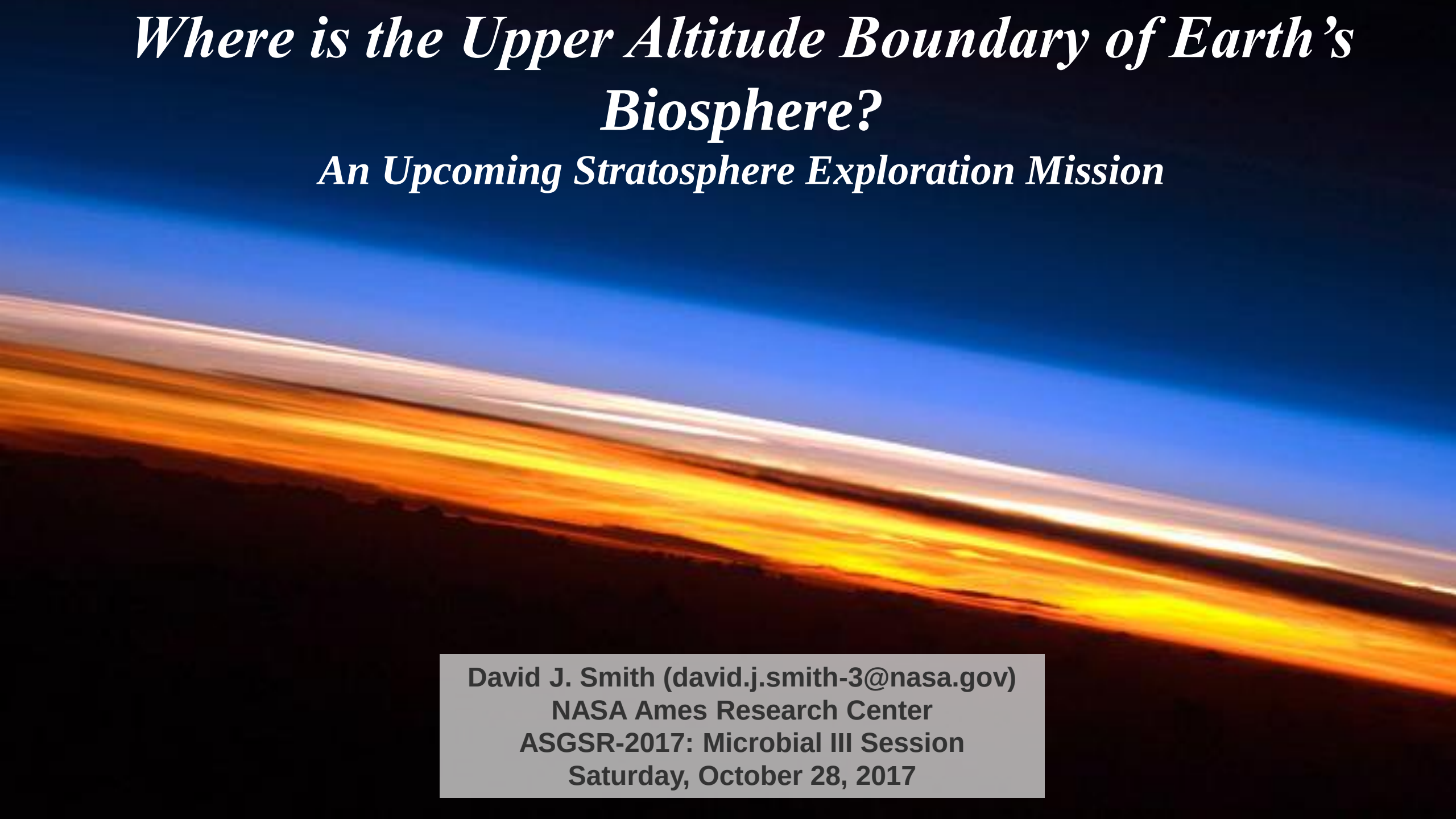
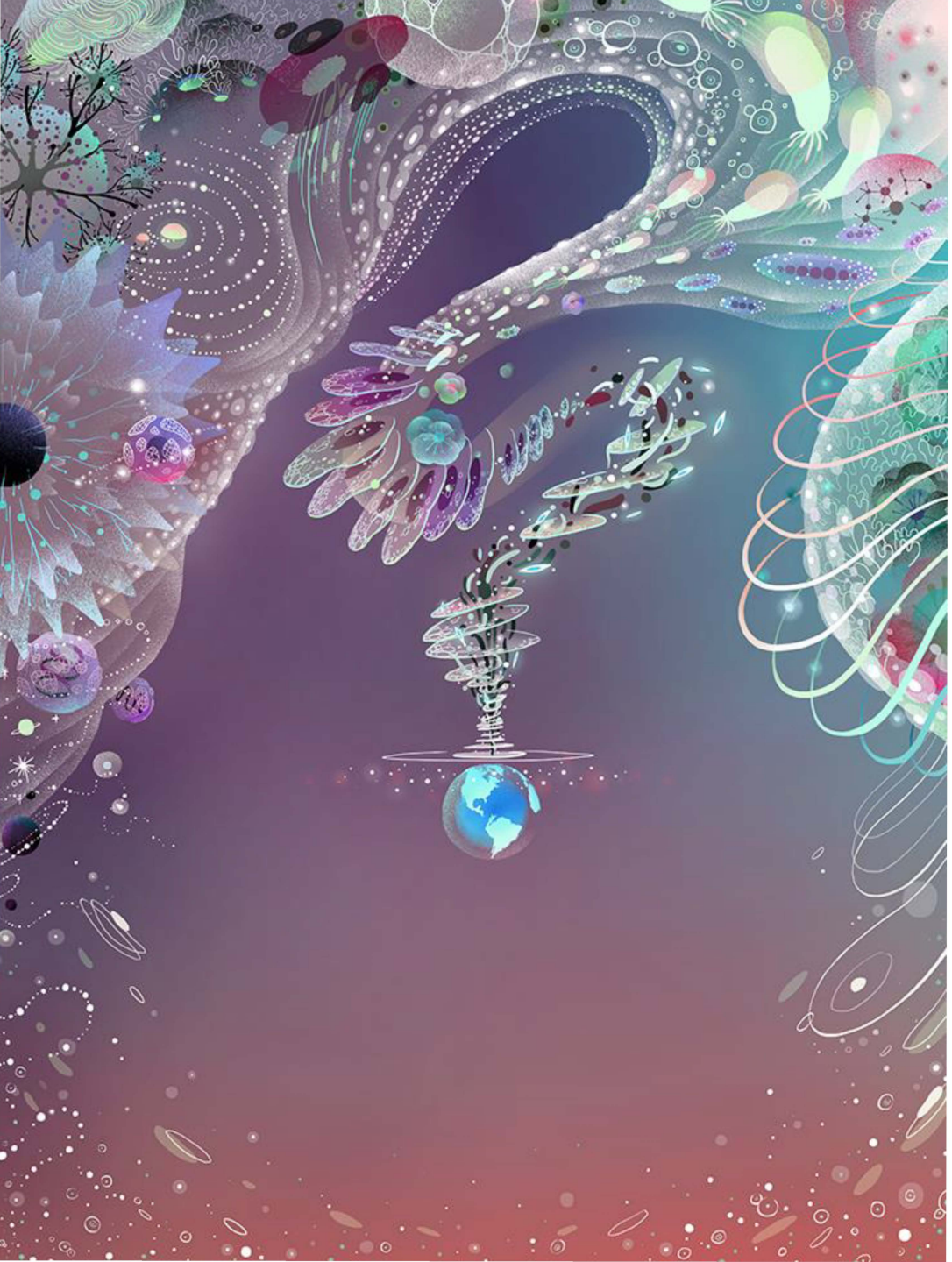
Where is the Upper Altitude Boundary of Earth's Biosphere?

