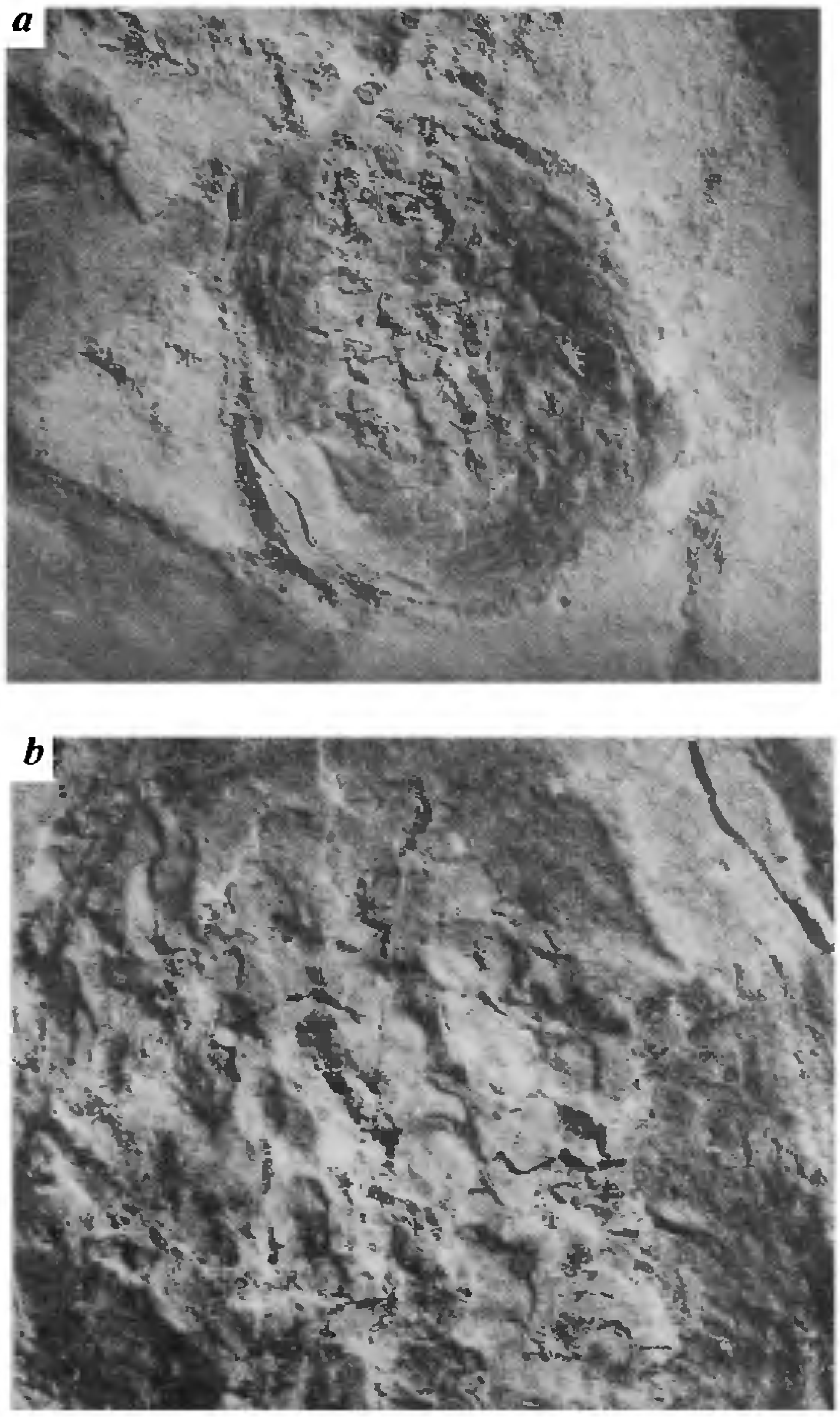

Figure 1. *a*, *Nesowalesia pantii* sp. nov. Specimen showing a concave disc with sporangia. Holotype specimen no. 36869 (×3), *b*, Enlargement of a portion of specimen in *a* showing numerous sporangia (×8)

mode of preservation in the two is different, the Indian ones being impressions while the Australian ones are carbonized. Indian specimens from Handapa are designated as a new species *Nesowalesia pantii* (holotype specimen number 36869, Birbal Sahni Institute of Palaeobotany, Lucknow) with a diagnosis 'concave disc-shaped organs with *Arberiella* type of sporangia' (Figure 1). The Indian specimens closely resemble the specimen described by Pant[4], photographed as Figure 7 on Plate 21.

