BACTERIA AND ARCHAEA

Aharon Oren
The Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel.

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Contents

Summary

With an estimated number of 4-6x10^{30} cells, the prokaryotes are the most numerous organisms on earth. They inhabited our planet long before the eukaryotes evolved, and metabolically they are the most diverse group. Compared to the number of eukaryotic taxa the number of prokaryote genera and species described is surprisingly small. A little over 9,000 different species of prokaryotes have been named, and classified in nearly 2,000 genera. The naming of prokaryotes is regulated by the International Code of Nomenclature of Prokaryotes. Internationally approved rules for naming species exist, but there is no universally accepted species concept for the prokaryotes. For the description of new representatives a polyphasic approach is used, which includes determination of numerous phenotypic and genotypic properties. If necessary, the genomes of related strains are compared by DNA-DNA hybridization or full or partial genome sequence comparison. Since the 1970s comparative sequence analysis of small-subunit ribosomal RNA has revolutionized our views of prokaryote taxonomy. Two phylogenetically unrelated groups were recognized, now known as Bacteria and Archaea. Far-reaching differences exist between the two 'domains' of prokaryotes, such as the presence or absence of peptidoglycan in the cell wall, presence or absence of ether linkages and the nature of the hydrophobic chains in the membrane lipids, sensitivity to certain antibiotics, and more. The domain Bacteria (29 phyla described) is the most diverse; most cultured representatives of the domain Archaea (5 phyla described, about 4% of all described species of prokaryotes) are extremophiles, living at high temperatures, high salt concentrations, and/or low or high pH. Analysis of rRNA genes isolated from DNA extracted from different ecosystems without prior cultivation

of the organisms shows that we know only a small fraction of the existing prokaryote species. Archaea are not restricted to extreme environments and abound also in seawater and in soils. Most of the extant diversity of Bacteria and Archaea remains to be characterized.

1. Prokaryotes: The Unseen Majority

In 1998, Whitman et al. published an essay titled: "Prokaryotes: the unseen majority" (for further details see the Bibliography section). In this paper they presented an inventory of the number of prokaryotes on earth, estimated at 4-6x10^{30} cells. Most of these occur in the open ocean, in soil, in the oceanic subsurface (sediments below 10 cm) and terrestrial subsurface (below 8 m depth) (1.2x10^{29}, 2.6x10^{29}, 3.5x10^{30} and 0.25-2.5x10^{30} cells, respectively). These numbers by far exceed the estimated number of eukaryotic cells in microorganisms and macroorganisms. The amount of organic carbon stored in these prokaryotes (Bacteria and Archaea combined; see below) was estimated at 60-100% of the carbon found in plants. Due to their large biomass, as well as their rapid growth and turnover rates, the prokaryotes are the major driving force for life on earth. This chapter provides an overview of the diversity within the world of the prokaryotic microorganisms, their taxonomic classification, and their phylogeny.

The development of the electron microscope in the 1930s-1940s led to the recognition that bacteria have a cellular structure that differs fundamentally from that of other microorganisms such as protozoa, unicellular algae, and yeasts. This resulted in the concept that life on earth can be divided into prokaryotes (a paraphyletic group that includes the Bacteria, including the 'blue-green algae', later renamed cyanobacteria, and the Archaea) and the eukaryotes – a clade that includes all organisms with a complex cell structure that includes a membrane-surrounded nucleus and organelles such as mitochondria and chloroplasts. Molecular studies based on the determination of the nucleotide sequence of ribosomal RNA, initiated in the 1970s, showed that the prokaryotes are not a phylogenetically coherent group, but instead two fundamentally different types of cells should be recognized. These are now named Bacteria and Archaea. The concept that the living organisms can be classified in three 'superkingdoms' or 'domains', the Archaea, the Bacteria, and the Eucarya (the eukaryotic world of animals, plants, algae, fungi and protozoa), is now generally accepted.

Without the help of the methods of molecular biology and bioinformatics it is impossible to obtain a reliable picture of prokaryote phylogeny and evolution. The geological record is of little use when attempting to reconstruct the evolution of prokaryotic microorganisms. Prokaryotes have inhabited our planet for at least 3.5 billion years, and they had already developed their tremendous metabolic diversity long before the first eukaryotic cells appeared. Morphological characters, so useful in the systematics of eukaryotes, are of little help in the classification of prokaryotes, and a useful fossil record is altogether lacking. Those early microfossils that were preserved provide little information, if at all, about the mode of life of the organisms. Reconstruction of the position of the Bacteria and the Archaea in the tree of life can therefore only be based on the study of the currently living species.

