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BLOSSOMING TREASURES OF BIODIVERSITY

35. Mosquito Ferns (*Azolla* species) – tiny ‘super plants’

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Introduction

Biodiversity is threatened by humankind's continuing usurpation of our planet's physical space and resources, and the associated damage to the landscape and atmosphere. Food production is widely acknowledged to be the most important human activity that needs to be addressed in order to mitigate the current biodiversity and environmental crises. In this contribution we feature obscure plants called Mosquito Ferns, whose minute size belies their incredible potential. Nicknamed ‘super plants’, these can not only produce food more rapidly than virtually any other life form, but do so in biodiversity-friendly ways that minimise use of space and energy resources. Mosquito Ferns are poised to become leading players in the search for sustainable relationships with the natural world.

The plants

Azolla, with about five extant species, is the only genus in the Azollaceae, a small family of ferns (which is sometimes combined with the genus *Salvinia* in the family Salviniaceae). The delimitation of species of *Azolla* and determination of their correct names is rather unsettled. The species reproduce primarily by budding off new plants. Sexual reproductive structures (described below) are usually absent, and without them it is very difficult to identify the species with certainty. Mosquito Ferns are sometimes mistaken for Duckweeds (Lemnaceae), a family of about five genera and 40 species of flowering plants, which are also tiny aquatic plants of worldwide distribution. Some species of Duckweeds are floating, whereas others are submersed in the water. Duckweeds are the smallest of all flowering plants, but they rarely produce flowers and seeds and, like Mosquito Ferns, reproduce mostly by budding off new plants.

Mosquito Ferns (often “Mosquito-ferns”) are also frequently called *Azolla*, Duckweed Fern, Fairy Moss (the plants have a moss-like appearance), Mosquito Plant and Water Fern. Species of *Salvinia* are more frequently called Water Ferns, so this name should be avoided. The name ‘Mosquito Fern’ is said to have arisen because thick mats of floating plants have a reputation for preventing mosquitoes from laying eggs. The curious genus name *Azolla* is based on the Greek *azo*, to dry + *olloyo*, to kill, an allusion to death from drought, which occurs should the plants lose their supply of water.

Azolla species are diminutive, delicate, free-floating, annual plants. The extremely small leaves (technically ‘fronds’ in ferns), no larger than 2 mm or 0.08 inch across, are alternately attached to the branching stems, often overlapping in two ranks. Mosquito Ferns are much reduced in form, and do not exhibit the characteristic highly dissected (‘ferny’) foliage of most ferns. The leaves have an upper green (i.e. photosynthetic) lobe, which bears hydrophobic hairs, and a smaller, usually colourless lobe, which is buoyant and often submersed. Thread-like, unbranched roots are produced from the axils of branches. Under good conditions the plants can grow over each other in layers and develop mats up to 5 cm (2 inches) thick. Most species of *Azolla* produce reddish pigments when stressed, for example by temperature extremes or feeding damage by herbivores, and this may result in a pinkish or reddish carpet of plants covering expanses of water. *Azolla filiculoides* has been called Red Water Fern because of the tendency to become reddish.

Perhaps the most remarkable feature of *Azolla* species is their symbiosis with a bacterial partner. The upper leaf lobes house the bacteria in a pouch on their lower side. This tightly integrated relationship is unique in the plant world.

Ferns reproduce by spores, not seeds. Most fern species have just one kind of spore, but *Azolla* has two

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Figure 1. Mosquito Fern (*Azolla caroliniana*) plants. Photos by S. Darbyshire.

kinds, in reproductive structures termed sporocarps, which are developed at the junctions of the lower leaf lobes with the branches. Male sporocarps are minute (about 2 mm or 0.08 inch in diameter) and produce male spores, which adhere to each other in clumps; female sporocarps are smaller than the male sporocarps, each producing just one viable female spore, which is much larger than the individual male spores. Arrowhead-like barbs on the clumps of male spores seem to assist in adhering to the female spores. Each male spore produces eight swimming sperms, some of which succeed in fertilising the females.

In China and Vietnam, particular strains of *A. pinnata* are cultivated in rice culture systems, as discussed below. Whether such strains have been ‘domesticated’ (changed genetically from wild ancestors), or simply represent selections of forms that exist in the wild, is undetermined. Some recent success has been achieved in selecting and recombining more productive forms of fern-bacterium partnerships.

