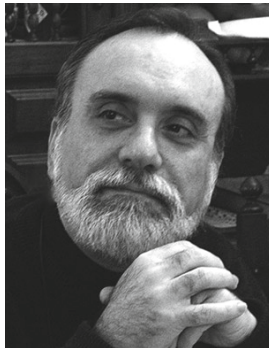


Biodata of **Francisco Carrapiço** author of “*Azolla as a Superorganism: Its Implication in Symbiotic Studies*”

Dr. Francisco Carrapiço was born in Lagos (1951), Portugal, and has a B.Sc. in Biology from the University of Lisbon, a Ph.D. in Cell Biology (1985) from the same university and a post-doc from the Arizona State University, USA. Being an Assistant Professor at the Faculty of Science of the University of Lisbon, and researcher of the Centre for Environmental Biology, his main field of research is Symbiomics, namely the *Azolla-Anabaena*-bacteria symbiotic system. Currently, he is part of the research team based at Utrecht University in The Netherlands studying the *Azolla* discovered in the Middle Eocene marine sediments from the Arctic and incorporated in the Azolla Darwin Project (<http://www.bio.uu.nl/~palaeo/Azolla/Azolla.htm>). He also the position of Secretary of the International Symbiosis Society (ISS) during the last 3 years.

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AZOLLA AS A SUPERORGANISM. ITS IMPLICATION IN SYMBIOTIC STUDIES¹

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1. Introduction

The symbiosis history begun many million years ago, probably even before the first manifestation of life arose in our planet (Carrapiço et al., 2007). But it was only in the nineteenth century with the presentation in 1867, by the Swiss botanist Simon Schwendener, of the “dual hypothesis” related to the lichens structure, that this “real story” had a scientific starting point for society (Boucher, 1985; Sapp, 1994; Honegger, 2000). Although Albert Bernhard Frank introduced the term “symbiotismus” in 1877, the word “symbiosis” was credited to Anton de Bary who, 1 year later, defined it as “the living together of unlike named organisms” (Frank, 1877; De Bary, 1878; Sapp, 1994; Sapp et al., 2002). This concept was presented in a communication entitled “Ueber Symbiose” (On Symbiosis) during a meeting of the Congress of German Naturalists and Physicians, at Kassel in Germany (De Bary, 1878). One of the biological materials used by this author to explain and characterize the symbiotic phenomenon was the *Azolla-Anabaena* association (De Bary, 1878, 1879).

Although this symbiotic association was previously studied by the German botanist Eduard Strasburger in 1873 (Strasburger, 1873), De Bary noted that no stage of the fern’s life cycle was free from cyanobacterium and that the latter was in no way harmful to *Azolla*. He considered this association as an example of a mutualistic case applied to the Plant Kingdom and based on the definition introduced in 1875 by the Belgian zoologist Pierre-Joseph van Bénéden (De Bary, 1878; Sapp, 1994, 2003). In 1895, the Danish botanist Eugenius Warming published “Plantesamfund” (Oecology of Plants), considering the *Azolla-Anabaena* association as an example of mutualism and as an exception to the normal behavior in plant communities – “in plant community egoism reigns supreme” (Sapp, 1994).

¹This article is dedicated to **Prof. Maria Grilli Caiola** life’s work and her contribution to the construction and development of the modern symbiotic studies, namely on the *Azolla-Anabaena*-bacteria research.

All these ideas and studies, at the beginning of the symbiotic research, reflect the importance of this fern and their symbionts for a more complete understanding of organisms' biology. All this, reinforced by the research developed nowadays and by the new data obtained, allows us to have a more broad and dynamic vision of the characterization of the *Azolla-Anabaena*-bacteria symbiotic system.

