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# IS THE *AZOLLA-ANABAENA* SYMBIOSIS A CO-EVOLUTION CASE?

Carrapiço F., University of Lisbon, Faculty of Sciences, Department of Plant Biology, Centre for Environmental Biology, 1749-016 Lisbon, Portugal, f.carrapico@fc.ul.pt

## Abstract

*Azolla* is an heterosporic floating fern presenting leaves overlapping, each with a floating and a submersed lobe. In the chlorophyllous dorsal lobe exists an ovoid cavity with a filamentous nitrogen-fixing cyanobacteria, usually referred to as *Anabaena azollae*, and several genera of bacteria. This leaf cavity behaves as both the physiological and dynamic interface unit of this symbiotic association where the main metabolic and energetic flows occur. In this sense, it can be considered as a natural microcosm. Traditionally considered as a 'lower' vascular plant, *Azolla* exhibits symbiotic characteristics more evolved than other vascular plant-cyanobacterial symbioses. This symbiosis is sustained throughout the fern's life cycle, where the cyanobacteria and bacteria are always present, in contrast to the more evolved vascular plant groups that associate with cyanobacteria. In this sense, we can consider this symbiotic association as a successful co-evolved system that also makes important contributions to the ecological, biofertilization and biotechnological fields. The concept of *Azolla* as a superorganism is discussed.

**Key words**: *Azolla-Anabaena*, symbiosis, evolution, superorganism

## Introduction

In some way, and despite the evolutive history, the origins, functional significance and taxonomic relationship of nitrogen-fixing vascular plant cyanobacterial symbioses remain almost unknown and a better understanding of these associations is required. In this sense, the *Azolla-Anabaena* research is a fine contribution for this goal.

*Azolla* is a small-leaf floating fern, whose genus was established by Lamarck in 1783 with a fossil record dating back to the mid-Cretaceous. The first description of this plant in the taxonomic literature was made in 1725 by the French Priest Louis Feuillée (1660-1732) from a Peruvian specimen (Evrard & Van Hove, 2004). According to this reference, the plant was used for improving chicken egg production. The leaves are overlapping, each with a floating and a submersed lobe. The chlorophyllous dorsal lobe (floating) has an ovoid cavity with 20-25 simple hairs, which develop during the differentiation of the leaves, one primary branched hair and one secondary branched hair. Inside this cavity exists a permanent endosymbiotic prokaryotic community of a filamentous nitrogen-fixing cyanobacteria, usually referred to as *Anabaena azollae*, and several genera of bacteria, embedded in a mucilaginous fibrillar network that immobilizes all of them, and which fills the peripherical area of the cavity. The centre of this cavity is apparently empty, devoid of mucilage, cyanobacteria and bacteria, and is probably filled by gas or liquid. The *Azolla* leaf cavity behaves as both the physiological and dynamic interface unit of this symbiotic association where the main metabolic and energetic flows are located, and where molecular recognition between the symbionts and the host occur. In this sense, it can be considered as a natural microcosm, a special micro-ecosystem which reveals a self-organization and an ecological defined structure (Carrapiço, 2002; Carrapiço & Pereira, 2005).

## *Azolla*, a co-evolved system

Although traditionally considered as a lower vascular plant (e.g. presence of a protostelic stem), *Azolla* exhibits symbiotic characteristics more evolved than the other vascular plant-cyanobacterial symbioses - cycads (Cycadophyta) and *Gunnera* (Anthophyta). There appears to be no direct correspondence between the fern's evolutionary phylogeny and the complexity of the symbosis. This unique symbiosis is sustained throughout the fern's life cycle, where the cyanobacteria and bacteria are always present (fig.1), either in the dorsal leaf cavities or in the sporocarps, in contrast to the more evolved vascular plant groups that associate with cyanobacteria. In fact, the *Azolla* plants are never infected *de novo*, since the cyanobiont is transferred between generations as akinete inocula. The presence of *Anabaena* throughout the life cycle of the fern favours the obligatory nature of the symbiosis and suggests a parallel phylogenetic evolution of both partners. In this sense, we can consider this symbiotic association as a successful co-evolved system that also makes important contributions to the ecological, biofertilization and biotechnological fields. This micro-ecosystem can also be considered as a natural photobioreactor (Shi & Hall, 1988), with millions of years of evolution, where the symbionts are immobilized and driven by the fern into increasing some of its own physiological and metabolic activities. It is the case of high performance for some specific metabolic reactions of the symbionts, namely nitrogen fixation, ammonium and hydrogen production enabled by the immobilized cyanobiont (Carrapiço, 2002).

