

The Zoo In the Mouth

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There's a philosophical quandary breeding in your mouth.

Ever since Aristotle, philosophers and scientists have searched for the right way to classify living things. We call living things with feathers “birds,” but we can also divide birds up into smaller groups, like pigeons and storks. We can drill down even further, to different species of pigeons. But it doesn't feel right to classify birds all the way down to every individual feathered creature on Earth. The fundamental unit of life's biodiversity has long been the species. Charles Darwin named his book *The Origin of Species* for a reason.

Darwin threw older notions of species into doubt, challenging the idea that they were fixed since creation or only able to change slightly over time. adjusted over time. In fact, old species give rise to new ones like shoots from a tree. Darwin concluded that where we choose to draw a line to mark the boundary of a species is a matter of convenience.

While Darwin created the modern foundation for biology with his theory, biologists didn't abandon the word *species*. It's still a helpful term for describing how life evolves—even if it's left scientists arguing about which definition can hold up best against the churning complexity of evolution.

In the 1940s, the biologist Ernst Mayr declared that “species are groups of interbreeding natural populations that are reproductively isolated from other such groups.” In other words, it's all about sex.

Other scientists didn't like Mayr's definition, because reproductive isolation is a squishy term. Stick a squirrel on a remote island, and it can't reproduce with its fellow squirrels on the mainland. Have you made a new species? Biologists now know that populations gradually become more and more reproductively isolated over thousands or millions of years. Do we have to wait till they're totally isolated before declaring the two populations new species?

Some scientists suggested that we think of a species in terms as a branch of the tree of life—as the smallest group of organisms that all descend from a common ancestor, and which we can distinguish from other groups. (I wrote a feature a few years ago about these debates over species in *Scientific American*, which you can read [here](#).)

These definitions work tolerably well for animals and plants. But they've turned out to be pretty lousy for microbes. And that's left scientists in a quandary, because it's now clear that the vast majority of the genetic diversity on Earth belongs to bacteria, viruses, and other life forms invisible to the naked eye.

When microbiologists started to study microbes in the nineteenth century, they went about their business like zoologists or botanists. They'd describe a microbe based on its appearance, what it fed on, and other features they could study in their laboratory. If it seemed different from other microbes that had been previously described, they'd give it an official species name, like *Escherichia coli*. When later microbiologists would come across a microbe—say, in the blood of a sick patient—they'd identify its species by systematically inspecting its traits, in much the same way a bird-watcher would look at a bird's plumage and listen to its song.

In the late 1900s, microbiologists were abandoning this method in favor of a new one: identifying species by their DNA. Zoologists and botanists were making the shift, too, but microbiologists were in for a particularly big shock. The diversity of microbes turned out to be mind-bogglingly exuberant. Microbes that had been considered almost identical before the age of DNA turned out to be more genetically different from each other than maple trees and penguins.

As I describe in my book *Microcosm*, a single species—*E. coli*—turned out to contain multitudes, from beneficial bacteria that can heal a baby's dysfunctional gut to all sorts of pathogens that make us sick in frightfully different ways, from making our cells spill their contents to slithering into them like tapeworms.

Making matters even more confusing was the discovery that microbes don't simply pass down their genes to their offspring the way we do. They are constantly swapping genes with each other, with little respect for any so-called species barrier. Scientists sometimes try to represent this gene traffic by drawing a web of life instead of a tree of life.

Trying to fit microbes into a definition of species based on animals and plants is a bit like trying to herd a flock of flamingos into a school bus. Mayr's definition is not much help, since microbes don't have sex like animals do. The branch-of-the-tree-of-life definition is not much help, either. Theoretically, any microbe that picks up a mutation is distinct from other microbes and should be considered its own species. And if it passes that particular mutation to a distantly related microbe, the whole tree-based approach collapses.

The situation is such a mess that some microbiologists have given up on species altogether. In 2012, W. Ford Doolittle wrote an awesomely titled commentary called, "How Bacterial Species Form and Why They Don't Exist."

This confusion can make life hard for microbiologists when reporters call. Like many other journalists, I've been reporting a lot on the remarkable explorations scientists have been making of the human microbiome—the collection of germs that call us home.