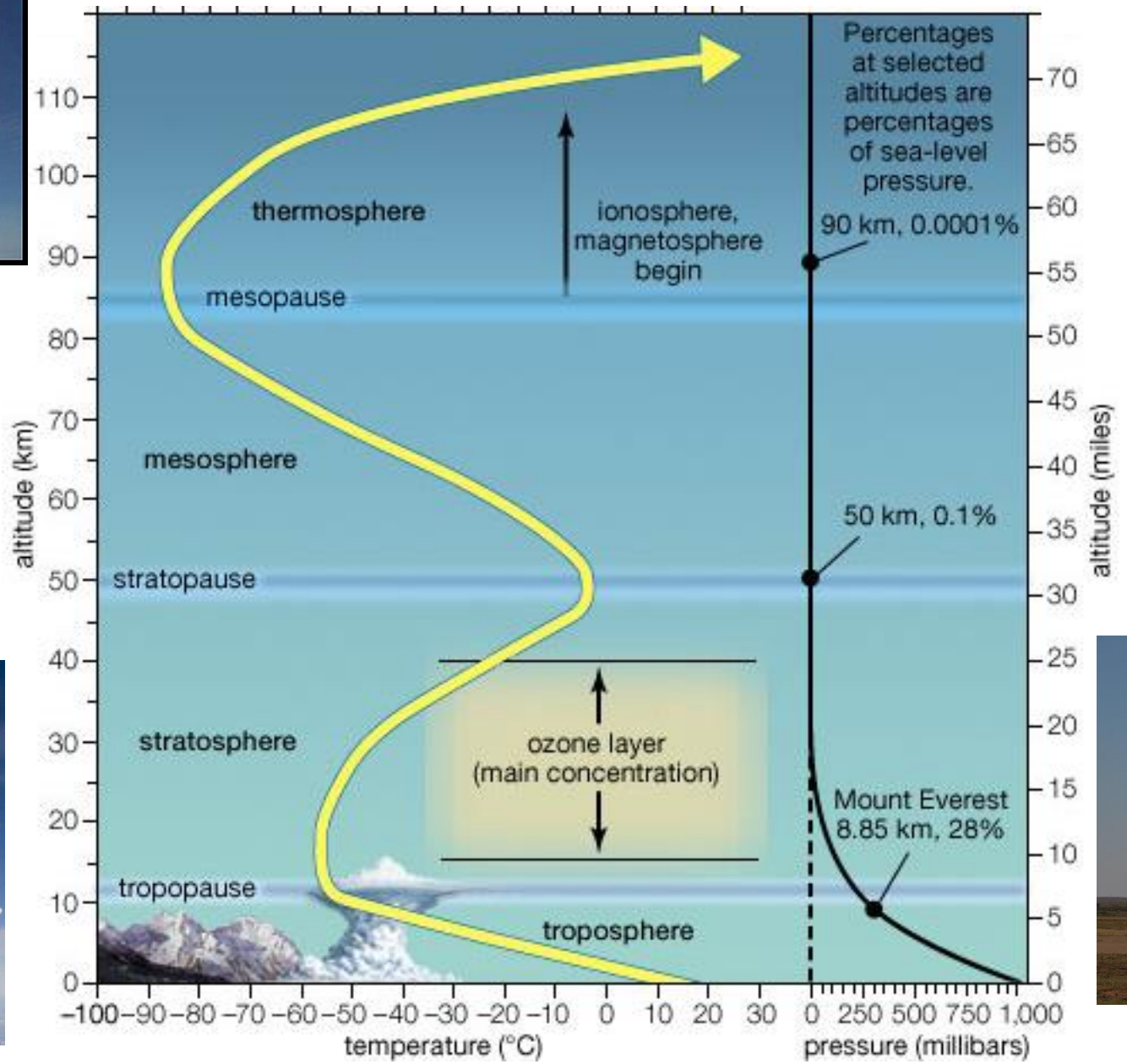
An Upcoming Stratosphere Exploration Mission

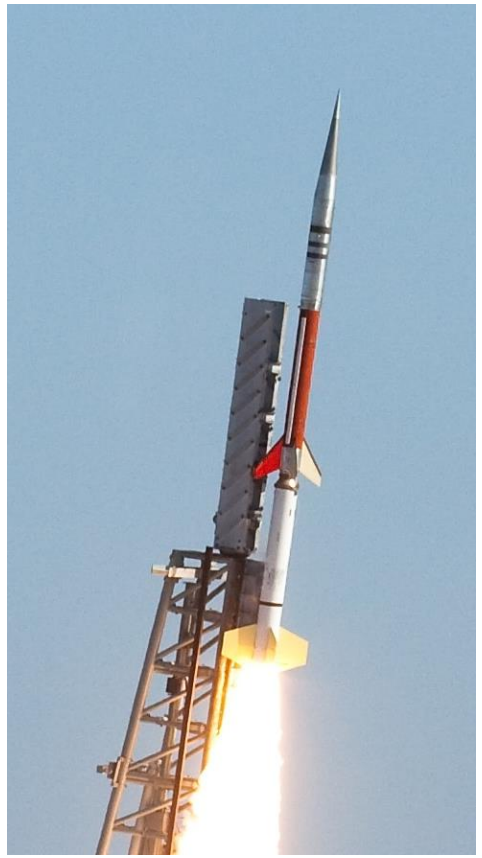


David J. Smith (david.j.smith-3@nasa.gov)
NASA Ames Research Center
ASGSR-2017: Microbial III Session
Saturday, October 28, 2017