Report of *Nesowalesia pantii* from Handapa bed is very significant as it is the first report of this sporangiate fructification from India.

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COMMENTARY

Whither microbiology?

Ramesh Maheshwari

A distinguished American microbiologist, C. R. Woese, has expressed deep concern at the present state of microbiology. He is of the opinion that all biology should be reorganized around microbiology. The merit of this view is examined. Some examples are given to illustrate the contributions of microorganisms to the understanding of biological principles and formulation of concepts. A possible area of microbiological research is identified which could become the focus of activity involving many young scientists in our country and contribute to the vitalization of microbiology.

In a recent commentary on the state of microbiology, Carl Woese expressed concern thus[1]: 'University microbiology departments are withering. We are eliminating them as entities in their own right, being demoted to intellectual "programs" within departments whose names typically begin with "molecular" or "cellular" .... In those that remain as microbiology departments in name, the emphasis in both teaching and research is shifting away from microbiology *per se*....' Increasingly, microbiology in these departments is becoming confined to that of growing of microorganisms for 'cloning, sequencing and expressing genes'. Elsewhere[2], Woese says: 'Microbiology is inadequately represented in the funding agencies. Students (and professors) genuinely interested in microbiology are increasingly hard to find. Microbiologists are afraid to teach real microbiology any more.... If microbiology isn't dead today, it definitely is moribund.' One may dismiss these criticisms as prompted from some occupational frustrations, such as the lack of sufficient funds for work, or lack of success in scientific research or the desire to attract attention. I am certain that none of these apply in the case of Woese.

Many biologists would recall that in the last decade somebody had pulled off a coup in biology. We had learned that all living organisms can be classified into two superkingdoms based on fundamental differences in cellular features: the prokaryotes (cells without a nucleus) and the eukaryotes (cells with a true nucleus). The tree of life had two main stems – one stem prokaryotic comprising the bacteria, and the other eukaryotic comprising plants and animals, the latter derived from the former. Woese's studies brought a fundamental revision of this picture. Examining the relationship between prokaryotic microorganisms by comparing the sequences of ribosomal RNA, Woese made the surprising discovery that the world of prokaryotes comprises unusual microorganisms, very different from the typical bacteria found in soil and water. (Imagine Woese's disbelief when, for example, a methane-producing microorganism which had all along been considered a bacterium turned out to be not a bacterium at all!) These microorganisms, which Woese named the archaebacteria (now called the Archaea), are found in unusual places such as hot springs, rumen of cattle, salt pans and brines. They not only differ from most bacteria in sequence of ribosomal RNA but also in biochemical features, such as the absence of muramic acid in cell wall and the presence of ether-linked membrane lipids. The archaebacteria are closer to eukaryotes than to typical bacteria. Their discovery showed that life on earth consists of not two but three primary domains: the Bacteria, the Archaea and the Eucarya. The new tree of life (Figure 1) is a framework for analysing the evolution of all life on earth. Woese placed the study of microbes in an ecological and evolutionary context. In recognition of his contributions, a hyperthermophilic archaeon, *Pyrococcus woesei*, which can grow at near 100°C, was named after him.

The concern expressed by this dedicated and distinguished microbiologist merits serious consideration. Woese contends that all of biology should be reorganized around microbiology. Surely, nobody will deny that microorganisms are important. Microbes cause diseases, make soil fertile, they allow cattle to digest grass, they clean up the environment, and they are used in the manufacture of antibiotics and alcohol. Beer and wine making, which involves alcohol fermentation by the yeasts, are very profitable industries! But, is the general, medical or industrial importance of microorganisms the compelling reason for biology to be reorganized around microbiology? Is the study of microorganisms crucial for learning and formulating biological mechanisms? Although I have no pretensions of being a real microbiologist, I believe that this narrative would demonstrate, at least approximately, the importance of microbiology in *all* biology. The report identifies a possible area of activity which could involve many microbiologists in our country and contribute to the vitalization of microbiology.