I've repeatedly asked, "So, how many species are in us?"

And each time, the microbiologists I talked to would squirm out of a real answer.

As deeply uncomfortable as microbiologists may be with the whole idea of species, they still need some way to measure the diversity of, say, the microbiome. Instead of species, they typically end up using something species-ish.

When microbiologists were shifting to using DNA, they chose one gene as a way to distinguish different kinds of microbes from each other. The gene, found in all living things, is called 16S rRNA. Microbiologists compared the variations in 16S rRNA genes from individual microbes that belonged to a single species—that is, a species as microbiologists would traditionally identify it.

They found that the gene varied by up to three percent. So they decided that if two microbes were 97% identical in this one gene, they belonged to the same group. Instead of a species, they called this group an operational taxonomic unit.

But it eventually turned out that this 97% cut-off was wrong. A good example of how it can lead scientists astray is the case of two species of bacteria that live on our bodies, *Streptococcus pneumoniae* and *Streptococcus mitis*. Back in the barbaric pre-sequencing days, microbiologists decided they were two separate species, deserving of two separate names. When you look at how these two microbes make a living, that decision makes ample sense. *S. pneumoniae* causes pneumonia, while *S. mitis* can live harmlessly on teeth.

Yet the 16S rRNA sequences of these two “species” are over 97% identical. In fact, they’re 99% similar. While their 16S rRNA genes have remained nearly identical, some of their other genes have veered off in different directions.

In the face of all this delicious confusion, microbiologists need a more powerful way to sort microbes into groups. But there’s been a limit to how fine they can draw their distinctions. Even the best DNA sequencers are not perfect. Here and there, they will make an error in reading a microbe’s genes. When microbiologists are distinguishing different kinds of bacteria with 97% thresholds, those little errors don’t matter. But if microbiologists want to distinguish microbes by tiny differences, making those errors would be akin to mistaking a wolf for a chihuahua.

A. Murat Eren, a microbial ecologist at Marine Biological Laboratory in Woods Hole, Massachusetts, and his colleagues have come up with a powerful new way to chart the diversity of microbes. They call it oligotyping. They line up the 16S rRNA genes from a group of microbes, and then they look for spots in the gene that have the most differences from one microbe to another. The scientists narrow down their search to the fewest spots that can distinguish the largest number of groups, which they call oligotypes. This approach allows them to avoid depending on tiny, error-prone differences that might only be present on a single microbe’s gene.

Recently, Eren and his colleagues tested out oligotyping on the microbiome. They searched the DNA sequences collected by the Human Microbiome Project, a massive survey of

microbial genes from over 200 people. Eren and his colleagues limited their search to just the microbes from people’s mouths. That still left them with over 10 million sequences to sift through.

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All told, they found at least 362 oligotypes of bacteria.^{*} Most of their oligotypes belonged to conventional species, but often the scientists found that a single so-called species contained several distinct oligotypes.

These oligotypes are not just minor variations on a theme. They lead different lives. Our mouths are like jungles, with lots of ecological niches. The environment on a tooth is very different from that on the tongue,

which is different in turn from the gums. Eren and his colleagues found that each oligotype dwelled in just one or a few parts of the mouth—and did so consistently from one person to the next. Some oligotypes only live on teeth, for example, while others live mostly on the tongue. The evidence for all this diversity already existed in the Human Microbiome Project's databases, but until now it was hiding in plain sight.

There are plenty of solid, practical reasons for inventing a better way to measure the diversity of microbes—even just in our mouths. Some bacteria that live there can potentially make us sick—not just by causing cavities, but by causing diseases in other parts of the body, such as heart disease. Old-fashioned definitions of species can lead scientists to overlook these pathogens, lumping them into the same group as harmless microbes.

But this research is also important for a more basic reason—because it helps us to wrap our minds around the staggering diversity of life on Earth. One way to start appreciating how vast that diversity is to just open our mouths.

*The paper will be posted at some point this week by the journal. The reference is: Eren et al., "Oligotyping analysis of the human oral microbiome," PNAS. www.pnas.org/cgi/doi/10.1073/pnas.1409644111