2. Un Peu d'Histoire

The genus *Azolla* is referred to have been established by the French naturalist Jean-Baptiste Lamarck in 1783 based on a specimen collected by the French botanist Philibert Commerson and his assistant Jeanne Baret in the Magellan region, during his voyage around the world in the Bougainville's expedition (1766–1769) (Svenson, 1944; Lamarck, 1783; Monnier et al., 1993; Schiebinger, 2003). However, Lamarck included it as a new genus of the family Naiadaceae (Lamarck, 1783), which is a family of flowering aquatic plants and not of pteridophytes. Recent data obtained from the Herbarium of the Botanical Garden of Lyon (Jardin botanique de la ville de Lyon, herbier LYJB, France) seems to indicate another possibility. It shows one specimen collected in Argentine (Buenos Aires region) by Commerson and incorporated in 1779 in the herbarium of Claret de la Tourrette, which was later identified as *Azolla magellanica* (currently *Azolla filiculoides*) by another researcher, and was probably the first to be collected (Fig. 1). This supposition is based on the journey on board the vessel the *Étoile*, where Commerson had made the voyage. Is it possible that Lamarck was not the first to



Figure 1. The *Azolla* sample collected by Philibert Commerson in the Buenos Aires region and existing in the Herbarium of the Botanical Garden of Lyon (LYJB) (photo courtesy of Frédéric Danet).

identify and to describe the genus? We do not have a final answer, but in the original description made by Lamarck in the *Encyclopédie Méthodique* (1783) about *Azolla filiculoides*, he refers: “C’est une petite plante aquatique, qui paroît flotter à la surface des eaux à la manière des Lenticules, (Lemna) avec lesquelles elle semble avoir beaucoup de rapports, et qui a néanmoins l’aspect d’une très petite fougère.” (*It is a small aquatic plant, which appears to float on the water surface in the manner of Lenticules, (Lemna) with whom it seems to have a great relation, and which nevertheless looks like a very small fern*).

All these data are correct, but how could Lamarck have all this information, considering that he was not on the trip, that he only saw the plant in the dried form, and *Azolla* did not exist in Europe at that time? The answer can probably be found in the information included by Commerson with the collected plant about its ecology, which was observed by this naturalist *in loco*. Curiously, there is a reference to *Asplenium* with a question mark in the *Azolla* specimen label existing in the Herbarium of the Botanical Garden of Lyon (LYJB). It seems that Commerson considered that the plant was probably a fern, but ignoring the correct genus he decided to do further studies later, considering that the plant resembled an *Asplenium*. In the 1783 work, Lamarck did not refer to the origin of the name, only saying that “Cette plante a été rapportée de Magellan par M. de Commerson” (*This plant was brought from Magellan by Mr. Commerson*).

Traditionally, the term *Azolla* is referred to be formed by two Greek words: *azo*, to dry, and *olloyo*, to kill, alluding to death from drought (Lumpkin, 1993). However, another possibility can be considered, that the term was adapted from a word used by the local population when Philibert Commerson collected it in Argentine or Chili. The first description and crude illustration of this plant in the taxonomic literature was made in 1725 by the French priest and naturalist Louis Feuillée from a Peruvian specimen in the book *Journal des Observations Physiques, Mathématiques et Botaniques* (Fig. 2). The plant under the name of *Muscus squamosus aquaticus elegantissimus* was mentioned to be used for improving chicken egg production (Feuillée, 1725; Evrard and Van Hove, 2004).

3. The Basic Biology

Azolla is a worldwide heterosporous floating or semi-aquatic pteridophyte, presenting overlapping scale-like bilobed leaves covering a slender and branched stem (rhizome) that floats horizontally on the water surface, with single or fasciculate pendulous roots (Carrapiço et al., 2000). This genus is placed in the Azollaceae family and includes monoecious plants that possess dimorphic sporocarps, whose micro- and megaspores develop in a leptosporangiate way (Carrapiço et al., 2000), with a fossil record dating back to the mid-Cretaceous. *Azolla* usually reproduces vegetatively by fragmentation of the abscission layer, at the base of each branch. Sexual reproduction is not very common and seems to be influenced by environmental factors. Sporocarps occur in pairs of either microsporocarps, megasporocarps,



Figure 2. The *Azolla* drawing presented by the naturalist Louis Feuillée in the year 1725 and under the name of *Muscus squamosus aquaticus elegantissimus* (courtesy of the Bibliothèque Centrale du Muséum National d' Histoire Naturelle, Paris).

or one of each, with the exception of *Azolla nilotica* that shows tetrads, in the place of the first leaf lower lobe of the sporophyte branch. Megasporocarps are much smaller than microsporocarps and contain only one megaspore (Carrapiço et al., 2000).