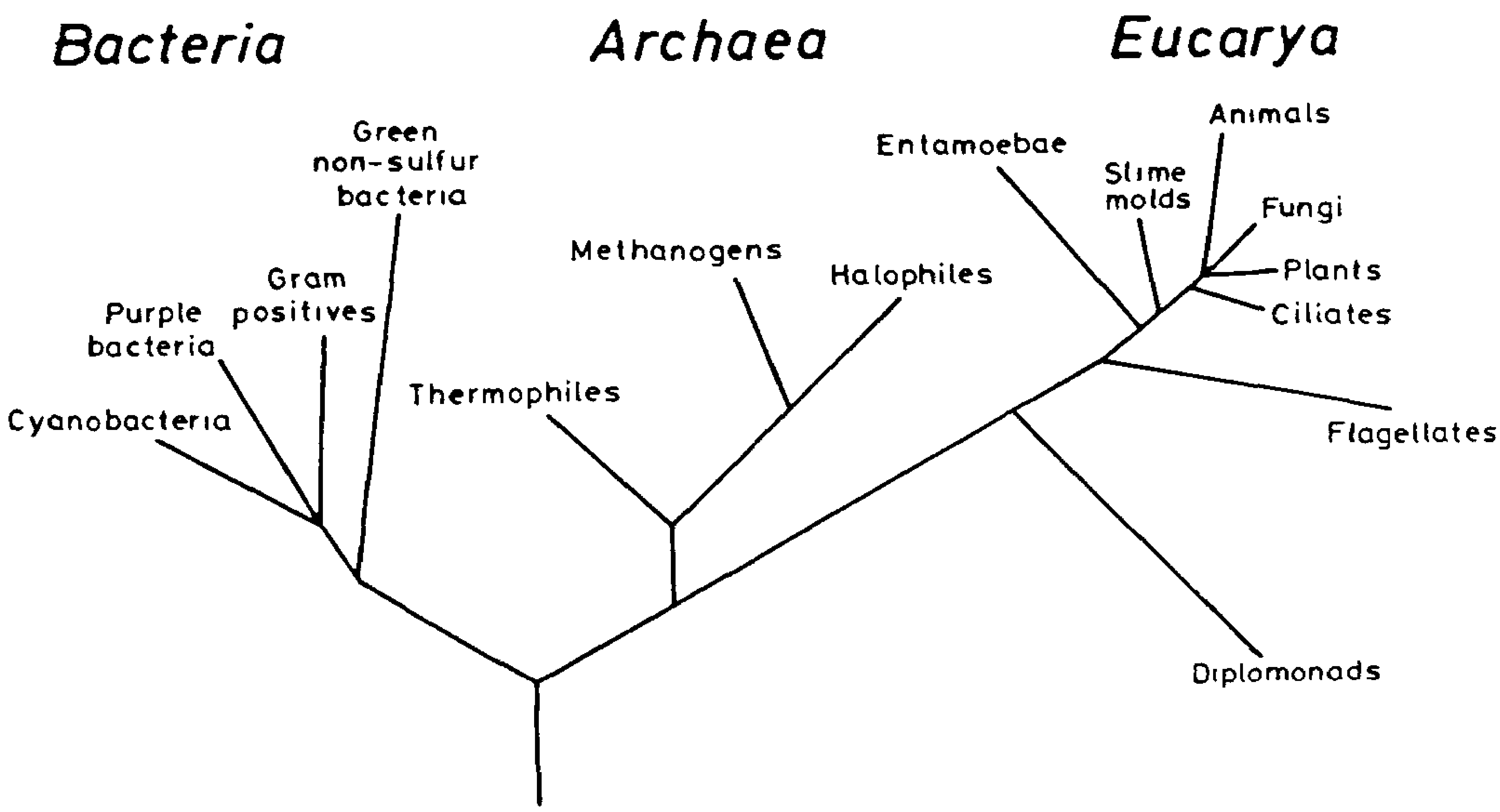

Figure 1. Universal phylogenetic tree determined from ribosomal RNA sequence comparisons showing the three domains, Archaea, Bacteria and Eucarya (based on ref. 2).

Teaching of microbiology

Microbial growth. We begin by the gesture of putting a few cells of a bacterium or yeast in a nutrient medium and measure cell numbers at regular intervals. Let us plot the logarithms of the numbers of cells as ordinate against time. The population displays a characteristic pattern of increase, the S-shaped sigmoid curve. We observe that after a short lag, the amount of living matter increases not in direct proportion to time, but according to geometric progression with time (exponential growth). In other words, the living matter multiplies itself by a constant factor in each successive unit of time. We learn that life is an autocatalytic process. However, the growth which started unrestrictedly stops abruptly when the population runs out of resources[3]. In this planet with dwindling supply of resources, the microbial growth curve has an obvious message for the humans.