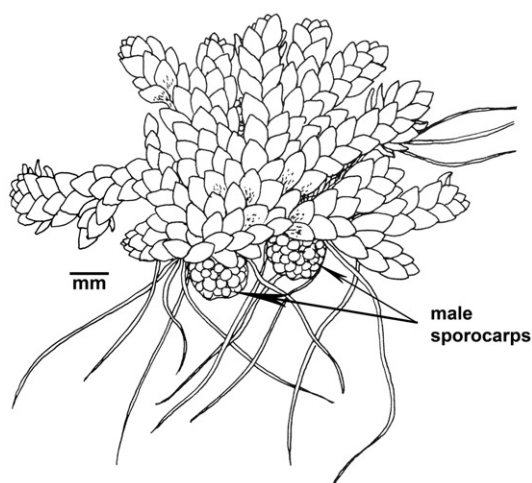


Figure 3. *Azolla microphylla*. Source: Cody, W.J. and Britton, D.M. 1989. Ferns and fern allies of Canada. Agriculture Canada, Ottawa.



Figure 2. Floating mats of Mosquito Ferns. Left: *Azolla caroliniana*; photo by S. Darbyshire. Right: *Azolla pinnata* var. *imbricata*; credit: Show-ryu (public domain photo).

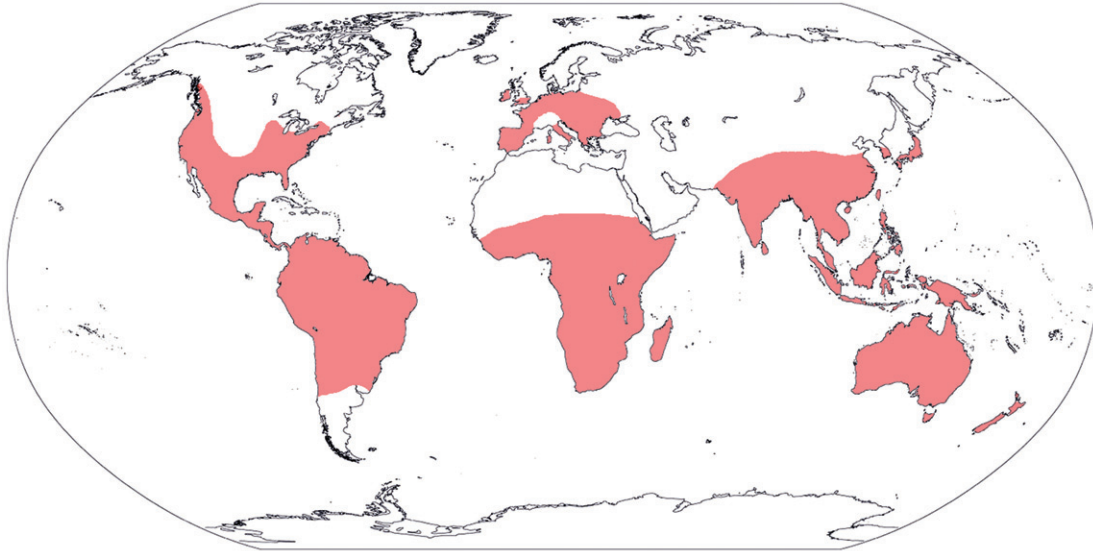


Figure 4. World distribution of *Azolla* species.

The North American species of *Azolla* have not been employed to any appreciable extent in agriculture, but it is possible that strains that grow well in aquaria have been unconsciously selected.

Geography and ecology

Mosquito Ferns are freshwater aquatic plants of temperate and tropical regions. Generally, the species occur in stagnant or slow-moving waters, such as found in ponds, lakes, marshes, swamps and some streams. Occasionally the plants are stranded on wet mud, but normally they float on the surface of water, the roots hanging down. The plants have limited tolerance of freezing temperatures and saline water (although some species can tolerate about 1% salt concentration and *A. filiculoides* can grow in 2.5% saline solution).

