Geobiology

Animal Roots and Branches

By Martin Thurau

Source: Wörheide/LMU

Sponges are one of the most ancient of all extant animal lineages. LMU geobiologist Gert Wörheide studies the group to learn about early animal evolution and how new species evolve.

There are clumps of yellow "stuff" on the reef-face – a familiar sight for LMU geobiologist Gert Wörheide, for he has often encountered such critters on his undersea expeditions into narrow crevices of coral reefs. These dimly lit spaces are often "nearly completely covered with them," he says. Some are bright yellow in color, others paler and less striking. These tuffets of porous material reminiscent of polystyrene foam are in fact living organisms. They may not look impressive, but they are highly successful. One of them, the so-called "lemon sponge", occurs all over the Indo-Pacific, on coral reefs in the Maldives and Polynesia, on the Great Barrier Reef off the Northeast coast of Australia, and even in the Red Sea and has been one of Wörheide's favourite study objects for more than 15 years.

And "they all look alike," even to the trained eye of an expert such as Wörheide. "If you were to put a specimen down on the table in front of me, I couldn't tell what part of the world it comes from." Focusing on their morphology – the shape of the tiny spicules that serve as the sponge's skeleton, for instance – doesn't get one very far. Accordingly, all lemon sponges are assigned to the same species, *Leucetta chagosensis*, irrespective of their geographic origin. "But the genetic data we have analyzed reveal," – and here Wörheide pauses for emphasis – "pronounced divergences between populations. They likely belong to different species."

One might be tempted to dismiss this finding as being of interest only to taxonomists. After all, it is estimated that there are around 15,000 species of sponges in the world today, and just over half of them have been classified. But Wörheide's case actually raises difficult definitional issues that are at the heart of our picture of biological evolution: How did animals such as sponges evolve in the first place? What is a species? How does one distinguish any given species from its closest relatives? How do new species arise? What mechanisms drive 'speciation', the splitting of one species into two?

The first sponges we know of are well over half a billion years old, and some of the fossil forms dating from that period are virtually indistinguishable from some modern sponges. It is as if morphological evolution in sponges came to a standstill very early on. All sponges belong to the phylum Porifera, a group that lies very close to the root of the phylogenetic tree that encompasses all multicellular animals or 'Metazoa'. As they are among the earliest known metazoans, sponges are crucial for our under-standing of the transition from single-celled amoeboid protozoans to multicellular animals. And this momentous phase in evolution is still the subject of controversial debate among evolutionary and developmental biologists.

What mechanisms drive 'speciation', the splitting of one species into two?

Needless to say, this topic is also of intense interest to Gert Wörheide. He has held the Chair of Paleontology and Geobiology at LMU since 2008, and also serves as Director of the Bavarian State Collections in Paleontology and Geology. Since he began his career more than 20 years ago, Wörheide has been fascinated by the ecosystems of the Great Barrier Reef and the adjacent Coral Sea. These biotopes are extremely species-rich, and represent hotbeds of diversity and ongoing evolution. Donning his diving gear, he has scoured the seaward slopes of the reefs, which are riddled with crevices and cavities, collecting biological material for closer study. And at some point he turned his attention away from the light-loving and colorful corals to the sponges – less eye-catching denizens of reef ecosystems, with their long and largely obscure evolutionary history. The realm of the deep sea, however, which harbors its own unique inventory of species, remained a closed book for him.

But in 2009 he returned to the Great Barrier Reef with an international team, and a remote-controlled submersible. The undersea vehicle was deployed to explore the reefs and seamounts to depths of about 1,000 meters on the Queensland Plateau, a submerged carbonate platform in the Coral Sea to the northeast of the Great Barrier Reef. The submersible enabled the marine scientists to journey back through time. The monitors aboard the mother ship revealed a previously unknown fauna and, using a joystick to control the submarine's mechanical arms, the team was able to sample material from the seabed. The expedition broached a lost world populated by unique invertebrates. Here ecosystems were found that have remained largely unchanged for tens of millions of years, and harbor a remnant fauna that dates back to the end of the Mesozoic Era 65 million years ago. Among the "living fossils" that move like wraiths though these waters is the graceful chambered *Nautilus*, an archaic cephalopod. The biologists were able to collect cold-water corals, crinoids (sea lilies), brachiopods, mollusks – and sponges.

Sponges are represented in virtually all marine habitats, from the tidal zone to abyssal depths. A few hundred species have adapted to freshwater habitats, but the vast majority is restricted to the oceans, and Porifera are especially diverse in the Indo-Pacific. Sponges come in all shapes – including beaker-, knob- and club-like forms – and in sizes ranging from less than a millimeter to the 2-meter diameter reached by the giant barrel sponges, which may live for hundreds of years. Many species possess a complex secondary metabolism and synthesize pigments, poisons to deter predators, or other unusual compounds. Pharmaceutical companies have begun to explore this reservoir of chemical structures in quest of promising drug candidates. Indeed, a number of therapeutics based on natural products isolated from sponges are now on the market.

Sponges possess relatively few of the kinds of distinctive morphological characters which are a hallmark of animal species that are more familiar to us. However, the glass sponges (Hexactinellida) at least are characterized by the highly complex and unique shape and architecture of their spicules, and the calcareous (Calcarea) and demosponges frequently also support their tissues by means of a mesh of spicules, made of calcium carbonate or glass, respectively. Their detailed architecture has provided many of the characters used by taxonomists to classify sponge species. DNA sequence data have now begun to complement and test traditional systematics.