2. The Species Concept for the Prokaryotes

In comparison with the numbers of plant and animal species described, the number of recognized and named species of prokaryotes is surprisingly small. We know more than a quarter of a million species of flowering plants, nearly thirty thousand species of fish, nearly a million different insects, and about 75,000 species of fungi have been described. At the time of writing (November 2011), the number of species of different prokaryotes described, Bacteria and Archaea combined, with standing in the nomenclature (but not including most cyanobacteria – see in the following) was less than ten thousand. These are classified in nearly two thousand genera. A complete list of the numbers, including those of the higher taxa – families, orders, classes and phyla, is given in Table 1. This list is based on the "List of Prokaryotic names with Standing in Nomenclature", maintained as a web site (http://www.bacterio.cict.fr) by Prof. Jean Euzéby of the National Veterinary School of Toulouse, France. This list is updated monthly.

Rank	Number	Comments
Kingdom	1	Category not covered by the Rules of the Bacteriological Code
Subkingdom	2	Category not covered by the Rules of the Bacteriological Code; equivalent to Domain
Division or Phylum	29	Category not covered by the Rules of the Bacteriological Code; Phyla proposed in the Approved Lists of Bacterial Names and in the International Journal of Systematic Bacteriology / International Journal of Systematic and Evolutionary Microbiology
Class	79	
Order	129	130, of which 1 is illegitimate
Family	291	297, of which 6 are illegitimate
Genus	1,937	2,065, of which about 108 are considered as synonyms, and 20 are illegitimate
Species	9,375	11,033, of which 31 are later homotypic synonyms cited in the Approved Lists of Bacterial Names, 1,257 are new combinations, 13 are *nomina nova*, about 290 are considered as later heterotypic synonyms, and 67 are illegitimate. Of the 11,033 names, 10,601 belong to the Bacteria and 432 to the Archaea

Table 1. The number of different species of prokaryotes described (Bacteria and Archaea combined) with names with standing in prokaryote nomenclature, and the number of higher taxa in which these are classified, as of November 4, 2011. Derived from http://www.bacterio.cict.fr.

The nomenclature of the prokaryotes is regulated by internationally agreed-upon rules fixed in the International Code of Nomenclature of Bacteria (The Bacteriological Code), now renamed as the International Code of Nomenclature of Prokaryotes. This Code covers the nomenclature of Bacteria as well as Archaea. The International Committee on Systematics of Prokaryotes (http://www.the-icsp.org) is responsible for the Code, and considers amendments and exceptions that may be needed to specific Rules. The rules of the Bacteriological Code do not cover taxa above the rank of class, so that there is no officially accepted nomenclature of phyla (divisions) and domains (subkingdoms) of prokaryotes. It must be noted that nomenclature of the cyanobacteria is traditionally regulated by the International Code of Botanical Nomenclature, so that only very few species of Cyanobacteria were named using the provisions of the Bacteriological Code. Accordingly, the above-mentioned numbers of species and genera of prokaryotes do not include most cyanobacteria. The proposed International Code of Phylogenetic Nomenclature (http://www.ohiou.edu/phylocode) has not yet been endorsed by the International Committee on Systematics of Prokaryotes.

In botany and zoology, priority of names goes back to the 18th century writings of Linnaeus, and new names published in any scientific journal or book may obtain standing in the nomenclature. Under the current system of the Codes there is no central system of registration of indexing of names, and as a result there is no clear picture of how many species of any group of plants and animals have actually been described and named. For the prokaryotes the situation is much simpler. In 1980 a new start was made in prokaryote nomenclature by the publication of the 'Approved Lists of Bacterial Names', which contained nearly 1,800 names of species. Since that time the only way to add names of prokaryotes with standing in the nomenclature ('valid publication of names') is by publication in the International Journal of Systematic and Evolutionary Microbiology (until 1999, the International Journal of Systematic Bacteriology), either in the form of an original article or as an entry in the 'Validation Lists' ('Lists of new names and new combinations previously effectively, but not validly published') that regularly appear in that journal. Thanks to the well-ordered framework established by the rules of the Bacteriological Code, the centralized registration and indexing of the new names in a single journal, and the establishment and maintenance of the http://www.bacterio.cict.fr web site, it is possible to obtain a complete overview of the number of prokaryotes with validly published names at any given moment.