EARTH'S ATMOSPHERE

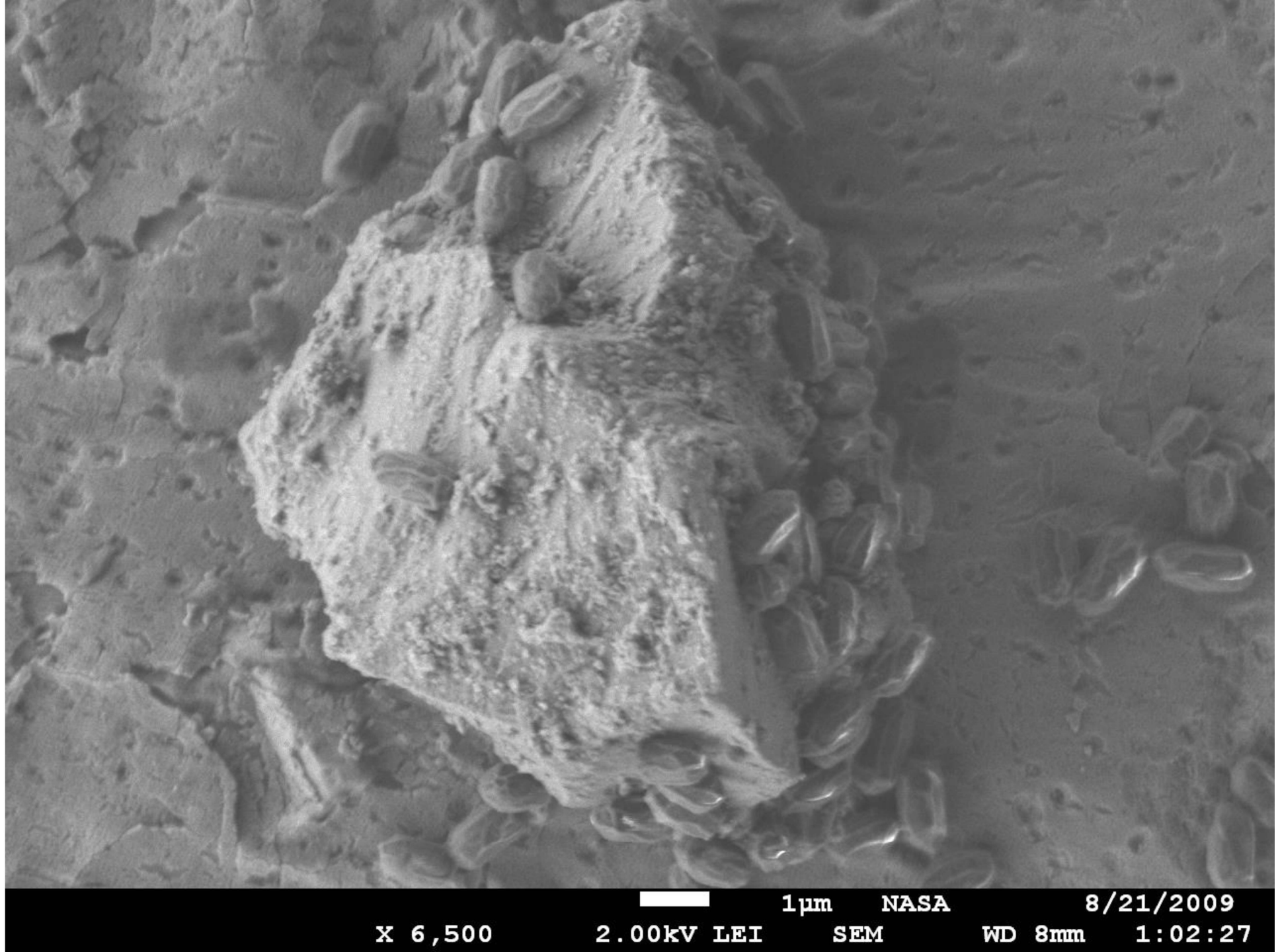


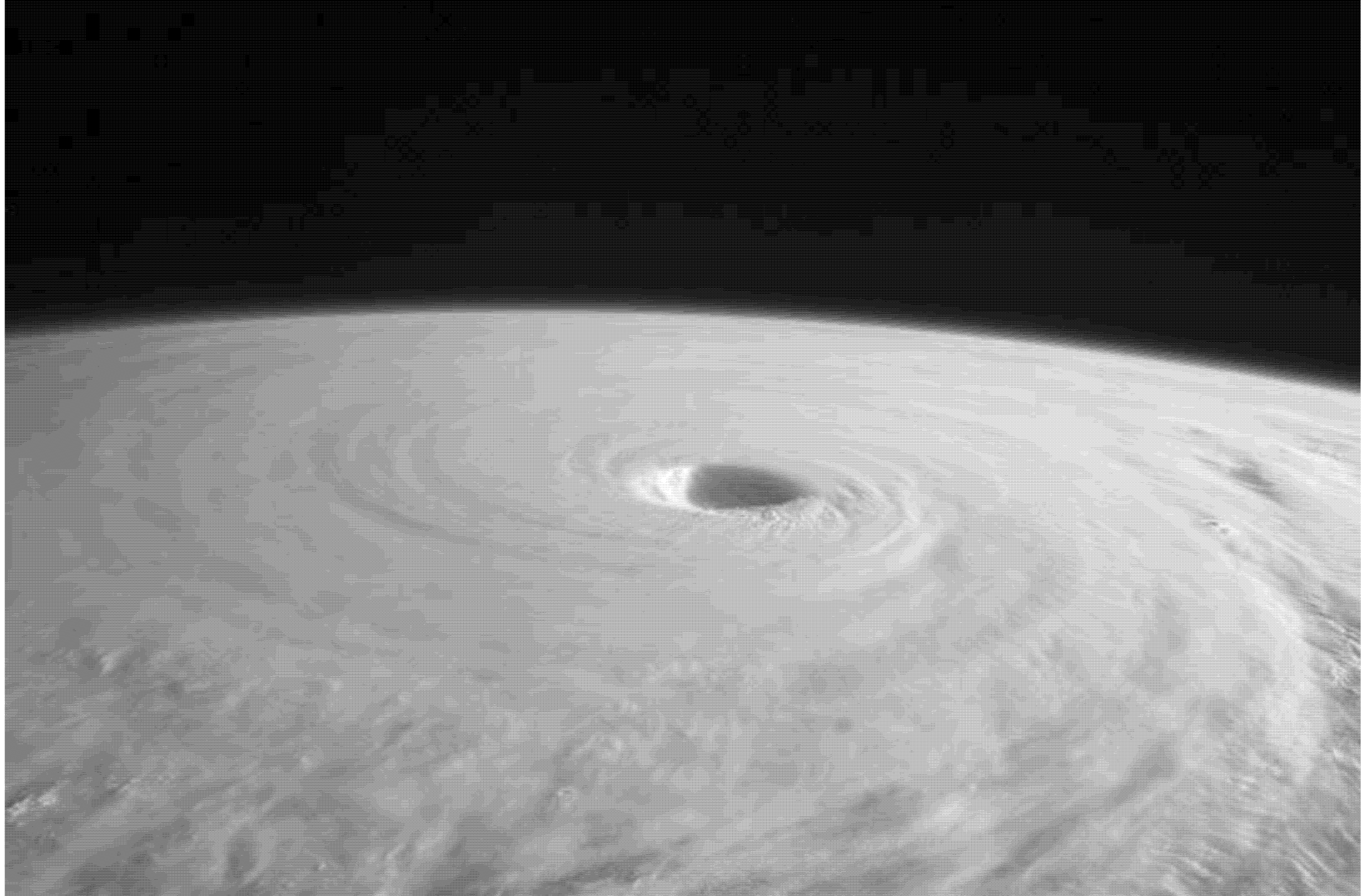


Felix Baumgartner

October 14, 2012

128,000 ft
(~39 km)


1µm NASA 8/21/2009
X 6,500 2.00kV LEI SEM WD 8mm 1:02:27



PNAS

Microbiome
composition
storms, and

Natasha DeLeon-Rodriguez[a]
Andreas J. Beyersdorf[d], Luk
and Konstantinos T. Konsta

[a]School of Biology, [b]School of Earth
Engineering, Georgia Institute of Te
Administration Langley Research Ce

Edited by W. Ford Doolittle, Dalhou

LETTER

LETTER

Inadequate methods and questionable conclusions in atmospheric life study

Our aerobiology team was pleased to read "Microbiome of the upper troposphere: Species composition and prevalence, effects of tropical storms, and atmospheric implications" by DeLeon-Rodriguez et al. (1). On the opposite side of the continent, at a different time of year, and using other molecular methods, we also found rich bacterial assemblages in the upper troposphere capable of long-range transport (2, 3). We were delighted to see the convergence and hope it will inspire follow-up investigations because the field truly needs a global monitoring network to enhance understanding of patterns and implications. However, we have some concerns about the work by DeLeon-Rodriguez et al. (1).