The gametophytic structures of *Azolla* show an endosporic development and are formed by the megaspore and microspore. These female and male structures produce an archegonium and antheridia. Fertilization occurs in the archegonium, where the egg-cell is fertilized by an antherozoid produced in antheridia, originating a zygote. The development of this structure gives an embryo and, by further development, a new sporophyte plant (Becking, 1987). The microspores remain embedded in a structure called massula, where occurs the development of the male gametophyte. The female gametophyte (megagametophyte) also remains completely concealed in the megasporocarpic complex and fertilization is facilitated by the close proximity of both structures (male and female gametophytes). In fact, massulae show glochidia, a specialized structure for anchoring to the megasporocarpic complex that facilitates the fertilization process, enabling the massulae with microspores to become entangled in the filamentous appendages of the epispore wall of the megaspore (Becking, 1987).

The leaves are sessile, alternate, often imbricate, in two ranks along upper side of the stem, 0.6–2 mm wide (Lumpkin, 1993). Each leaf has an emerged, thick, greenish or reddish, and photosynthetic dorsal lobe and a very thin, immersed hyaline ventral lobe (Fig. 3). The dorsal lobe has an ellipsoid cavity, measuring approximately 0.15×0.30 mm, that opens to the external environment

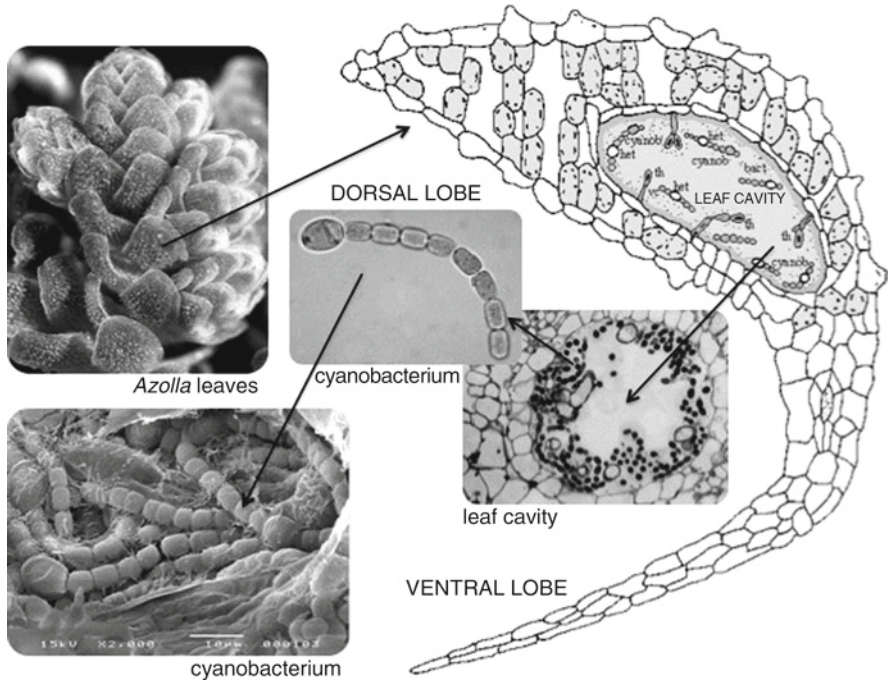


Figure 3. Location of the leaf cavity in *Azolla filiculoides* and *Anabaena azollae* (cyanobacterium).