Three species of Mosquito Ferns are native to the Americas: *A. caroliniana*, *A. microphylla* (including *A. mexicana*) and *A. filiculoides*. *Azolla caroliniana* is native to southeastern Canada, the eastern half of the US, the Caribbean, Central America and South America. It has also become naturalised elsewhere. This is the most cold-tolerant of *Azolla* species, capable of surviving hard frosts and prolonged coverage by ice. It is also the most adapted to survival on mud. *Azolla filiculoides* is native to western North America, generally in areas near the Pacific Ocean. It is also native to South America, and has been become naturalised in much of the remainder of the world. This species is also cold-tolerant, and can survive under thin ice. *Azolla microphylla* is native to southwestern Canada, the

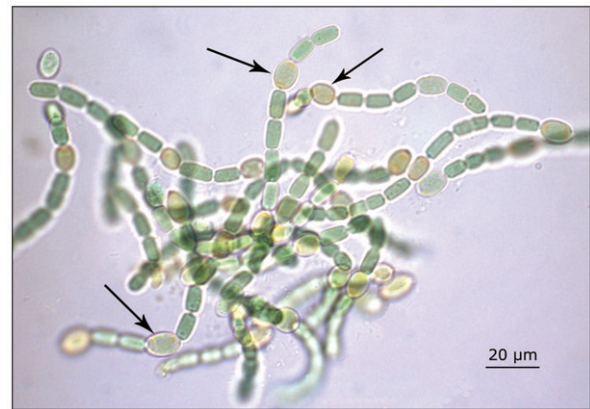


Figure 5. *Anabaena azollae*, the nitrogen-fixing cyanobacterium which forms a symbiotic relationship with Mosquito Ferns. The large cells within the filaments are 'heterocysts' (arrows point to three of these), special cells that synthesise organic nitrogen compounds from the nitrogen in the air. The smaller cells are photosynthetic. Photo by S. Darbyshire.

western US, Central America, South America, and the West Indies. *Azolla pinnata* (including *A. rubra*), called Water Velvet and Ferny Azolla, is native to Africa, Australia, and Asia. *Azolla nilotica* is native to Africa.

The weed problem

Azolla species are commonly grown as ornamentals in aquaria, where they provide hiding places for small fish. Plants discarded from aquaria account for the occasional appearance of Mosquito Ferns outside of their native distribution areas. In parts of the

United States, the introduced *A. pinnata* has been declared a noxious weed, and shops that market aquatic plants are subject to penalties for distributing it, even inadvertently. In some areas of the world, including Australia and New Zealand, Mosquito Ferns are significant weeds.

Nitrogen ecology

Mosquito Ferns are associated with a bacterium in a symbiotic relationship. The bacterium develops unbranched filaments which are divided into cell-like segments, with occasional segments that are larger and less pigmented, where nitrogen fixation occurs as described below. The bacterium is widely identified as *Anabaena azollae*, a member of an important group of photosynthetic bacteria called cyanobacteria (these bacteria are also known as blue-green algae because of the colour of the pigments responsible for photosynthesis). Controversy regarding the identification of the symbiotic associate of Mosquito Ferns as *Anabaena*, and whether there are other bacterial associates, is discussed below. The fern furnishes protection and probably some nutrients to the bacterium, while the latter provides nitrogen to the fern, as noted below. The bacterium occurs in cavities in the leaves, in the growing points of the stems, and on other parts of the plant. When the plant reproduces by spores, the bacterium becomes associated with a specific area (the 'indusial cap') on top of the megaspore (the female reproductive structure). Symbiotic associations between plants and nitrogen-fixing bacteria are common, but the *Azolla*–*Anabaena* association is unique in that the bacterium cannot reproduce in nature outside of its host, the fern. No other plant–cyanobacterium symbiosis is known with a comparably precise mechanism that guarantees transfer of the bacterium from one plant to its offspring (in other symbioses, plants developing from seeds must acquire their bacterial partner from free-living bacteria in the environment).

The bacterium fixes atmospheric nitrogen; that is, it converts inert gaseous nitrogen into a chemically combined form that the plants can metabolise. Since nitrogen is a critical nutrient for living things, and is almost always insufficiently available in soil (or water) for optimum plant growth, this provides Mosquito Ferns with a tremendous advantage. There are as many as 10,000 species of ferns, but only *Azolla* species are known to be symbiotically associated with nitrogen-fixing organisms. (Nitrogen-fixing microbial symbionts are best known in association with species in the Pea family, but a variety of unrelated plants and even some animals have such relationships.) As a result, Mosquito Ferns can grow very rapidly, under ideal conditions

doubling their biomass every two to five days. Insufficient phosphorus in water is usually the chief limiting factor for Mosquito Fern growth, and when chemical runoff (such as from fertilisers and sewage) enriches water with phosphorus, the result may be spectacular carpeting of bodies of water with Mosquito Fern plants (algal blooms similarly result from such 'eutrophication' of water). Phosphorus-based detergents are well known as a cause of eutrophication.