First, the *Materials and Methods* (both in the article and *SI Materials and Methods*) of DeLeon-Rodriguez et al. (1) were inadequate for follow-up studies. Publishing air-pump specifications is absolutely critical for standardizing aerobiology methods. No details or citations of the air pump used onboard the DC-8 aircraft were provided. In addi- in the atmosphere. Our recent work used filters and a high-volume air pump just like DeLeon-Rodriguez et al. (1). We processed on average 360 m^3 of ambient air per sample and measured about 6–20 cells (2). DeLeon-Rodriguez et al. (1), in comparison, only pumped on average 6 m^3 of ambient air per sample, yet the authors measured about 5,100 cells. It seems strange that a pump 60-times less efficient than ours would capture ~1,000 times more cells. Perhaps the air-filtered column values listed in Table S1 of DeLeon-Rodriguez et al. (1) were miscalculated? There seem to be other errors in the same table. For example, how could bacterial contribution be 276% on September 16, 2010?

Third, referring to the possibility of the atmosphere as an ecosystem, the authors concluded "...it is conceivable that these groups could remain metabolically active in clouds" (1). The line between viability and active growth/metabolism was blurred with this conclusion. There is no evidence in this report that microorganisms can metabolize at the extreme low temperatures in the up-

Finally, DeLeon-Rodriguez et al. concluded that "...bacteria are at least two orders of magnitude more abundant relative to fungi at high altitudes" (1). However, the timing of the sampling flights (August and September) was never discussed. Lower fungal numbers would be expected in the late summer, and a seasonal influence should have been addressed before arriving at such a conclusion. Most Northern Hemisphere fungi release reproductive spores in the springtime, and indeed our work during that season in 2011 measured a more even ratio of airborne bacteria to fungi (2).

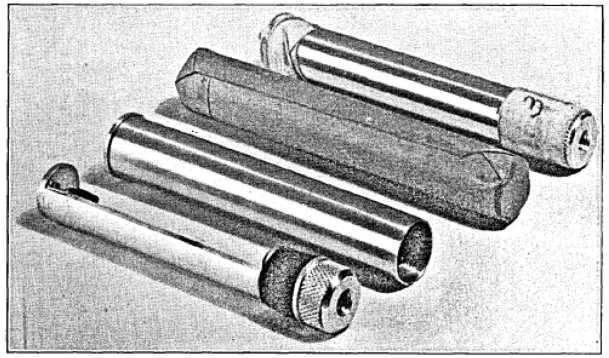
We were disappointed that these basic issues were overlooked during peer review.

David Joseph Smith[a,1] and Dale Warren Griffin[b]

[a]National Aeronautics and Space Administration Surface Systems Office, Kennedy Space Center, FL 32899; and [b]US Geological Survey, Coastal and Marine Science Center, St. Petersburg, FL 33701

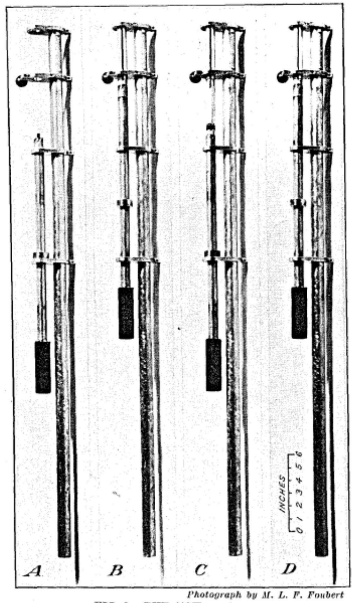
Photograph by Charles and Anne Lindbergh. Used by special permission. Copyright, National Geographic Magazine

FIG. 7. BLACK MOUNTAINS PUSH JAGGED POINTS THROUGH THE SNOW
A TIP OF THE WING SHOWS IN THE PICTURE AS THE PLANE SKIRTS THE ICE CAP SOUTHWARD FROM CLAVERING ISLAND TO ANGMAGSSALIK.

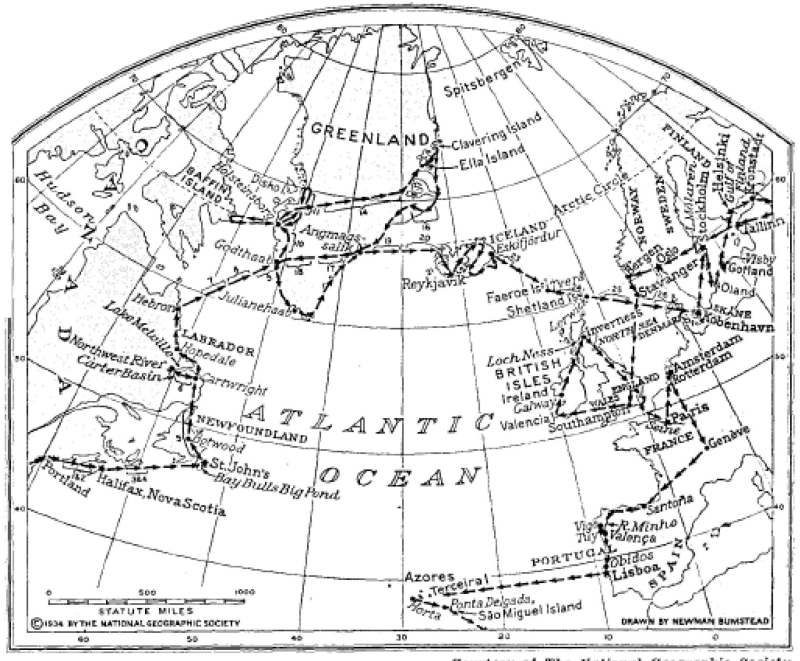


Photograph by M. L. F. Foubert

FIG. 4. EACH SLIDE CONTAINER OR CARTRIDGE CONSISTS OF TWO MAJOR PIECES



Photograph by M. L. F. Foubert



Courtesy of The National Geographic Society

FIG. 2. MAP SHOWING ROUTES FLOWN AND POINTS BETWEEN WHICH COLLECTIONS WERE MADE. NUMBERS INDICATE INDIVIDUAL COLLECTIONS AND REFER TO RECORDS SOME OF WHICH ARE GIVEN IN FIGURES 8 AND 9.

THE SCIENTIFIC MONTHLY

JANUARY, 1935

COLLECTING MICRO-ORGANISMS FROM THE ARCTIC ATMOSPHERE

By FRED C. MEIER

COOPERATIVE INVESTIGATIONS, BUREAU OF PLANT INDUSTRY AND WEATHER BUREAU, U. S. DEPARTMENT OF AGRICULTURE

WITH FIELD NOTES AND MATERIAL

By CHARLES A. LINDBERGH

WHEN the red-winged monoplane piloted by Charles A. Lindbergh soared away from Flushing Bay on July 9, 1933, bound for aerial exploration near the Arctic Circle, there began an unusual botanical collecting trip. Mrs. Lindbergh was prepared to fly the ship during intervals when her husband might be occupied with manipulation of an instrument new to transatlantic airplanes—so new, in fact, that it was completed just in time for the writer to carry it by plane from Washington to New York to be added to other scientific equipment which had been assembled for the expedition. With this new device, which, being untried, was noncommittally called the "sky hook," it was planned to make collections of microorganisms from the atmosphere along the course of flight. As an incidental feature of their aerial voyage, the two flyers were cooperating with the U. S. Department of Agriculture in its studies of the epidemiology of rusts and other plant diseases. It was also hoped that identification of materials collected at various altitudes between points on the course might contribute to our knowledge of the movement of air currents in northern regions.