through a pore, surrounded by two cell layers, located in the adaxial epidermis of the leaf cavity (Braun-Howland and Nierzwicki-Bauer, 1990; Veys et al., 1999; Lechno-Yossef and Nierzwicki-Bauer, 2002). This cavity, an extracellular compartment formed by an infolding of the adaxial epidermis during development (Peters and Meeks, 1989), contains an endosymbiotic community composed of two types of prokaryotic organisms: a heterocyst-forming, N_2 -fixing filamentous cyanobacterium – *Anabaena azollae* Strasburger – (first described by Strasburger as *Nostoc* in 1873 and re-named *Anabaena azollae* in 1884, and probably it may well belong to this genus rather than *Anabaena*) (Peters and Meeks, 1989; Adams, 2000) and a variety of bacteria strains mainly identified as members of the genus *Arthrobacter*, *Corynebacterium*, and *Agrobacterium*, associated with other bacteria showing the presence of nitrogenase (Carrapiço, 1991; Lindblad et al., 1991; Serrano et al., 1999; Lechno-Yossef and Nierzwicki-Bauer, 2002). These microsymbionts are specific of this association and live immobilized in a mucilaginous fibrillar network, which fills the peripheral area of the cavity. This mucilaginous material is delimited by two envelopes, an internal and external one, leaving the center empty and probably filled with gas or liquid (Carrapiço, 1991, 2002; Lechno-Yossef and Nierzwicki-Bauer, 2002).

Also present in the leaf cavity are three types of trichomes, which show an ultrastructure of transfer cells: about 20–25 simple hairs, one primary and one secondary branched hair in number of 2 (one primary and one secondary). Since the *Azolla* leaf cavity has no direct connection with the vascular system of the fern, the trichomes are involved in the transfer and uptake of metabolites from the fern to the prokaryote colony, and from this one to the plant (Braun-Howland and Nierzwicki-Bauer, 1990; Pereira and Carrapiço, 2007). A mixture of lipids, unsaturated lipids, polysaccharides, polyphenols (*o*-dihydroxyphenols, phenols with free –OH groups and tannins), and alkaloids or alkaloid-like compounds were detected in the vacuoles of simple hairs (Pereira and Carrapiço, 2007). The function of these bioactive metabolites in the *Azolla* symbiosis is still not well understood, but may play a role in the selection of the microorganisms that are not useful to the fern, in the control of the endosymbionts in the cavity and in the establishment and maintenance of the symbiosis (Pereira and Carrapiço, 2007). These data also suggest an exchange of chemical compounds at the level of the host–microbionts–host system, functioning as a biological and chemical communication language in this dynamic association (Pereira and Carrapiço, 2007). As we referred previously, the interior of the leaf cavity is lined by an outer and an inner envelope, creating a narrow space close to the periphery of the cavity where the bacteria, the cyanobacterium, and the trichomes are located. This results in an intimate contact between all the partners, helping in the recognition process as well as in the exchange of metabolites and efficient use of the nitrogen fixed by the cyanobiont (Carrapiço, 2002).

4. *Azolla*, a Scientific Curiosity?

For many years, this pteridophyte was seen as a botanical curiosity by Western researchers, since the presence of cyanobacterium inside the leaf cavity allowed it to be considered as a classical example of a mutualistic symbiosis. However, the complexity of the relationship between the host and the symbionts was later recognized as a new level of biological organization. In fact, 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). This micro-ecosystem can also be considered as a natural photobioreactor (Shi and 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.