Enrichment culture. Next, we prepare a synthetic medium containing a carbon source and mineral salts but lacking in nitrogen. We inoculate this medium with a pinch of garden soil and incubate it at room temperature. We note that microbes with specific metabolic properties develop in this medium. In this particular case, the medium selectively allows growth (enrichment culture) of microorganisms that can fix atmospheric nitrogen to the exclusion of numerous other microbial types present in the soil[3]. This simple exercise strikingly demonstrates the principle of natural selection in just a matter of few hours!

Microbial metabolism. We turn our attention to microbial metabolism and get acquainted with the sequence of reaction steps beginning from glucose and leading to the formation of ethyl alcohol, lactic acid, acetic acid or glycerol, depending upon the microbial species[3]. It suffices to note here that through a comparative, penetrating study of the dissimilatory pathways of microbial metabolism distributed among numerous types of bacteria, the great Dutch microbiologist A. J. Kluyver discerned that the recurring patterns in the variety of metabolic pathways are but few elementary reactions such as transhydrogenation, transphosphorylation, decarboxylation, etc[4]. From this was developed the concept of unity of biochemistry. A certain chemical conversion occupies a predominant position in a particular microorganism. For example, the conversion of pyruvate into acetaldehyde by pyruvate decarboxylase is predominant in yeasts which carry out alcoholic fermentation but is absent in lactic acid bacteria which carry out lactic acid fermentation.

Regulation. The existence of regulatory mechanism can be traced to the discovery in 1861 by Louis Pasteur, who observed that despite the lower consumption of glucose, more yeast is formed in the presence of air than in its absence (Pasteur effect)[5]. It stimulated intensive research on identification of the control sites in catabolism of glucose. Later, the observations that addition of certain amino acids to bacterial cultures shuts off the entire sequence of reactions, leading to the formation of the amino acid, led to the formalization of the concept of feedback inhibition[3]. The phenomena of induced synthesis of enzymes and the existence of specific membrane transport protein (permease) were discovered from studies dealing with growth and nutrition of bacteria[3].

Photosynthesis. The green cover on the earth has inspired many authors to comment that it is because of the remarkable process of photosynthesis invented by the plant chloroplast that man obtains food for himself and his cattle, fibre for his clothing, wood for fuel, his boat and his flute, and the paper for writing his poetry or scribbling his philosophy. A cover-up here? 'Chloroplasts did not evolve as a part of the plant cell; they were acquired by the (evolving) plant cell from the bacterial world through endosymbiosis', says Woese. 'In other words', he says, 'it is the bacteria that have invented photosynthesis, and plant chloroplasts are merely domesticated cyanobacteria'. The cyanobacteria are the most self-sufficient organisms in the biosphere. They not only carry out photosynthesis but also fix gaseous nitrogen from the atmosphere into biologically useful nitrogen compounds – a biochemistry which a vast majority of organisms are incapable of.

'We owe a debt of gratitude to the microorganisms whose peculiarities have provided a general insight into the understanding of photosynthesis', said van Niel[4]. What an ingenious use Engelmann (1880) made of certain motile bacteria as sensitive indicators of oxygen to demonstrate the direct connection between oxygen evolution and light absorption by chloroplasts in *Spirogyra* filaments! By illuminating the alga with light from a prism, he was able to determine the action spectrum of photosynthesis[4].

It was on the basis of the knowledge of a variety of microorganisms and the application of the concept of the unity of biochemistry developed by his master, A. J. Kluyver, and most importantly on the basis of the observations that (a) several types of bacteria can synthesize their cell material from CO_2 without the benefit of radiant energy, (b) the purple sulphur bacteria can grow anaerobically in a minimal medium supplemented with sulphide and bicarbonate, provided they are exposed to light, and (c) photosynthetic activity of the purple bacteria is not accompanied by evolution of O_2, that van Niel was led to conceptualize *photosynthesis* as a *photochemical oxidation–reduction process* and formulate his famous equation[4]:

$$\underset{\text{H donor}}{2H_2A} + \underset{\text{H acceptor}}{CO_2} \rightarrow \underset{\text{Oxidized H donor}}{2A} + \underset{\text{Reduced acceptor}}{(CH_2O)} + H_2O.$$

What this equation implied was that plant photosynthesis and bacterial photosynthesis are fundamentally similar light-dependent reactions. The difference is in the hydrogen donors they employ – being H_2S in the case of sulphur bacteria and H_2O in the case of the green plants. A direct consequence of the above-mentioned general formulation was the prediction that O_2 produced during photosynthesis by the green plants must come directly from water and not from CO_2.