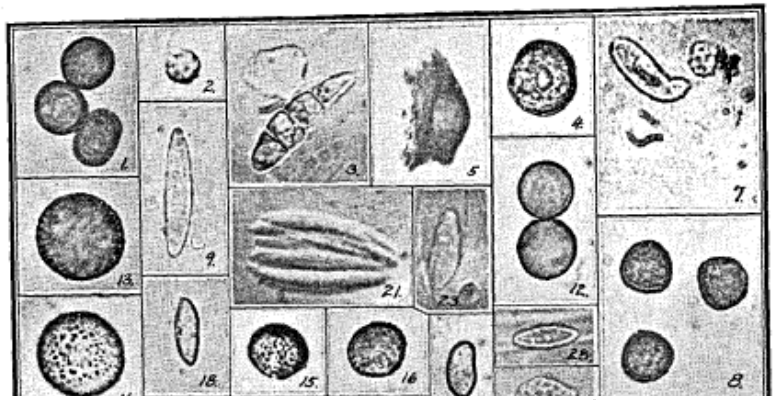
HISTORY OF AIR-CONTENT STUDIES

While it is generally known that bacteria, spores of higher fungi and pollen grains are present among dust particles in the atmosphere near the earth's surface, much detailed information of practical value remains to be revealed by further research. The aerial movement of pollen from certain flowering plants concerns the physician who deals with "hay fever" and related troubles. The plant pathologist and the medicopathologist are interested in obtaining facts concerning the part that air currents may play in disseminating reproductive bodies of organisms that cause specific diseases of plants and animals. Definite information of this sort is obviously an aid to a well-planned control program. As early as 1921 airplanes were used in making collections of rust spores as an aid to planning the barberry-eradication campaign for the control of stem rust of small grains. This work, by E. C. Stakman,[1] A. W. Henry, G. C. Curran, W. N. Christopher and pilots of the Army Air Corps, in the course of cooperative investigations of the U. S. Department of Agriculture and

[1] Stakman *et al.*, *Jour. Agr. Research*, 24, 1923.

The results of our new upper air studies bring to light fundamental principles lead to many practical applications, perhaps the most important of which are improved measures of control of diseases of plants and animals.” To get the hang of how to handle them we “exposed” a couple of aluminum cylinders before starting. It happened that Fred coughed upon a slide of one of these. “That’s ruined,” he said, starting to throw it away. “The collection of germs on that slide would look like a menagerie under a microscope.”

But I insisted on adding that cylinder to our collection. I thought it would give the laboratory workers something unique to ponder when they came upon its contents among the more innocent bacteria of the equatorial upper airs. Heaven knows what cosmic conclusions Fed’s contribution might help them reach! . . . such absurd procedure must be debited to a pilot’s perverted sense of humor.”



CHECK SLIDES

Six slide containers, returned unused after having been carried throughout the trip, were employed as checks. Careful microscopic examination of the slides within demonstrated these to be free from spores and pollen grains.

Numerous examinations of petrolatum from the lot used in preparing the slides for the trip, likewise gave confidence in results obtained.

Photomicrographs by F. C. Meier

FIG. 11. SOME OF THE MORE CONSPICUOUS OBJECTS FOUND ON SLIDE 9. X 660. (SEE FIGS. 2 AND 8.)

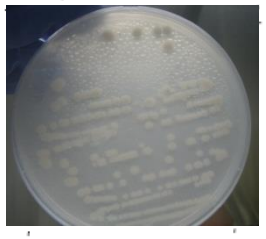
Aerobiologia (2010) 26:35–46
DOI 10.1007/s10453-009-9141-7

ORIGINAL PAPER

Stratospheric microbiology at 20 km over the Pacific Ocean

David J. Smith · Dale W. Griffin · Andrew C. Schuerger

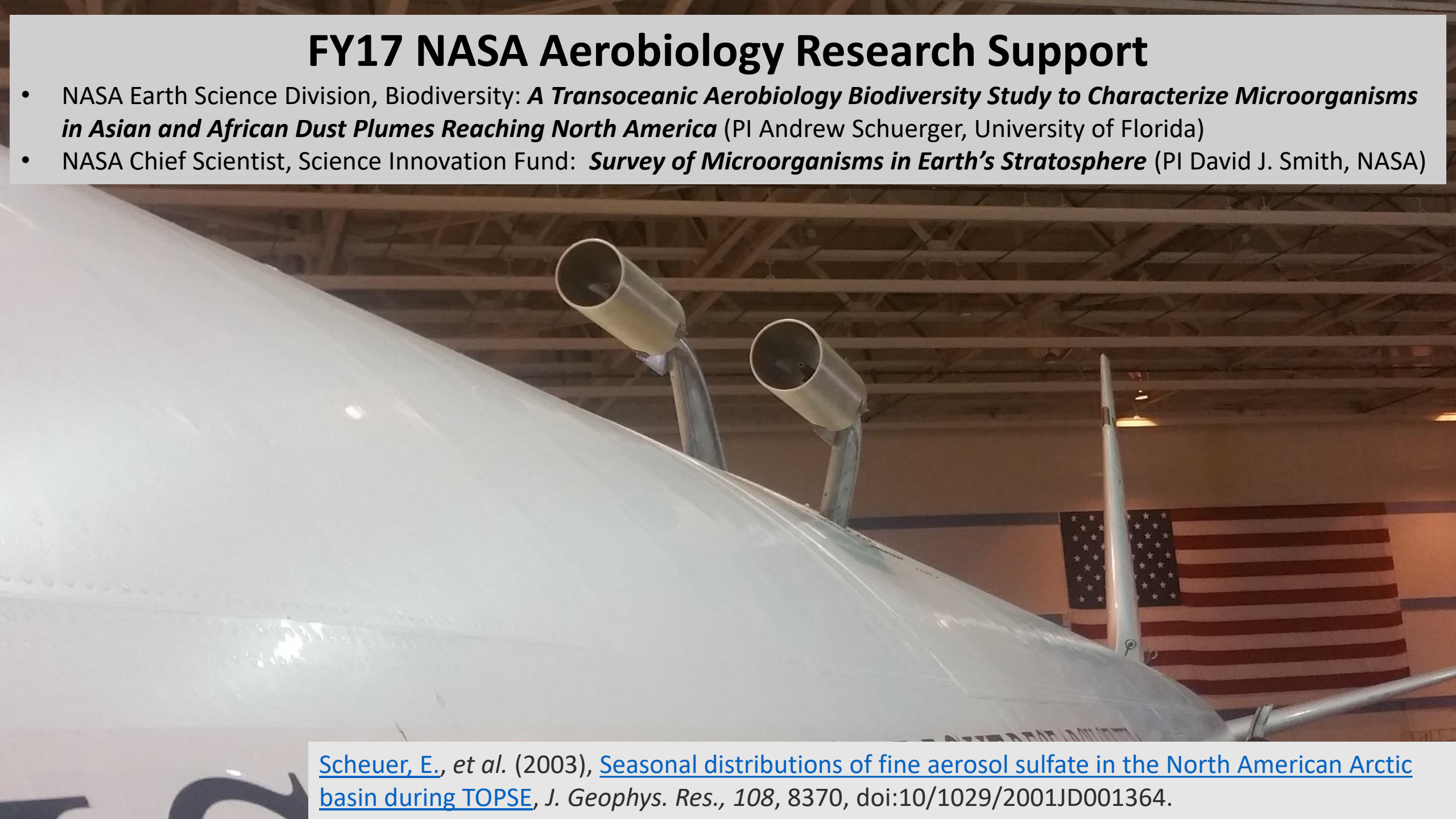
Bacillus endophyticus, *Bacillus subtilis*, *Bacillus* sp.



class *Eurotiomycetes* and *Penicillium* sp.