A journey back through time

Almost 10 years ago, Wörheide pioneered an international collaborative project that aimed to catalog all sponge species with the aid of DNA barcoding. Just as every product in a supermarket's inventory nowadays is designated by a unique barcode, the idea behind the Sponge Barcoding Project was to use the sequences of specific genes that all sponges share as identifiers with which specimens could be unambiguously distinguished from one another and later assigned to a genus and a species. The method is based on the principle that sequences of a given gene from close relatives will differ less than those obtained from their more remote kin. And ideally, the number and distribution of the differences between similar pairs will allow one to decide whether or not the donors belong to the same species. Wörheide's team has now analyzed material collected in the course of several expeditions, and studied thousands of specimens held in the Queensland Museum in Brisbane and in other collections.

The sponge's body plan is quite simple, and all body functions proceed at the cellular level. Sponges lack muscles, sensory organs, and a digestive tract. Simple diffusion suffices for gas exchange. Water is pumped by flagellated cells into the body through pores in the outer cell layer, the animal subsists mainly on food particles filtered from the stream of fluid through the body which is then expelled through a chimney-like body opening, the so-called osculum. Not much is needed to be an animal after all.

According to some evolutionary theory, simple structures have been thought likely to be older than more complex ones. Are sponges then the most basal animal lineage? In addition to the primitive sponges which, according to Wörheide's molecular genetic data, are all derived from a common ancestor, there are other candidates for this title. Take *Trichoplax adhaerans*, the only officially classified representative of the phylum *Placozoa*. These are planar amoeba-like animals made up of only four loosely associated cell types. They are, like sponges, devoid of organ systems and nerves, and they have a small genome compared to other animals.

However, given the length of time elapsed since the simplest animal phyla originated, genetic data from their modern descendants need to be analyzed with great care. Reports recently published in the leading journals *Science and Nature* have suggested that comb jellies (which superficially resemble true jellyfish, themselves related to corals) represent the most basal extant animal lineage, even though – unlike the sponges – they do have nerves and muscles. This would imply either that the common ancestor of animals already possessed these highly complex traits (and that sponges and *Placozoa* must have lost them) or that these systems arose twice independently during animal evolution. Both proposals are "quite interesting hypotheses" according to Wörheide. "They would overturn our whole understanding of evolution and overturn several hundreds of years' worth of comparative anatomy." He and his colleagues have been working on early-animal phylogeny for many years using genome-scale (phylogenomic) data and have since shown that the "comb-jelly basal" hypothesis might in some cases be caused by phylogeny reconstruction artefacts. To generate additional data, Wörheide and his team are currently sequencing and comparing the genomes of marine and freshwater sponges, work partly funded by a LMUexcellent Project. This data will contribute to answering some of the still controversially discussed questions about the early evolution of animals.

The reconstruction of molecular phylogenies is a much more complicated task than the barcoding of a species with the help of a few marker-gene sequences. For these phylogenomic studies, Wörheide uses well over 100 genes from each of several dozen species. "And the search for the most probable phylogenetic tree, which involves testing complex evolutionary models, is extremely time-consuming," he says. "It's a good year's work for the cores in our computer cluster."

The so-called "lemon sponge", one of Wörheide's favourite study objects since more than 15 years. *Source: Wörheide/LMU*

Wörheide's team has now used a molecular systematics approach to establish a new family tree for 'lithistid' sponges. These 'rock sponges' share a characteristically robust skeleton, and have traditionally been assigned to a single taxonomic group. But the genetic data reveal that they do not actually form a natural group, i.e., not all are derived from the same common ancestor. Their characteristic spicule architecture actually evolved independently in several different lineages.

So what exactly is a species? That's a good question, Wörheide responds. There are myriads of species concepts. The biological species concept, in spite of its deficiencies, remains the most coherent: Individuals belong to the same species if they can mate and produce fertile progeny. Members of different species, on the other hand, are "reproductively isolated" from each other. The problem is that this definition is of little practical use in the case of sponges – not least because this group of relatively simple organisms uses a wide range of reproductive strategies. "There are asexual and hermaphrodite forms, species with separate sexes and species that nourish their embryos." So sponge specialists are frequently "still stuck," says Wörheide, with the classical morphological species concept, which defines species on the basis of differences in their external structures.

And how do such differences arise? How can the genetic variation that leads to morphological differences develop? What accounts for the splitting of one species into two? The classical model posits that speciation begins when genetic and geographical barriers divide subpopulations of the same species from one another, so that they have a chance to follow different developmental trajectories. Over hundreds of millions of years, tectonic forces have torn landmasses apart, fragmenting populations and disrupting ocean currents and dispersal patterns.

Not only large-scale geological processes, but also geographic barriers, climatic factors and fragmentation of habitats by human activities can lead to the divergence of once cohesive populations. Even changes in migratory behaviors can lead to permanent separation of subpopulations. Most adult sponges are sessile, but their motile larvae “can disperse for a period of time in the water column,” says Wörheide, albeit for shorter times than, for example, coral larvae. Some sponges reproduce asexually by means of buds, which can attach to flotsam and/or are dispersed by currents to colonize new habitats.

Dissociated subpopulations diverge because the physical environments, the biological communities, the habitats, competitive interactions and ecological niches they encounter are likely to differ. Hence, they are exposed to different selection pressures. Chance too plays an important role. If a newly formed subgroup is much less numerous than its ancestral population, its gene pool is smaller, which reduces the range of variation that evolution can draw on. Geneticists call this the “founder effect”.

The genetic differences between populations of the widely distributed lemon sponges in different seas turn out to be quite extensive, despite their morphological conservatism. Wörheide’s painstaking genetic studies have demonstrated that a combination of isolation and founder effects has powered this remarkable example of morphologically cryptic speciation: Here too, evolution has not been idle.

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