Chemosynthesis. It was through the study of the life-style of a number of bacteria (nitrifying bacteria, sulphur bacteria, iron bacteria, etc.) found in nature under aerobic and anaerobic conditions that it was discerned that in addition to photosynthesis there is another process, chemosynthesis. In chemosynthesis the organic matter of the organism is built from CO_2 as the sole source of carbon without the supply of the radiant energy. As was first shown by the Russian microbiologist Winogradsky, a variety of bacteria can derive energy for growth from the oxidation of inorganic compounds[3,6]. That chemical energy can be derived from the biological oxidation of hydrogen, sulphide, sulphur, ammonia, carbon monoxide, ferrous, etc., was a revolutionary idea. It showed the apparently diverse ways in which microorganisms have solved the problem of providing for their indispensable energy requirements

Chemosynthesis had been thought to be a rare phenomenon until 1977, when scientists took a submersible at a depth of some 3000 m in the Pacific ocean. They discovered jets of superheated black water ejecting from the ocean floor and mixing with the cold sea water[7,8]. They were astonished to see a luxuriant fauna thriving in the warm water, in the vicinity of the hydrothermal vents (Figure 2) What is the food source for these new forms of animal life in the permanently dark environment? Pioneering work by Holger Jannasch showed that the ecosystems of animal populations are supported by microbial chemosynthesis rather than photosynthesis[7]. A variety of chemoautotrophic bacteria live in dense populations in the animals. The bacteria obtain energy by oxidizing geothermally provided reduced sulphur compounds and fix inorganic carbon through the Calvin–Benson pathway (Figure 3). The organic carbon is taken up by the animal hosts. The hosts are among the few animals on earth that are not fed directly by photosynthetically fixed carbon.

Thermophilic microorganisms. In the last decade another revelation occurred in microbiology. Karl Stetter isolated anaerobic archaea from sulphur-rich, marine volcanic environments that are able to grow at extremes of temperatures[9]. The upper temperature limit of life was pushed up to 110°C. Microbiologists are competing to see who can find microorganisms that beat this record!

The diversity of hyperthermophilic archaea has, in turn, started the race to discover the enzyme which has the highest degree of thermostability. Incredible it may seem, but an α-amylase from *Pyrococcus furiosus* retains enzyme activity even after autoclaving at 120°C for 5 h (ref. 10)! These microorganisms are the focus of the emerging area of biocatalysis at extreme temperatures and for biotechnological opportunities.

Early life. And yet another revelation. Thomas Gold predicted that microbial life is widespread at depth in the crust of the Earth[11]. He believes that in mass and volume the subcrustal life may be comparable with all surface life. Some microbiologists have sampled fluids from borewells drilled some 4000 m into the granitic rock or into the sea bed and have reported finding thermophilic anaerobic bacteria[12,13]! These surprising findings have fundamentally altered the views on the physical setting under which life arose and of the nature of early life on earth. Life probably arose in a hot environment. The ancestor may have been a chemosynthetic thermophile[14].