FY17 NASA Aerobiology Research Support

- NASA Earth Science Division, Biodiversity: ***A Transoceanic Aerobiology Biodiversity Study to Characterize Microorganisms in Asian and African Dust Plumes Reaching North America*** (PI Andrew Schuerger, University of Florida)
- NASA Chief Scientist, Science Innovation Fund: ***Survey of Microorganisms in Earth's Stratosphere*** (PI David J. Smith, NASA)



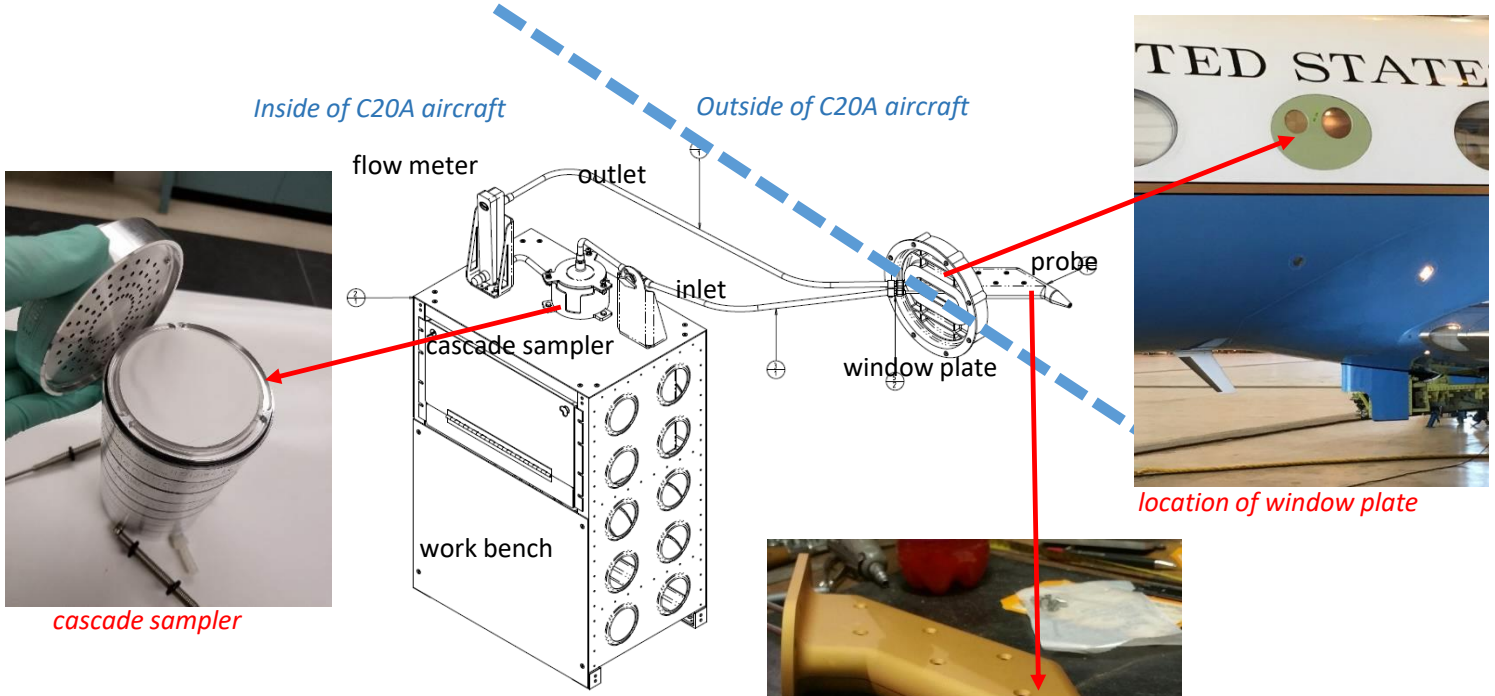
Scheuer, E., *et al.* (2003), Seasonal distributions of fine aerosol sulfate in the North American Arctic basin during TOPSE, *J. Geophys. Res., 108*, 8370, doi:10/1029/2001JD001364.

Platform Name	Center	Duration (hours)	Useful Payload (lbs)	GTOW (lbs.)	Max Altitude (ft)	Air Speed (knots)	Range (n. mi)
C-20A (G-III) - Armstrong	NASA AFRC	7.00	2,500	69,700	45,000	460	3,400

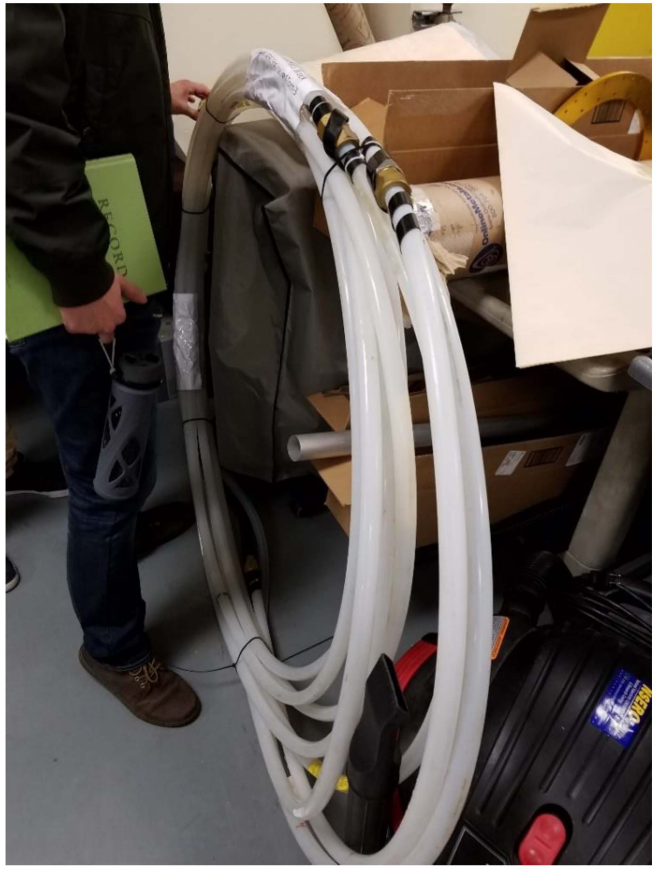
The NASA C-20A (Gulfstream III) is a business jet that has been structurally modified and instrumented by NASA's Armstrong Flight Research Center to serve as a multi-role cooperative research platform for the earth science community and a variety of flight research customers. This particular aircraft, which carried the military designation of C-20A, was obtained from the U.S. Air Force in 2003.