Azolla has been used as green manure for rice cultivation and animal feed in China and in Vietnam, during several centuries, and more recently in Africa (Carrapiço et al., 2000) and in Central and South America. The use of *Azolla* as

biofertilizer can avoid the adverse effects of chemical fertilizers on long-term soil fertility, thus improving soil productivity and environmental safety. The new advances in the investigation of this symbiosis have contributed to a more comprehensive perception of the complexity between the host and the symbionts. This complexity has been translated into new models of knowledge and new areas of application. The biotechnology and environmental engineering are some of these main fields, which have and can still profit with these new data. New bioreactors can be developed if we consider these results, namely those related with the living conditions existing in the leaf cavity of the fern. The latter enable high performance for some specific metabolic reactions of the symbionts, namely nitrogen fixation, ammonium and hydrogen production by the immobilized cyanobiont. Further, the use of *Azolla* as wastewater biofilter (Costa et al., 1999; Forni et al., 2001) and in biologically based life-support systems (BLSS) incorporated in bioregenerative space devices are now in progress in several laboratories (Carrapiço, 2002).

Recent data from the 2004 Arctic Coring Expedition (ACEX) cores, drilled in the central Arctic Ocean near the North Pole, show the presence of fossil *Azolla* in Eocene sediments (~48,5 Ma) (Brinkhuis et al., 2006). The plant's remains occur as laminations, reflecting seasonal or longer cycles and they have also been observed in more than 50 Arctic wells from northern Alaska, the Canadian Beaufort, and the Chukchi Sea (Bujak, 2007). According to this author, these data can be used to determine the maturation level (amount of heating) and type of hydrocarbons produced by the *Azolla* remains, suggesting that the *Azolla* interval may be an Arctic-wide petroleum source rock (Bujak, 2007). The presence of repeated *Azolla* laminations in the central Arctic Ocean also indicate that the *Azolla* plants grew *in situ* on freshwater layers that repeatedly developed on the surface of the Arctic Ocean, rather than being transported from freshwater bodies, such as lakes, on the surrounding land. Brinkhuis et al. (2006) and Bujak (2007) also suggested that the enormous quantities of *Azolla* inhabiting the Eocene Arctic Ocean for almost a million years may have triggered the initial shift from the Mesozoic greenhouse world towards our present icehouse state. According to their model, CO₂ absorption by the fern resulted in an abrupt reduction in this atmospheric gas with critical consequences in the climatic change and implications for the global biogeochemical cycles (Brinkhuis et al., 2006; Bujak, 2007). A very recent work presented by Collinson et al. (2009, p. 155) indicates that the Arctic *Azolla* can be included in a new fossil species, *Azolla arctica*.

5. *Azolla*-*Anabaena*-Bacteria Association, a New Level of Biological Organization

Although traditionally considered as a lower vascular plant, *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 symbiosis. In fact, this symbiotic system is sustained throughout the fern's life cycle, where the cyanobacterium and bacteria are always present (Fig. 4), either in the dorsal lobe leaf cavities or in the sexual structures (sporocarps) (Carrapiço, 1991, 2002). The *Azolla* plants are never infected de novo, since the cyanobiont is transferred between generations as akinete inocula. The presence of *Anabaena* and bacteria throughout the life cycle of the fern favors the obligatory nature of the symbiosis and suggests a parallel phylogenetic evolution of both partners, and can be considered a successful co-evolved system. Bacteria was first observed and described to be present in the *Azolla* leaves cavities by Grilli (1964). The presence of these prokaryotes in the leaves and also in the cavity below the megasporocarp's indusium in association with cyanobacterium cells suggests a behavior pattern similar to the cyanobiont and can be considered the third partner of this symbiotic system (Carrapiço, 1991).

The *Azolla* leaf cavity can also be considered as the basic physiological unit of this 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. This information is