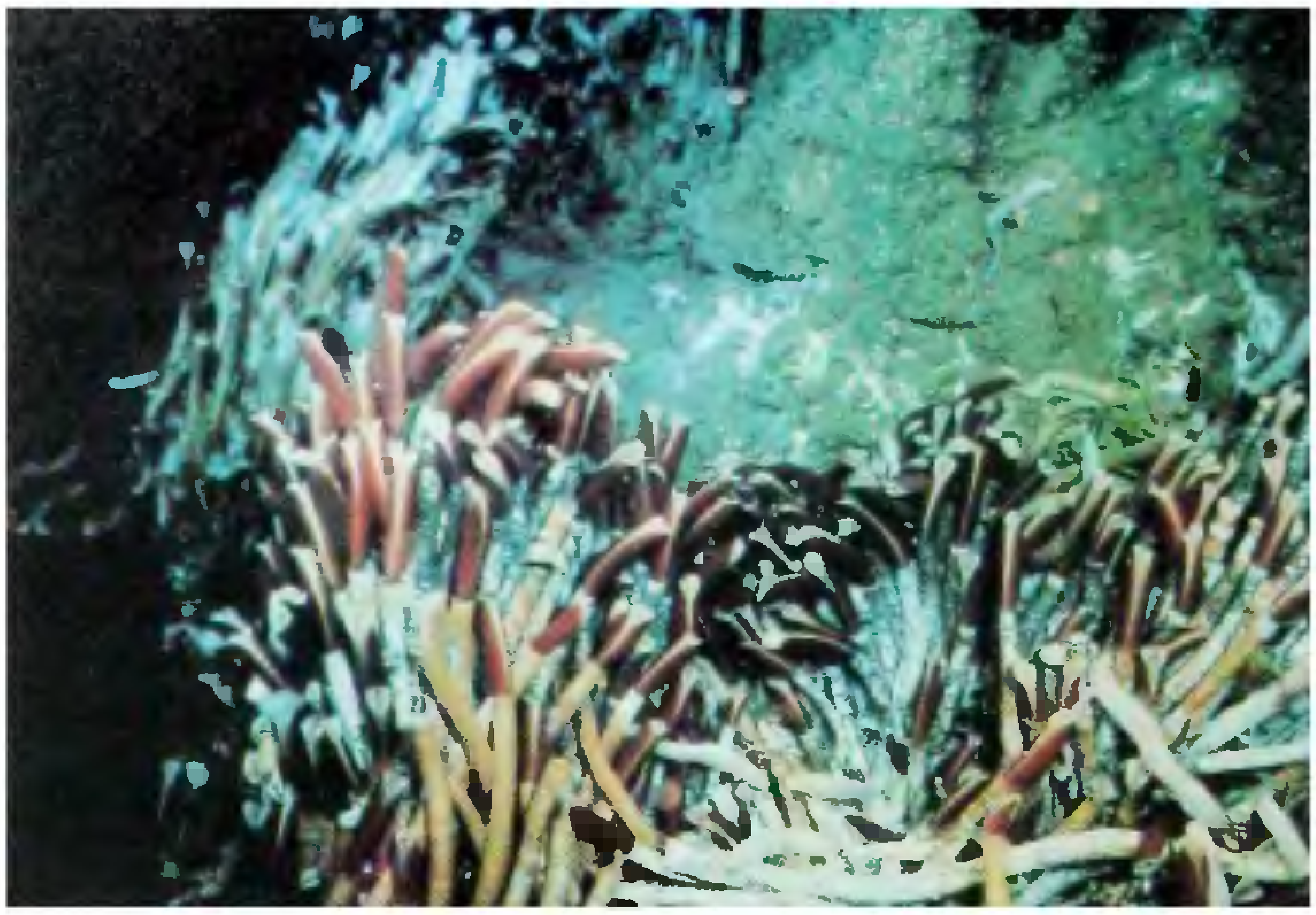

Figure 2. Giant tubeworms, *Riftia pachyptila*, at hydrothermal vent (courtesy of IFREMER, France).