NASA C20A Aircraft Flights

Aim 1: Measure microbial abundance and diversity in the lower stratosphere using state-of-the-art metagenomic methods
Aim 2: Isolate & archive viable microbes collected from stratosphere
Aim 3: Characterize atmospheric transport history of bio-aerosols

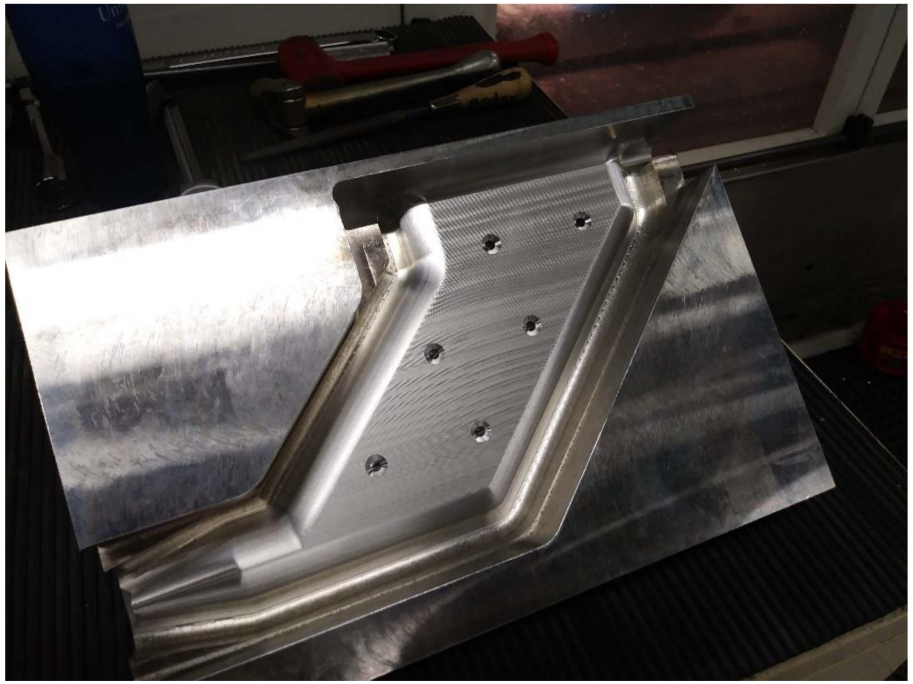


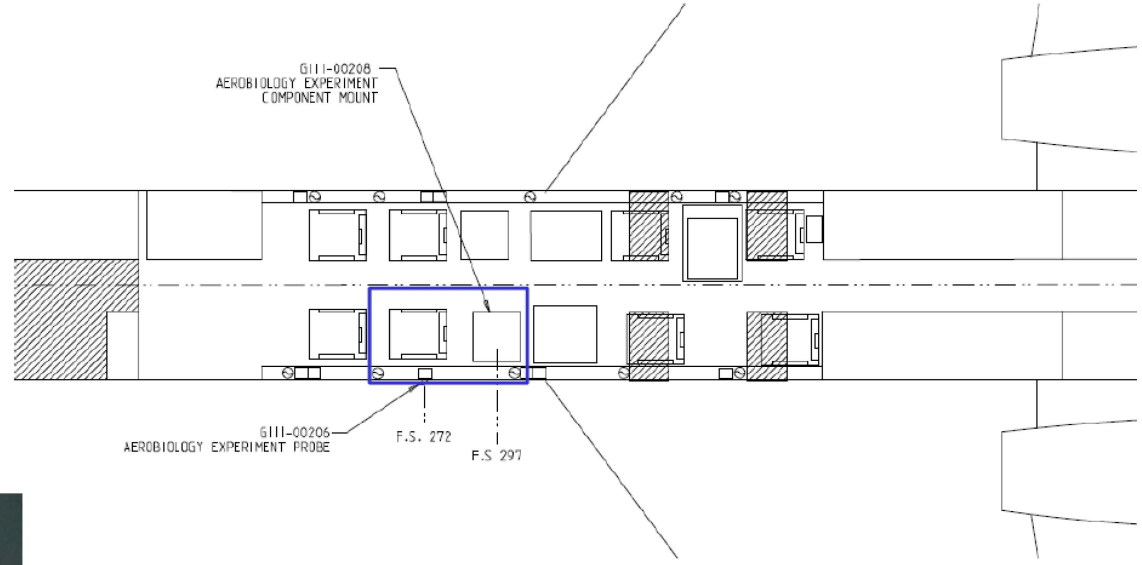

OMAX
5555



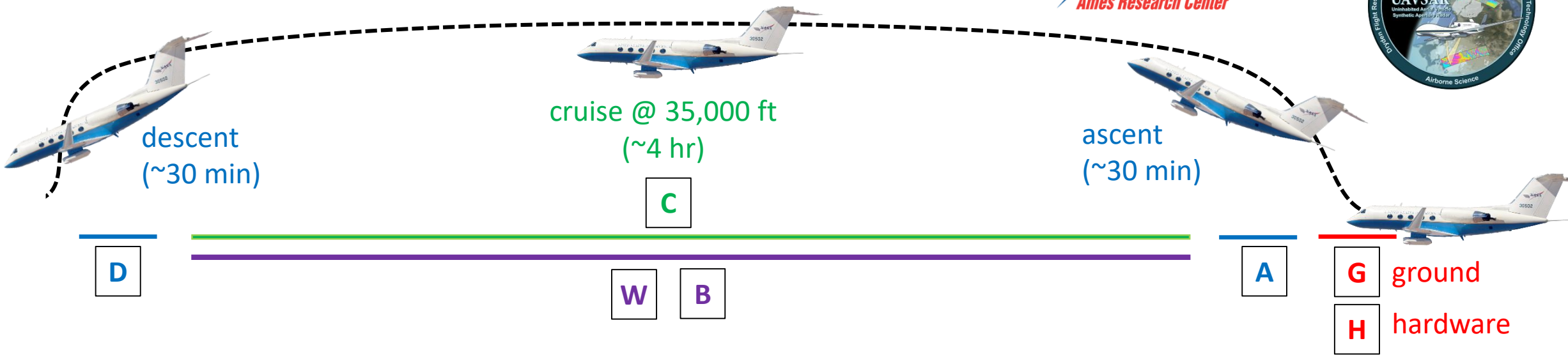

TED STATE


OF AMERICA




GIII-00208
AEROBIOLOGY EXPERIMENT
COMPONENT MOUNT
GIII-00206
AEROBIOLOGY EXPERIMENT PROBE
F.S. 272
F.S 297

Aerobiology Mission – Overview



Ground (G) / Hardware (H) – Exterior surfaces on aircraft around external probe prior to take-off each day. Hardware swabbed after daily sterilization. The purpose of this control sample type is to identify any contaminants associated with our operations.

Ascent (A) / Descent (D) – Air will be collected from ground up to tropopause; air will be collected from cruise down to the ground. The purpose of this sample type is to make comparisons between tropospheric and stratospheric taxa.

Cruise (C) – Air will be collected at cruise altitude continuously. **This is our primary science sample type.**

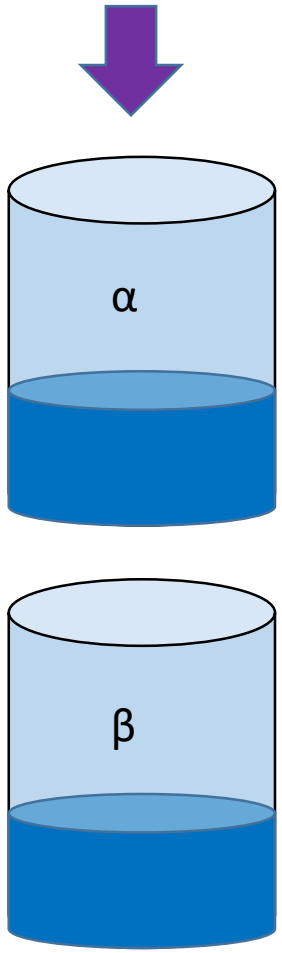
Witness (W) / Blank (B) – Adjacent to the air sampler inside the aircraft there will be a "witness" plate passively collecting any microbes in the cabin air. For blank, plates loaded/removed into sampler with plumbing lines closed. The purpose of this control sample type is to identify any potential contaminants associated with our operations.