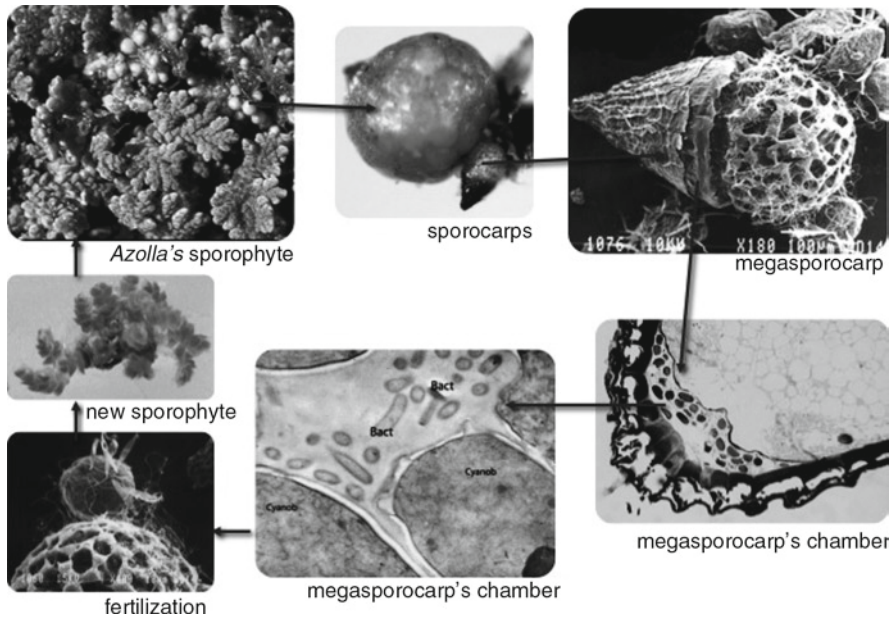


Figure 4. *Azolla*'s life cycle, showing the permanent presence of bacteria (bact) and cyanobacteria (cyanob) throughout the fern's life cycle.

supported and agrees with the concept introduced by René Dubos and Alex Kessler in 1963 related to the creative manifestations of symbioses, where the nutritional effects of symbiosis are not its most interesting manifestation. More important is the fact that many symbiotic systems produce substances and structures that neither one of the two components produces when growing alone (Dubos and Kessler, 1963). This is emphasized by Angela Douglas in her book “Symbiotic Interactions” referring that the common denominator of symbiosis is not mutual benefit but a novel metabolic capability, acquired by one organism from its partners (Douglas, 1994). Also, Douglas Zook reinforces these principles referring that symbiosis is the acquisition and maintenance of one or more organisms by another that results in novel structures and metabolism. Some symbiotic evolutions may involve partner genetic exchanges (Zook, 1998).

These ideas can be found and translated in a clear way in the metabolism of nitrogen associated to this symbiotic system shared by the host and partners. The atmospheric N_2 fixed by the cyanobacterium through the heterocysts is converted into ammonia and released into the leaf cavity. It has been shown that intracellular ammonia pools of symbiotically associated *Azolla* are five times greater than those of endophyte-free *Azolla* (Braun-Howland and Nierzwicki-Bauer, 1990). The activities of ammonia-assimilating enzymes in the isolated trichomes of the dorsal leaf cavity were much higher than those in *Azolla* leaves, while the activities in the *Anabaena* filaments were repressed to very low levels. For example, it was shown that the host accounted for at least 90% and 80% of the total glutamine synthetase and NADH-dependent glutamate dehydrogenase activities, respectively. These results suggest that hair cells play an important role in the assimilation of nitrogen, which the cyanobiont fixes and releases into the cavity and it is transferred to the pteridophyte (Uheda, 1986), and it was acquired during the development of the symbiotic process. Recent data published by Papaefthimiou et al. (2008) indicate the existence of different cyanobacteria strains or ecotypes inhabiting the fern species. These results reinforce our belief that the leaf cavity behaves as a micro-ecosystem or as a natural microcosm (Carrapiço, 2002) with a self-organization and an ecological defined structure, where natural selection acts to evolve different cyanobacteria ecotypes.

All this information agrees with the concept of superorganism referring that, in ecological terms, each plant and animal must be considered as “superorganism” – symbiome, which includes its own genes, those of cellular organelles (mitochondria and/or chloroplasts), as well as the genetic information of symbiont bacteria and virus living within the organism (Sapp, 2003). It is also important to take into consideration the relevance of the fitness and how we validate it in terms of symbiotic prevalence. It goes beyond the reproductive view of each individual and reinforces the ecological behavior of the symbiotic system as a whole (Bouchard, 2007).