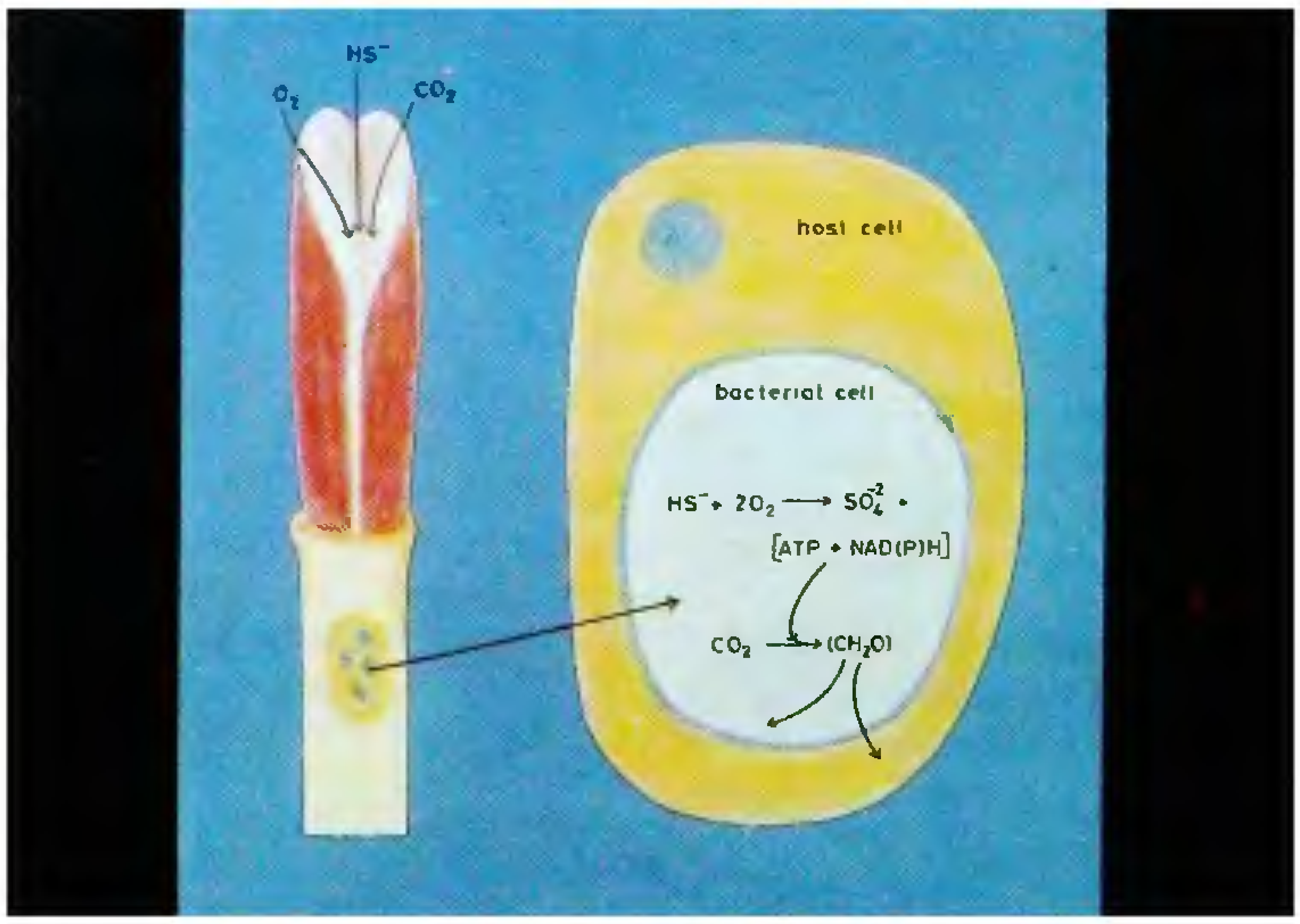

Figure 3. Schematic representation of symbiotic relationship between *Riftia* and chemosynthetic bacteria. Hydrogen sulphide serves as the electron donor, or energy source. The energy is produced when hydrogen sulphide and oxygen are combined and it is used for fixation of CO_2 (based on refs 7, 8).

Microbial diversity. 'The essence of the microbial world lies in its diversity', says Woese[2]. An innovative application of the techniques of molecular biology has been made to estimate how much of microbial diversity had escaped detection by conventional pure-culture technique in an

Mixed population comprising uncultivated species

↓

Total DNA

↓

Ribosomal DNA

↓

PCR amplification

↓

Recombinant DNA library of rRNA genes

↓

Gene sequencing of unique clone types

Figure 4. Sampling of microbial populations through isolation and sequencing of ribosomal RNA genes directly from the environment[15].

already well-studied place. Norman Pace took a census based on unique ribosomal RNA genes extracted and cloned directly from a niche (Figure 4)[15]. He demonstrated that more species of archaea were present than suspected.

It has been estimated that of the total number of species of bacteria (40,000) and the fungi (150,000), only about one-tenth have been discovered[16]. The fascination for microbial diversity comes from their strangeness and their exotic habitats. J. R. Postgate[17] gives a fascinating account of the specialized microbes which live at the bottom of deep oceans, in the mud in the estuaries of the rivers, in sewage treatment plants, in the interior of compost heaps, in the rumen of cattle, in near-boiling sulphuric acid conditions, in alkaline environments equivalent to a solution of caustic soda, in strongly salty places, in root nodules of leguminous plants, etc. A generalization that can be made from the results of microbiological sampling of diverse habitats is that no matter how harsh the conditions are in nature, so long as liquid water is available, microbial life will be found.

The importance of studying diversity has been beautifully stated by David Perkins[18]:

'Knowledge of a flute or a kettledrum is not sufficient to understand all the other instruments in a symphony orchestra or to predict their characteristics. Nor is a knowledge of a single species, however complete, adequate for understanding diverse species. Diversity of research organisms in the laboratory must at least dimly reflect the diversity of species in nature if the scope and beauty of evolutionary improvizations are to be appreciated and the genetic manipulation that brought them about are to be understood.'

Some other contributions of microbes. Before this 'refresher' course is closed, let us cast a final breezy look. Other examples where microorganisms contributed to new discoveries are: gene regulatory proteins[19], simplest light-driven proton pumps in halophilic bacteria[19], restriction modification system[19], random and directed mutations[20], checkpoints in cell cycle[21]. Lest we forget, the success in obtaining nutritional mutants in the fungus *Neurospora crassa* had led to the discovery of the relationship between genes and enzymes. The formulation of one-gene–one-enzyme hypothesis by Beadle and Tatum had sparked a revolution in biology.