The major problem in the taxonomy of prokaryotes is the lack of a clear species concept. Botanists and zoologists not always have well-defined ideas how to delineate species, genera and higher taxa, but delineating species is even more problematic for taxonomists who describe and classify Bacteria and Archaea. Prokaryote systematics still lacks a firm theoretical basis. There is an official nomenclature, but there is no official classification of prokaryotes, and concepts how to sort the organisms in groups are constantly modified as new scientific methods are being developed and ideas about classification are changing.

A prokaryote species can be operationally defined as a monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity in many independent characteristics, and is diagnosable by at least one discriminative phenotypic property. However, there is no strict consensus on what characteristics are

important to determine the degree of similarity and how high this overall similarity must be. The way to delineate genera, families and higher taxa are even less well defined, as for eukaryotes. Using this so-called phylo-phenetic species concept, delineation of species is based on a 'polyphasic' approach, using as many characteristics as possible, phenotypical as well as genotypical. Individual phenotypic or genotypic properties are insufficient as parameters for species delineation, but the combination of many such tests yields information that can be used for classification. Relevant phenotypic parameters include the shape and size of the cells, motility, the mode of flagellation, the ability to produce endospores, presence of cellular inclusions, color, colonial morphology, ultrastructure, and Gram-staining behavior. Chemotaxonomy adds important data such as the chemical composition of the different types of lipids in the cell membrane, the fatty acids present in these lipids (in the case of the Bacteria that have fatty acids linked by ester bonds that can be hydrolyzes), the types of respiratory quinones, the presence or absence of certain polyamines, the exact chemical structure of the peptidoglycan in the cell wall (in the Bacteria domain), teichoic acids and mycolic acids in those organisms that possess them, and the presence of exopolysaccharides. Physiological properties to be included in species descriptions are among others the mode of energy metabolism, nutritional requirements, presence or absence of activity of certain diagnostic enzymes, ecological parameters (requirements for temperature, pH, redox potential, salinity, etc.), and susceptibility to different antibiotics and other antimicrobial agents. The above list is by no means exhaustive.

Genomic properties important in the characterization of prokaryotes include the DNA base ratio (expressed as the mol% guanine + cytosine), the sequence of the gene encoding small-subunit (16S) ribosomal RNA (see section 3), sequence comparisons of other 'housekeeping' genes (e.g. genes encoding elongation and initiation factors, RNA polymerase subunits, DNA gyrases, heat shock and recA proteins) in 'multilocus sequence analysis' (MLSA) in order to make robust estimates of bacterial species phylogenies, DNA-DNA hybridization tests comparing the total genome with that of the phylogenetically closest neighbors, and if necessary even complete genome sequencing. Additional information can be obtained using techniques such as restriction fragment length polymorphism (RFLP) and other methods of DNA fingerprinting.

A widely accepted pragmatic definition of a prokaryotic species defines a species as a group of strains, including the type strain, that share at least 70% total genome DNA-DNA hybridization and have less than 5^{o}C ΔT_{m} (= the difference in the melting temperature between the homologous and the heterologous hybrids formed under standard conditions). These properties depend on the entire genome and are not determined by the features of one or more single genes. Values between 30 and 70% DNA relatedness reflect a moderate degree of relationship. This operational definition was proposed about 25 years ago and remains mainly satisfactory also today. At the level of genera and families, DNA-DNA hybridization has limited resolving power. Based on DNA-DNA hybridization values the species concept for the prokaroyotes is much broader than that for higher eukaryotic organisms. For example: humans and chimpanzees are 98.4% related on the basis of DNA-DNA hybridization, even lemurs (78% DNA relatedness with humans) should be included in the same species as humans if based on the criteria used in prokaryote taxonomy!