These ideas and concepts, especially the superorganism one, can be applied to *Azolla* and its symbiotic association, a good example of a synergistic biological system. In this association, complex ecological communities of permanent microorganisms co-operate along with the fern in the maintenance of the whole.

New metabolic and organic capabilities are acquired and developed by the partners, which establish a new level of organization that goes beyond the individual capabilities of any individual partner, suggesting that the synergies associated to symbiosis had and have a leading role in the morphological, reproductive, physiological, and metabolical complexification of the organisms.

The *Azolla-Anabaena*-bacteria symbiotic association can also be considered as a successful co-evolved system, with the symbionts always present in the fern's life cycle, indicating a phylogenetic parallel evolution of the relation partners, and a typical example of a hereditary symbiosis.

6. *Azolla* in Stress

As we referred previously, *Azolla* can develop as a free floating or semi-aquatic plant. These two ecotypes are present in different but complementary ecological conditions. The first one is present in freshwater aquatic environment, where *Azolla*'s sporophyte shows a horizontal growth with normal rate of sporocarps formation, and roots growing freely in the aquatic medium. The second one, more rare, can be found in sand wet banks, when the water level goes down and where *Azolla* is fixed to the substrate by the root system. It shows a sporophyte vertical growth with a high rate of sporocarps formation, and turning reddish very quickly due to the anthocyanins production and accumulation in the vacuoles of the epidermal cells of the leaves. The presence of these phenolic compounds in the *Azolla* sporophyte leaves is a normal consequence of a stress condition related to high temperatures and phosphorus deficiency (Tung and Watanabe, 1983) and to light intensity. The sporophyte does not present the typical growth it has in water, but resembles a kind of small "bunches," to prevent water losses and to maintain the humidity (Fig. 5). The root system is interconnected and forms a kind of complex network, where the sand particles are trapped. Finally, the higher sporulation rate improving the fitness of the symbiotic system as a whole (symbiome) is a response to the survival in dry environments. This stress condition is a unique situation that needs to be further studied for a full understanding of the plant behavior.

Azolla can grow in natural and artificial media without the presence of a nitrogen source, forming extended, colored mats that cover the freshwater surfaces, where phosphorus is the main limiting factor to its growth. When a phosphorus level over $0.4 \text{ mg} \cdot \text{l}^{-1}$ occurs in nature, an *Azolla* bloom can be the result. It was what happened in April 1993, when a massive fern bloom occurred along several kilometers of the international Guadiana river in Portugal (Fig. 6). In this situation, phosphorus acted as an environmental stress factor and the main trigger for the uncontrolled growth of this plant in the river. In the period 1990–1993, southern Portugal experienced low rainfall with long dry seasons. This factor, combined with several dams along the river, caused low water flow ($3.64\text{--}1.13 \text{ m}^3 \cdot \text{s}^{-1}$) during 1993. Moreover, farming and industrial activity in the upper area of the Guadiana, together with untreated domestic effluents from several towns and



Figure 5. *Azolla*'s sporophyte living in the wet sand banks of the Golegã lagoon (Portugal) and submitted to natural stress conditions, shows small bunch arrangements to prevent water losses (detail in the lower right corner).



Figure 6. Photo of the *Azolla* mat taken in a boat placed in the middle of the Guadiana river, Portugal.

villages, contributed to the organic contamination of the river that year. Lower flows also promoted very high nutrient concentrations. At different river sites during the first months of 1993, the phosphorus levels changed, with maximum concentration values in April between 5.36 and $0.63 \text{ mgP} \cdot \text{l}^{-1}$. This massive *Azolla*

bloom represented the first occurrence in Portugal and Europe of such a large scale uncontrolled growth of this fern in a river (Carrapiço et al., 1996).