The study of microorganisms has been central to the effort to understand life. We are still searching for answers to numerous fundamental problems. Where will the answers come from? As in the past, in large part from the study of microorganisms.

Investigation and inquiry

How is it then that in spite of an impeccable record of microbiology, its flag is at half-mast? Surprising as it seems, but professors and students genuinely interested in microbiology are indeed very few, as observed by Woese[2]. Is it that the contributions of microbes to biology is consciously or unconsciously covered up in the teaching of biology? Or, is it that the repeated assertion that 'what is true for *E. coli* is also true for elephants' generated the impression of the futility of gaining fundamentally new information, particularly when encyclopaedic information is already available on *E. coli*[18]?

The major fault in the teaching of microbiology (and other sciences) is that emphasis is placed on facts, instead of on observations and experiments. Invariably, the eminent microbiologists record that their curiosity and interest in microbiology were aroused from the excitement in collecting samples of some pond water or marine sediments or soil from some tropical forests or from some frigid place, from the suspense in waiting for microorganism to appear in an enrichment medium, from the fascination of the diversity of their types, from the microscopic observations of motile bacteria or of the intricate configuration of fungal spore walls, from the challenge of designing conditions for mass culturing a tractable organism, or from puzzling over the special features of the biochemistry which allows a particular microorganism to live in its ecological niche and cope with the environmental stress[22–25]. The eminence of these microbiologists comes from their realization that each microbial species is a unique, intrinsically valuable and a source of new knowledge.

Microbiological research

Extremophiles. Given the widely different level of scientific and technological development, the limited funds for science in our country, can we contribute to the vitalization of microbiology? The answer is a clear yes. I propose that research on extremophiles – the microorganisms which are indigenous to unusual habitats – may be a starting point. Perhaps, a fascination for the extremophiles as source of good biological questions and as new materials for new products and processes may usher a renaissance in microbiology in the country. India, with its diversity of natural habitats (desert, alpine and tropical soils, hot springs, porous rocks, glaciers, lakes, sediments of streams, caves, alkaline soils, brackish marshes, coastal lagoons, ocean, etc.) and a variety of man-created unusual habitats (composts, municipal waste dumps, sewage digestors, stagnant waters, oil-contaminated areas, areas receiving industrial waste discharge, effluents of acid mines, corroded materials, etc.), presents great opportunities for the investigations of extremophiles. Because unusual habitats occur across the length and breadth of the country, research on extremophiles may well be one of the best ways to reach out and encourage scientists in the remote and less endowed institutions. Moreover, extremophile research is an area in which, because of the paucity of information, these scientists can contribute to the generation of knowledge, accomplish something and not feel frustrated. The pioneering studies of Thomas Brock on thermophilic micro-organisms were done in remote places with simple facilities[26]. Could any one have predicted that the bacterium *Thermus aquaticus*, isolated in 1969 by Brock and Freeze from a hot spring in the Yellowstone National Park, USA, would in less than 20 years be exploited as a source of thermostable DNA-copying enzyme (*Taq* polymerase) and make the PCR technology an enormously successful technique? This enzyme and many restriction enzymes discovered from screening of thousands of bacteria and archaea are now churning millions of dollars annually in the developed countries. Witness the *Taq* polymerase controversy[27]. There was a time when people fought for the possession of women or for a piece of land. Now people are fighting for the rights to a bacterial gene product!

An understanding of life cannot be complete without a knowledge of the variations of biochemical design that evolved over many millions of years. Microbes offer unique experimental material for understanding the diversity of adjustments that they make to get adapted to their environment. Let us hope that the traditional areas of microbiology concerned with the study of the diversity of microbes and of their biochemical activities will be pursued vigorously.

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Finding *patterns* in nature's maze: An endless quest

K. Chandrashekara

I am writing in response to 'Are patterns a rule in nature?'[1] by K. N. Ganeshaiah (KNG). Without doubt, reading it was quite a stimulating experience. However, I feel compelled to respond to a few issues raised in the paper; as the saying goes, it is better to debate an issue and not settle it rather than settle an issue without debating it.

Patterns: a question of scale

Are patterns a rule in nature? I find the question rather incomplete and bordering on the philosophical. What sort of pattern does the author have in mind? At what level of organization? At what temporal and spatial scales? To a physicist, chemist or for that matter any scientist, the answer to the question will be an assertive 'yes', given the fact that all matter in nature,