An essential part of any description of a new species of prokaryotes is the designation of a type strain that will remain the nomenclatural type of the taxon, and deposition of a pure (axenic) live culture of that strain in at least two culture collections located in different countries. The isolate is thus made available to scientists all over the world. This is, however, not always possible. In case a novel type of prokaryote cannot yet be cultured or cannot be obtained in axenic culture but still can be studied in sufficient detail, it can be described with the status of 'Candidatus', as a candidate taxon to be later described in full, including valid publication of the name. The status of 'Candidatus' is not covered by the rules of the Bacteriological Code. As of November 4, 2011, 100 such 'Candidati' had been described in the International Journal of Systematic Bacteriology / International Journal of Systematic and Evolutionary Microbiology, and more such descriptions can be found in other journals.

3. Bacteria and Archaea, the Two Domains of the Prokaryotic World

As stated above, the determination of the sequence of the gene encoding 16S ribosomal RNA is one of the key elements in any polyphasic characterization of a novel prokaryote. When testing the properties of a putative new species, most investigators will even start with the 16S rRNA sequence analysis, as this provides a rapid means of placing the new isolates within a phylogenetic framework and enables a comparison with earlier described species. Determination of phylogenetic relationships, based on 16S rRNA, and to a lesser extent also on 23S rRNA sequence similarities, is now a routine procedure in bacterial taxonomy. Most prokaryote taxonomists will embark upon a full polyphasic characterization of novel strains only when the 16S rRNA sequence is sufficiently different (e.g. <97% sequence similarity) from the 16S rRNA of earlier described species with standing in the nomenclature. The current taxonomy of classification schemes for the prokaryotes, as given e.g. in the latest edition of Bergey's Manual of Systematic Bacteriology (see the Bibliography below), are primarily based upon the phylogenetic framework deduced from small subunit rRNA sequence data.

The recognition that phylogenetic information can be obtained by sequence comparison of nucleotides in DNA and RNA or of amino acids in proteins originated in the 1960s. In a key paper published in 1965, Zuckerkandl and Pauling argued that today's organisms are the products of historical events, and that it may be possible to reconstruct such evolutionary events from sequence comparisons of homologous and phylogenetically informative molecules: DNA, RNA, and proteins. 'Molecular clocks' should be searched for – molecules with a conserved function, in which the sequence of amino acids or nucleotides changes randomly at a more or less constant rate, so that the number of changes should be approximately proportional to evolutionary time. Phylogenetic trees can then be reconstructed that show the evolutionary relationship between the different organisms.

For comparative studies of prokaryotes, the 16S and the 23S ribosomal RNA molecules fulfill the basic requirements for such 'molecular clocks' better than any other molecule: ubiquitous distribution, functional constancy, common ancestry, genetic stability, appropriate size, and the presence of independently evolving domains within the molecule. The 16S rRNA (length 1542 nucleotides in *Escherichia coli*) has become the most popular molecule for routine testing of phylogenetic relationships in the

prokaryote world ever since the techniques for the analysis of its sequence were first developed in the 1970s. Curated databases are available that can be used to compare newly obtained sequences not only with those of earlier described and named organisms, but also with sequences retrieved from DNA extracted from natural environments without prior cultivation of the organisms. Thus, the latest update (release 10, August 9, 2011) of the Ribosomal Database Project (http://rdp.cme.msu.edu) contains over 1.9 million entries of prokaryote small-subunit ribosomal RNA genes. Most of these gene sequences belong to yet undescribed species (see Section 6). Other useful databases for rRNA gene comparisons are the SILVA project (http://www.arb-silva.de) and Greengenes (http.//greeengenes.lbl.gov).

The recognition that the prokaryotes are not a monophyletic group but should be split into two domains came from the work of Carl Woese who pioneered methods to obtain sequence information for ribosomal RNA molecules in the mid-1970s. Based on 16S rRNA sequence information it became clear that methanogens (*Methanococcus, Methanosarcina, Methanothermobacter*), red extreme halophiles such as *Halobacterium* and *Halococcus,* and extreme thermophiles like *Sulfolobus* and *Thermoplasma* are unrelated to the other prokaryotes known at the time. Therefore Woese proposed the new domains Archaebacteria and Eubacteria, later renamed Archaea and Bacteria; the name Eucarya was suggested for the domain that contains all eukaryotes: plants, animals, fungi, and protozoa.

