Molecular taxonomy and explorers of the microbial jungle

Water bears, roundworms, hairybacks and mud dragons teem amongst the sand grains. **Holly Bik** introduces us to the world of marine meiofauna and the new tools and techniques that are revolutionizing taxonomy.

Every patch of sea floor, every stretch of sandy beach, contains an invisible living world. Grab a handful of mud or sand and you won't see much at first glance—but look under the microscope, and a busy mix of worms, crustaceans, and odd-shaped creatures will quickly be revealed.

Benthic ecologists and taxonomists who study these microscopic marine animals group them according to body size. Collectively, these animals are named the 'meiofauna—an intermediate size class that typically includes all species with a body size between 45 µm to 1 mm in length. In marine sediments, nematode worms (roundworms) are by far the most abundant group, but the meiofauna includes over 20 other animal phyla such as Tardigrades (water bears), Kinorhynchs (mud dragons), Gastrotrichs (hairybacks) and Platyhelminthes (flatworms), as well as fungi, protists, and eggs and larval stages of larger organisms which fall within this size class. As you may have guessed, the meiofauna is a somewhat arbitrary conglomerate of organisms. Size classes of benthic species have been historically defined by taxonomic protocols that rely on a stack of steel sieves with decreasing mesh sizes (the standard equipment that marine scientists use to collect and separate animals from sediment particles). The meiofauna comprise all species that do not pass through a 45 µm fine mesh sieve—which generally translates into animals with a body size of 1–3mm in length, about the same size as a grain of sand.

Nematode worms represent one of the most abundant and diverse groups within the meiofauna. Any given patch of marine mud (whether in a coastal estuary or in the deep sea) contains hundreds of thousands—and sometimes, up to tens of millions—of roundworms per metre squared. Very little is known about the ecology or evolutionary history of most of these species. For nematode worms, the total number of species on earth has been estimated to be anywhere between one million to 100 million species, yet only ~4,000 marine nematode species have formal taxonomic species descriptions. Nematodes and most other meiofaunal groups have a persistent 'taxonomic deficit'. The pace of traditional morphological taxonomy is too slow to keep up with the astounding number of meiofaunal species on the planet. Most species are never described, and new species are almost certainly lurking in every patch of sea floor.

On a glass coffee table, most marine nematodes would look like small specks of dust when viewed with the naked eye. To transfer these worms from a petri dish to a glass microscope slide, taxonomists use an eyelash glued on a long wooden handle (think: a paintbrush stripped of all bristles but one) or a fine metal wire. Studying meiofauna requires both patience and fine motor skills, and honing this skill set can take years if not decades. Specimens must be carefully mounted on glass microscope slides and viewed under high power (typically 100 x) in order to discern small and hidden anatomical features. Mouthparts, body ornamentation, tail shape, and sensory organs are all used to differentiate amongst nematode species. However, because the nematode body plan is so simple—a hollow tube pointed at both ends—distantly related species can have almost identical morphological features. Meiofaunal taxonomists must use high-power electron microscopes to look for small differences in morphological features, or more commonly in recent years, they must harness the power of DNA.

Genomics and DNA sequencing have upended meiofaunal taxonomy over the past two decades. But, instead of making taxonomy obsolete (as many scientists initially feared), the ability to investigate a species' genome has revolutionized marine science. Instead of sitting at the microscope for months on end, molecular taxonomists can now use genetic information to quickly obtain a species identification. Visual morphology and DNA barcodes can both be obtained from the same specimen, allowing taxonomists to more deeply examine the relationship between the observed anatomical structures and the evolutionary history of that species inferred from the genetic 'family trees'. Molecular biology has (unsurprisingly) spurred massive reorganizations in the taxonomic classifications of many marine meiofaunal groups, proving that visual morphology is often not the best indicator of species relatedness.

Another important advancement has been the development of high-throughput sequencing platforms, such as Illumina's MiSeq and HiSeq machines, which can generate hundreds of millions of DNA sequences in the course of a few days. With these newer types of DNA sequencing technologies, visual identification can be bypassed altogether. All the animals in a handful of mud can be ground up simultaneously to extract their genetic material, and a common DNA barcode is generated from all the species present in this mixture. These so-called 'metabarcoding' studies of marine meiofauna usually rely on a short fragment of the 18S ribosomal RNA gene (a conserved nuclear gene that encodes the recipe for making a ribosome, the protein factory of cells). A high-throughput dataset containing millions of DNA barcodes is then compared to online genetic databases to deduce the meiofaunal species present in your original dollop of mud. However, sparse or incomplete databases for some meiofauna groups can still make it difficult to identify many species—meaning that the 'taxonomic deficit' continues to exist even in the genomic age.

Genomics is empowering modern taxonomy, and vice versa. Random pieces of DNA are rarely useful without context—and the elegant morphology of microscopic meiofaunal species is perhaps the perfect type of complementary data, especially given the strong historical traditions of taxonomic disciplines and the roughly 280 years of scientific effort since the time of Linnaeus! Similarly, modern taxonomists recognize the limits of morphology, and are increasingly excited to incorporate cutting-edge genomic techniques to answer long-standing questions about the ecology and evolution of microscopic species. Microbial 'dark matter'—meiofauna, protists, bacteria, viruses, and other hidden forms of life—are globally ubiquitous but too often overlooked in favour of larger and more charismatic marine species. Meiofaunal biodiversity may rival that of insects on earth, and marine sediments are an unexplored microbial jungle—where important scientific discoveries are still hiding amongst the sand grains.

Holly Bik (holly.bik@ucr.edu) is Assistant Professor, Department of Nematology, University of California at Riverside.

Nematode worms are one of the most abundant and diverse groups within the meiofauna. The nematode body plan is simple, and distantly related species can look almost identical. © Tiago José Pereira.