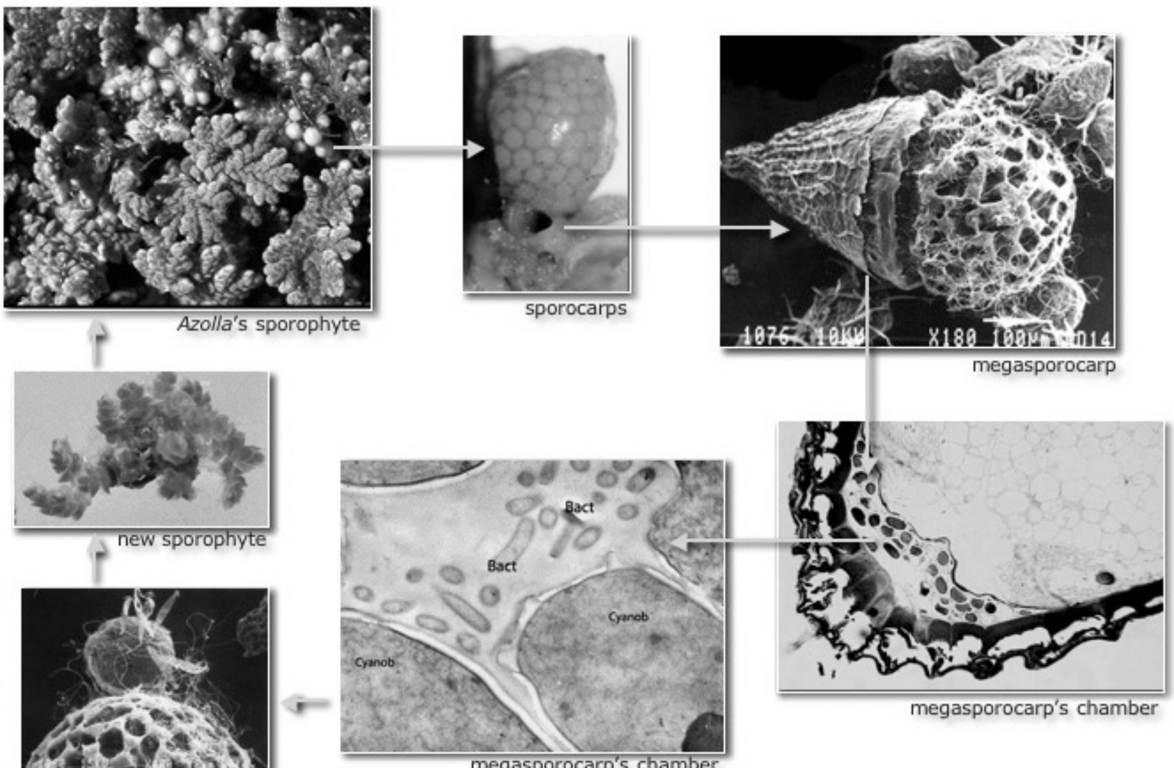


Fig. 1- *Azolla*'s life cycle, showing the permanent presence of bacteria and cyanobacteria throughout the fern's life cycle.

## *Azolla* as a superorganism

The concept of symbiosis was introduced by Anton de Bary in 1878 as "*the living together of unlike named organisms*" in a communication entitled “Ueber Symbiose” (On Symbiosis) during a meeting of the Congress of German Naturalists and Physicians, at Cassel in Germany (de Bary, 1878). De Bary used this term when discussing the presence of the cyanobacteria in the leaf cavity of *Azolla*, as well as the nature of lichens and the role of the algae and fungi in this association. Numerous works about the nature of this symbiosis, which is still not fully understood nowadays, followed this innovative approach. In 2003, Jan Sapp (Sapp, 2003) introduces the concept of superorganism, referring that “*every eukaryote is a superorganism, a symbiome composed of chromosomal genes, organellar genes, and often other bacterial symbionts as well as viruses. The symbiome, the limit of the multicellular organism, extends beyond the activities of its own cells. All plants and animals involve complex ecological communities of microbes, some of which function as commensals, some as mutualists, and others as parasites, depending on their nature and context*”. In the same sense, we believe that this idea can be applied to the *Azolla-Anabaena*-bacteria symbiosis. The *Azolla* leaf cavity can be considered as the basic physiological unit of the symbiotic association (Grilli Caiola and Forni, 1999), where complex ecological communities of permanent microorganisms co-exist with the fern to maintain the whole. New novel metabolic and organic capabilities are acquired and developed by the partners to establish a new level of organization, extending beyond the capability of each individual forming the association.

## Conclusion

Since the introduction of the symbiosis concept by Anton de Bary, *Azolla* studies made an important and innovative contribution to a new approach in biology, namely in symbiomics. In this sense, the *Azolla-Anabaena*-bacteria symbiotic association can be consired a successful co-evolved system and a superorganism that also makes important contributions to the ecological, biofertilization and biotechnological fields.

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