNA which cannot be currently linked to cultivable microorganisms. The use of isotope fractionation provides a possible means of determining whether the biological entities described here are of Earth or cosmic origin; we eagerly look forward to performing this test on our stratosphere samples.

In conclusion, we accept that it is difficult to believe that biological entities are continually raining down to Earth from space. Doubters who claim that the particles shown here are not biological in nature need to provide evidence that inorganic particle can match their form and complexity. Similarly, those who claim that stratosphere-derived biological entities like the ones of shown here, by necessity, came from Earth, must show how established physics can be circumvented in order for particles which generally exceed 10 microns and can reach 300 micron in size, can be elevated from Earth to 22-27km. In the absence of such alternatives and mechanisms we will continue to assert that the findings presented here show that biological entities are continually arriving to Earth from the cosmos.

Acknowledgements: Photographs from the *Journal of Cosmology*, with permission.

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