Salinity is another stress condition for *Azolla* growth. The presence of 10 mM NaCl in the environment affects the growth of *Azolla pinnata*, becoming lethal at 40 mM (Rai et al., 2006). Plants exposed up to 30 mM NaCl exhibit longer roots than the control and their number is reduced. The salinity sensitivity associated with *Azolla-Anabaena* association results from the inability to maintain low Na and high Ca level under salt stress, since this symbiotic system lacks mechanisms of regulating ion transport, level of Na and NaCl-induced deficiency of Ca, when stressed with NaCl (Rai et al., 2006).

The different abundance of proteins in *Azolla* and in *Anabaena azollae* with functions related to protein assembly, modification and degradation (chaperones), and stress-related proteins, such as superoxide dismutase and peroxiredoxins, probably reflects the radically different growth conditions experienced in the symbiosis (Ekman et al., 2008). Some of these proteins, such as superoxide dismutase located in the heterocysts of the cyanobiont, suggests a role in protecting nitrogenase from superoxide radicals generated via respiration (Canini et al., 1992). Probably, the first stress conditions can be found in the cyanobacterium lifestyle present in the *Azolla* leaf cavity due to the accommodation in this symbiosis, with high heterocyst differentiation and nitrogen fixation elicited by N deprivation (Ekman et al., 2008).

7. Concluding Remarks

We believe that it is important to reconsider symbiosis as a general mechanism in heredity and development, in addition to gene mutations and recombination, as a source of evolutionary innovation, and to hold symbiotic processes as one of the main bases of biodiversity and evolution on Earth. This idea implies also the central role of interactions, in which individuality (new entity) emerges through incorporation. It involves horizontal mergers, which can be rapid, and, usually, discontinuous, creating permanent and irreversible changes, the ground for evolutive novelty. Something new arises through merging, being a unique or new metabolism or structure(s), which was not present before symbiosis (Carrapiço et al., 2007). In this context, we can consider this entity as a new taxonomic novelty or even as a new level of biological organization. Thus, biology must take into account this reality, integrating symbiosis, not only as a factor of evolutionary change, but also as a taxonomic element in the organization of the living world. A good example is the symbiotic system *Azolla-Anabaena*-bacteria, a successful co-evolved system that also makes important contributions to the ecological, biofertilization, and biotechnological fields. The importance of symbiosis must be understood in a global perspective, with consequences not only at the scientific level, but also to develop and improve the adequate tools for teaching symbiomics

in schools and universities. As Joseph Seckback refers in the preface of his book “Symbiosis: Mechanisms and Model Systems,” we must have an “open-minded and science-orientated reader to the global importance of symbiosis and to new aspects of symbiotic relationships among living organisms” (Seckback, 2002).

8. Summary

Symbiosis is one of the main processes responsible for the biodiversity and evolution on Earth, which has had a leading role in the morphological, physiological, and metabolic complexification of organisms. *Azolla*, a heterosporious floating or semi-aquatic fern, constitutes a good example of a synergistic symbiotic system. In the chlorophyllous dorsal lobe leaf, there is an ellipsoid cavity with a filamentous nitrogen-fixing cyanobacterium, usually referred to as *Anabaena azollae*, and several genera of bacteria. This leaf cavity behaves both as the physiological and dynamic interface unit of this symbiotic association where the main metabolic and energetic flows occur, and as a natural microcosm. This symbiosis is sustained throughout the fern's life cycle, where the cyanobacteria and bacteria are always present. In the *Azolla-Anabaena*-bacteria association, complex ecological communities of permanent microorganisms cooperate along with the fern in the maintenance of the whole, which leads to the idea that this symbiotic system can be considered as a superorganism in ecological terms. New metabolic and organic capabilities are acquired and developed by the partners, which establish a new level of organization that goes beyond the individual capabilities of any individual partner, suggesting that the synergies associated to symbiosis had and have a leading role in the morphology, reproduction, physiology, and metabolic complexification of the organisms.

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