Sequencing of the 16S rRNA genes of nearly all the over 9,000 known species of prokaryotes (Table 1) and their comparison with representatives of eukaryote lineages has supported the three-domain concept. Of all prokaryote species, about 4% belong to the domain Archaea and 96% to the Bacteria. Considerable sequence information on large subunit (23S) rRNA molecules has accumulated as well. Its primary structure is at least as conserved as that of the small subunit rRNA, but it contains more and longer stretches of informative positions. However, the database of 23S rRNA sequences is far less complete than that of the 16S rRNA. 23S rRNA-based phylogenetic trees generally have a similar topology as 16S rRNA trees and the same branches (phylum-level subdivisions) can be recognized, but minor differences do exist. Unfortunately, rRNA based trees still do not enable the exact determination of the relative branching order of the phyla in most cases. The Chloroflexi and the "Thermus-Deinococcus" phylum appear to share a common root, and Chlamydiae and Planctomycetes as well as the Bacteroidetes and the Chlorobi represent two phylum clusters. Most other phylum clusters that can be recognized in some trees are rather unstable when slightly changing the parameters or the underlying data set for tree reconstruction.

Figure 1 shows the result of an attempt made in 2006 to reconstruct the universal tree of life with the three domains: Archaea, Bacteria, and Eucarya. This tree was based on sequence comparisons of 31 universal protein families rather than on small-subunit rRNA sequences alone, and only included organisms for which the complete genome had been sequenced at the time. It largely confirms the topology of rRNA-based trees. However, the more new information becomes available, the more difficult it becomes to produce satisfactory trees. Even today there still is much discussion about the true topology of the universal tree, and some question whether it is possible to reconstruct such a tree at all.