Post Flight Processing

α 2-4 µm

β < 2 µm

Our cascade air sampler collects microbes on 2 separate stages with DNA-free filters; we will assay both stages and keep the samples separated



For every flight (3 separate flights at each location), each sample type will be concentrated into 6 mL tubes with Tris. Tubes will be shipped back to our labs on ice

2 ml
N = 30

metagenomic analysis *for identity and abundance.*
Bacteria and archaea

2 ml
N = 30

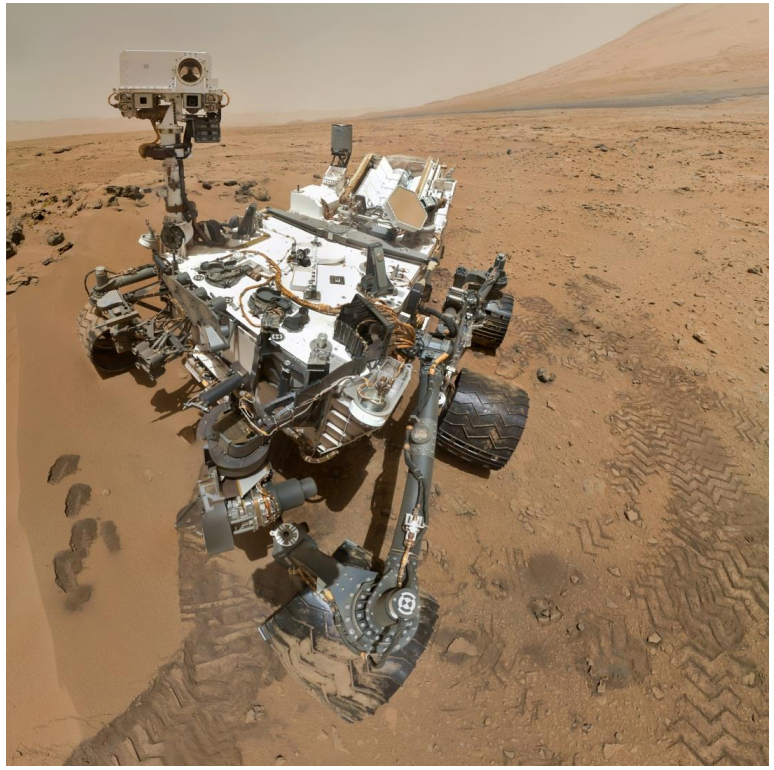
culture *for identity and identifying any novel species*
Bacteria and fungi

2 ml
N = 30

microscopy *analysis and/or sequencing*
T.B.D.

Searching for extraterrestrial life on other worlds presents an obvious (and unsolved) problem:

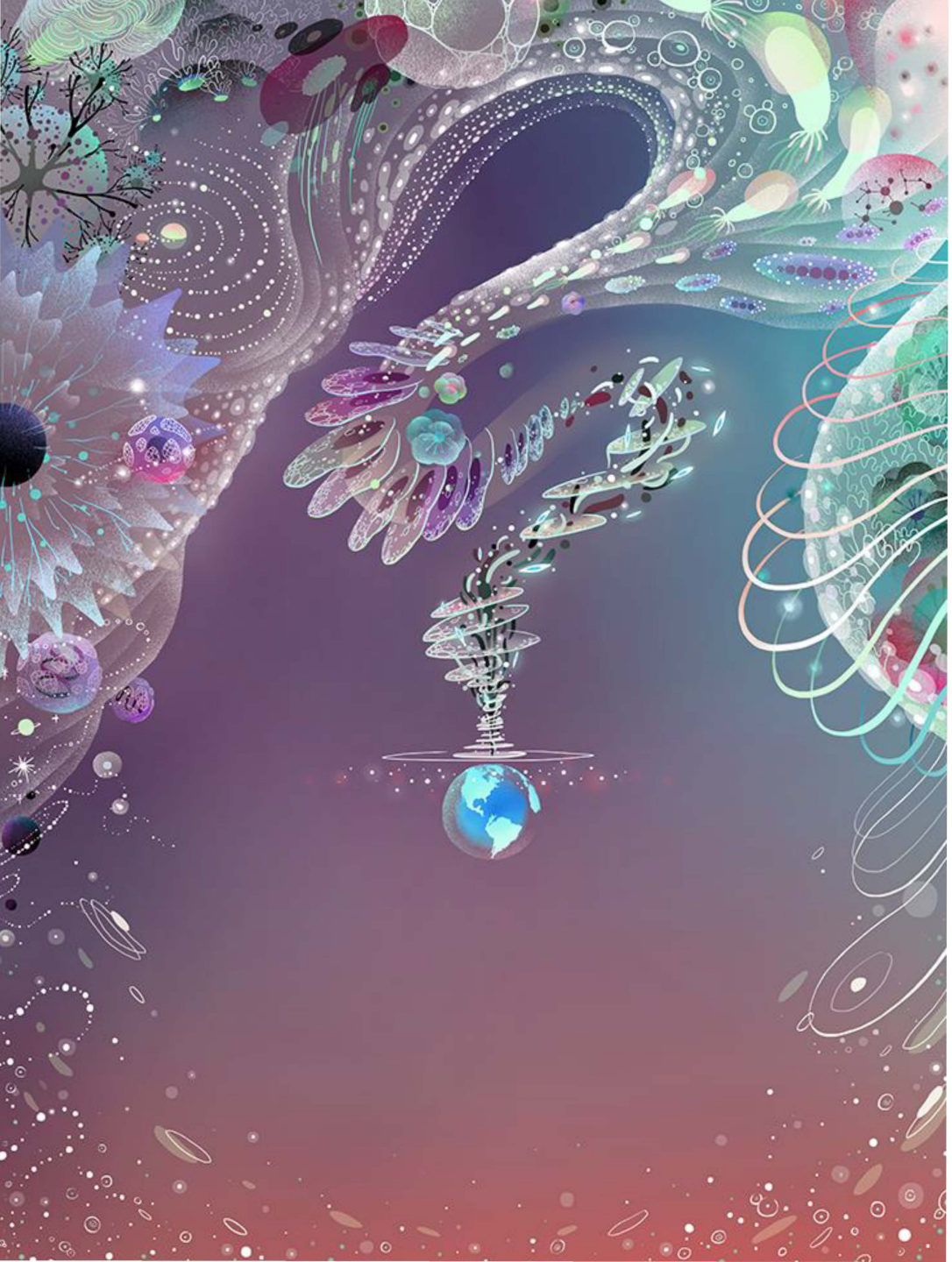
Is it possible to collect samples without terrestrial contamination from microbes or biomolecules on sampling instruments?



We face the same fundamental challenge in the upper atmosphere

Microbial “prospecting” in extreme environments pays off!

Taq polymerase → PCR



Acknowledgements

NASA Ames Office of the Chief Scientist (FY17 Science Innovation Fund Award); NASA Biodiversity Grant; NASA Armstrong Flight Research Center (C20A Aircraft Team)

Terry Lusby, John McGrath, Paul Martinez, Alex Osario