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**Diversity in the globally intertwined giant barrel sponge species complex**  
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## SUMMARY

Sponges are the oldest multicellular animal lineage that occurs on Earth today. Despite their special place in evolutionary history, sponges have received relatively little attention in scientific research compared to other 'charismatic' organisms such as corals and fish. Among other things, this is due to the logistical problems of working under water and the lack of clear distinctive features between sponge species. However, it is becoming increasingly clear that sponges play a central role in marine ecosystems, such as coral reefs. Sponges, for example, are important in nutrient cycles, such as nitrogen, carbon, silicon and phosphorus. In addition to this, sponges provide habitat for other organisms and can erode and consolidate coral reef structures depending on the species, thereby influencing biodiversity in the system. In view of the changing conditions in oceans and seas, which are related to climate change and other human-induced stressors, it is important to thoroughly understand the role of sponges in coral reefs. This knowledge is essential in order to predict how coral reefs will respond to future conditions and to take appropriate measures to preserve these rich ecosystems. In this thesis, an attempt is made to better understand the evolutionary history of sponges and to gain a better understanding of the cooperation between sponges and the various microorganisms that they harbor. To achieve this goal, the giant barrel sponge has been used as a model organism. This group of sponges is widespread and occurs in the Caribbean and Indo-Pacific, from the east coast of Africa and the Red Sea to Taiwan and New Caledonia. Moreover, giant barrel sponges are abundant components of many coral reefs and are among the largest sponges in existence.

8

The central questions of this thesis are: 1. How many types of giant barrel sponges exist? 2. What is the evolutionary history of the extant giant barrel sponges? 3. What drives variation in the composition of the prokaryotic community of giant barrel sponges? 4. How do richness, diversity and composition of the prokaryotic communities in (giant barrel) sponges relate to those of other organisms in coral reef ecosystems?

To answer these questions, samples from a large number of giant barrel sponges (*Xestospongia* spp.) have been collected. A family tree of all specimens was constructed after DNA-analysis. Although it was originally believed that the giant barrel sponges in the Atlantic Ocean and the Indo-Pacific Ocean were two different species, our analyses showed that there are several species in both ocean basins. Even more remarkable, is that each species is most closely related to a species in the other ocean basin. The relationships and patterns in the family tree do not correspond with the geographical distribution of the giant barrel sponge species as presently understood. In other words, a giant barrel sponge from Curaçao, in the Caribbean can be more akin to a specimen in Indonesia,  $\pm 18,000$  km apart, than to a specimen one meter away on the same reef. According to our analysis, there are three giant barrel sponge species in the Atlantic Ocean, and, at least, six in the Indo-Pacific Ocean. The intertwined evolutionary history of the species in both oceans shows us that there were

already several giant barrel sponges before the Atlantic Ocean and the Indo-Pacific Ocean became separated. Each of these species was split into two populations during the collision of the European and African tectonic plates about 40 million years ago and subsequently developed into different species.

Although the giant barrel sponge species look alike, there are subtle differences in some places. In North Sulawesi (Indonesia), one species has a smooth appearance, while the other is covered with spiky or digitate structures. In Tanzania, one type is bronze-colored and another blue-purple. However, these distinctions are location-specific, and there are no distinctive features that occur ocean-wide. This makes it impossible to distinguish between the species in the field without the ability to perform DNA analysis.

Sponges house a large and diverse community of microorganisms. It is generally believed that these microorganisms play a central role in the physiological processes in the host. Modern DNA-analysis has made it possible to make an extensive screening of the microorganisms that live in giant barrel sponges. At present, millions of microorganisms can be identified from a single individual. It appears that the region in which a giant barrel sponge occurs mainly determines the composition of their microbial community. However, if you look at a smaller scale within a region, for example within one reef or around an island such as Curaçao, it appears that not only is the geographical location important, but the host species to which an individual belongs also plays an important role. The composition of the microbial community in giant barrel sponges is, therefore, dependent on a combination of factors, and the influence of these vary across spatial scales.

Sponges were generally seen as one of the richest sources of microbial organisms on coral reefs, and the composition of sponges was believed to be very different from other hosts such as corals, sea cucumbers, fish and lobsters. An extensive analysis of 216 hosts, across a range of host taxa, showed that sponges are indeed a rich source of microorganisms on coral reefs, but not the richest. In addition to this, many microorganisms from sponges are also found in other host species. It appears that the microbial communities of different hosts on a coral reef are much more closely connected than previously thought.