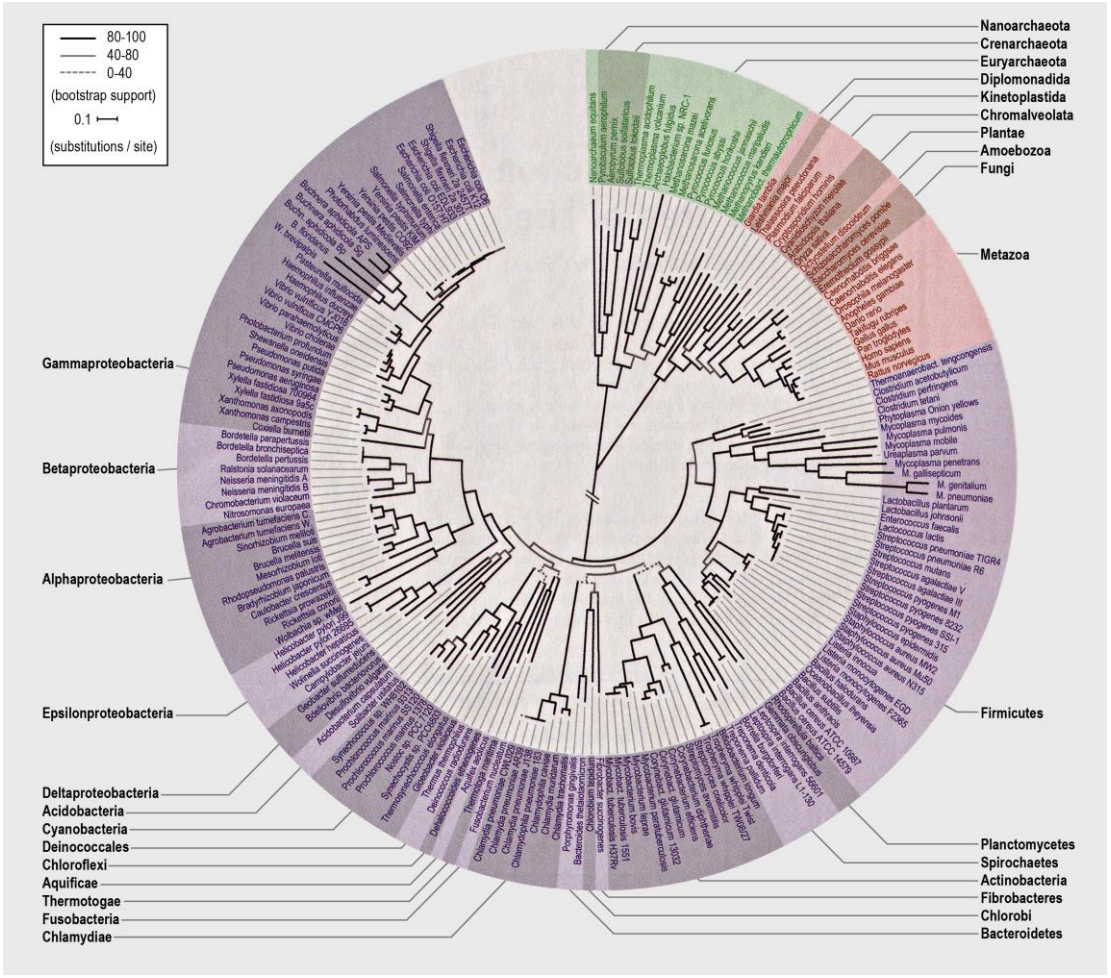

Figure 1. Global phylogeny of fully sequenced organisms. The phylogenetic tree has its basis in a cleaned and concatenated alignment of 31 universal protein families and covers 191 species whose genomes have been fully sequenced. Green section, Archaea; red, Eucaryota; blue, Bacteria. Labels and color shadings indicate various frequently used subdivisions. The branch separating Eucaryota and Archaea from Bacteria in this unrooted tree has been shortened for display purposes. (Modified fom Ciccarelli et al. (2006). Toward automatic reconstruction of a highly resolved tree of life, *Science* **311:** 1283-1287; reproduced by permission.)

Another problem that has not yet been definitively solved is how to properly root the tree. The first three-domain trees reconstructed by Woese were unrooted. In 1989 the first attempts were made to determine the location of the root, using ancient gene duplications to root the tree. Typically, a phylogenetic tree is rooted by the inclusion of an outgroup, a related group which is less similar to other members of the tree than they are to one another. However, by definition, no outgroup exists for a universal tree containing representatives from all three domains. Most experts place the presumed root of the tree somewhere between the Archaea and the Bacteria, so that the Archaea and the Eucarya share one major branch. This is also the topology suggested in Figure 1, in which the branch separating Eucaryota and Archaea from Bacteria has been shortened.

A major factor that complicates the reconstruction of phylogenetic trees is the abundant horizontal gene transfer now known to occur between often completely unrelated microorganisms. Bioinformatical analyses of prokaryote genomes showed that up to 20-30% of the genes, and sometimes even more, may have been derived from phylogenetically unrelated organisms, often even belonging to different domains. Prokaryote genomes are more and more considered as mosaics of genetic elements derived from different sources. The elucidation of the true relationships thus becomes difficult. Horizontal gene transfer may to a large extent be responsible for the apparent lack in physiological consistency of many phyla and other higher taxa.

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Biographical Sketch

Aharon Oren is Professor of Microbial Ecology at the Hebrew University of Jerusalem, Israel. He obtained his M.Sc. degree (microbiology and biochemistry) (1974) from the University of Groningen and his Ph.D. degree in microbiology (1978) from the Hebrew University of Jerusalem. He has been on the staff of the Hebrew University as research fellow (1979-1982), lecturer (1984-1985), senior lecturer (1985-1991), associate professor (1991-1996) and professor of microbial ecology (since 1996). He has been post-doctoral fellow (1982-1983) and visiting assistant professor (1983-1984) at the University of Illinois at Urbana-Champaign and affiliate professor of George Mason University, Fairfax and Manassas, Virginia (1999-2010). He is an editor for International Journal of Systematic and Evolutionary Microbiology, FEMS Microbiology Letters, and Extremophiles. He is executive secretary/treasurer and past chairman of the International Committee on Systematics of Prokaryotes, and president of the International Society for Salt Lake Research. Prof. Oren was the recipient of the Moshe Shilo Prize (1993) and the Ulitzki Prize (2004) of the Israel Society for Microbiology, and was elected fellow of the American Academy of Microbiology in 2000. In 2010 he was awarded an honorary doctorate by the University of Osnabrück, Germany. His major interests are the microbial ecology of hypersaline environments, the physiology of halophilic microorganisms, and microbial taxonomy.