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# The Species Concept is Even More Problematic

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#### Abstract

The human microbiome is the ensemble of genes in the microbes that live inside and on the surface of humans. Because microbial sequencing information is now much easier to come by than phenotypic information, there has been an explosion of sequencing and genetic analysis of microbiome samples. Much of the analytical work for these sequences involves phylogenetics, at least indirectly, but methodology has developed in a somewhat different direction than for other applications of phylogenetics. In this article, I review the field and its methods from the perspective of a phylogeneticist, as well as describing current challenges for phylogenetics coming from this type of work.

## Introduction

Although there is something of a divide between phylogeny as practiced as part of microbial ecology on one hand and that for multicellular organisms on the other, there are many parallels between the two enterprises. Both communities struggle with issues of sequence alignment, large-scale tree reconstruction, and species delimitation [1]. However, approaches differ between the microbial ecology community and that of eukaryotic phylogenetic, in part because the scope of the former contains an almost unlimited diversity of organisms, leading to additional problems above the usual. The species concept is even more problematic for microbes than for multicellular organisms, and hence there is also considerable discussion concerning how to group them into species-like units. Organizing microbes into a sensible taxonomy is a serious challenge, especially in the absence of obvious morphological features [2].

The human microbiota is the collection of microbial organisms that live inside of and on the surface of humans. These organisms are populous: it has been estimated that there are ten times as many bacteria associated with each individual than there are human cells of that individual. The microbiota have remarkable metabolic potential, being an ensemble of genes estimated to be about 150 times larger than the human collection of genes. Much of our metabolic interaction with the outside world is mediated by our microbiota, as it has important roles in immune system development, nutrition, and drug metabolism. Our food and drug intake, in turn, impacts the diversity of microbes present. Traditionally, our microbiota have been transmitted from mother to infant in the birth canal and by breastfeeding. In this section, I will briefly review what is known about the human microbiota and its effect on our health.

It is now possible to assay microbial communities in high throughput using sequencing. One way is to amplify a specific gene in the genome for sequencing using polymerase chain reaction (PCR). Scientists typically pick a "marker" gene in that case that is meant to recapitulate the "overall" evolutionary history of the microbes. Another way is to randomly shear input DNA and/or RNA and then perform sequencing directly. We will consistently refer to the former as a survey and the second a metagenome, although these words have not always been consistently used in the literature [3].

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The Human Microbiome Project generated lots of survey, metagenome, and whole-genome sequencing data and these data are available on a dedicated website. The MetaHIT study also generated lots of data but it is not available to outside researchers [4].

As described above, "metagenome" means that DNA is sheared randomly across the genome rather than amplified from a specific location, and thus the genetic region of a read is unknown in addition to the organism from which it came. Because metagenomes do not proceed through an amplification step, they do not have the same PCR primer biases as a marker gene survey; however, extraction efficiency concerns remain and multiplex sequencing is known to have biases of its own.

It is possible to subset metagenomic data to marker genes. That is, one can use 16S reads that appear in the metagenome as well as reads from other "core" genes that are expected to follow the same evolutionary path and are present in a large proportion of microorganisms. This is proven to be a useful strategy, and several groups have built databases of core gene families as well as provided programs and/or web tools to phylogenetically analyze meta genomes subset to those core genes However, because of the variability of gene repertoire in microbes, this core gene set may be relatively small: even the largest collection of genes in these databases only recruits around 1% of a meta genome.

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