Most literature identifies the symbiotic nitrogen-fixing associate of *Azolla* as *Anabaena azollae*. However, since the 1990s this has been challenged. It has been claimed that the species belongs to *Nostoc*, another genus of blue-green algae. Others claim are that the cyanobacterium species present is neither *Anabaena azollae* nor *Nostoc*. It has been demonstrated that additional cyanobacteria are present in small quantities in some *Azolla* species, and some kinds of non-photosynthetic bacteria are also present; the roles, if any, of these other bacteria is uncertain.

Although *Azolla* species and *Anabaena azollae* are permanently associated with each other (the bacterium has not been found outside of the plant and the plant is almost always accompanied by the bacterium), Mosquito Fern roots have retained the capacity to extract nitrogen from their environment. Interestingly, when the bacterium has been experimentally killed off (using antibiotics), the plant compensates for the loss of bacterium-supplied nitrogen by developing extra roots.

'Because of the growing concern about conservation of the environment and the need for deploying renewable, sustainable resources, the application of *Azolla* as a biofertiliser on agricultural crops, in order to provide a natural source of the crucial nutrient, can be very beneficial to the future of our planet.'

—G.M. Wagner (1997)

Organic vs. chemical fertilisation

Nitrogen is the element that is most often the limiting factor in plant growth, and the addition of nitrogen is usually the most efficient way of increasing crop production. Although the atmosphere is almost 80% nitrogen, plants (and animals) cannot use it in the gaseous form, N_2 . Bacteria, particularly nitrogen-fixing bacteria (mostly free-living, some symbiotically associated with certain plant species), are able to convert gaseous, inert nitrogen to ammonium ions (NH_4^+) or nitrate ions (NO_3^-), which plants can then utilise.

In the early 20th century, chemists discovered how to generate nitrogenous fertilisers from atmospheric nitrogen, and such synthetic fertilisers now dominate agriculture. Unfortunately there are associated



Figure 6. Common species used in organic shallow-water food-producing systems in Asia. Rice, the chief crop, benefits from the presence of the other species, as discussed in the text. The Mosquito Ferns provide organic fertilisation for the Rice plants, and also provide food for plant-eating ducks and fish, and for livestock. All of these species ultimately are eaten by people. Prepared by B. Brookes.

negative effects: (1) the production of these fertilisers requires considerable expenditure of energy, including the need for transportation; (2) the production of chemical fertilisers is alarmingly consumptive of fossil fuels: for every unit of nitrogen fertiliser produced, two units of petroleum are required; (3) plants cannot absorb all applied fertiliser, so much of it leaches into the air, soil, and water, significantly reducing microbial activity in soil, and exposing numerous plants and animals to atmospheric and water-born toxins. Nitrogen fertilisers may account for about one-third of greenhouse gases. In particular, a significant proportion of the fertiliser volatilises as nitrous oxide (N_2O), contributing to atmospheric pollution and the global greenhouse gas problem. Nitrous oxide absorbs infrared radiation in the atmosphere, causing atmospheric warming, and it can also combine with oxygen in the stratosphere to form nitrogen monoxide, which reacts with ozone and decreases the ozone layer. Still another undesirable gas that results from the application of chemical fertilisers is gaseous ammonia (NH_3), which leads to eutrophication and acidification of natural ecosystems. It has been shown that the production of ammonia is substantially decreased when Mosquito Ferns are added to chemically-fertilised rice paddies.

The key to reducing the undesirable effects of synthetic fertilisers on ecosystems is their replacement with 'biofertilisers'. The use of natural, organic

material to encourage growth of plants amounts to mimicking nature, and has many advantages. Properly applied, natural fertilisers are non-polluting, supply a much wider range of nutrients to plants than do synthetic fertilisers, and improve soil structure and the welfare of soil biota. Moreover, organic material decays slowly, providing long-term benefits. Organic fertilisers require more elaborate storage and application, and increased labour compared to chemical fertilisers, explaining why synthetic fertilisers are widespread in industrialised nations, while natural fertilisers are popular in developing countries. However, the growing financial costs and increasing ecological and health damage associated with industrial fertilisers suggest that natural fertilisers will become increasingly important.

The use of *Azolla* as an organic fertiliser in place of synthetic fertilisers has great potential to reduce the world's reliance on fossil fuels. While Mosquito Ferns have many uses, they are most important as biofertilisers or 'green manure', especially in Southeast Asia and in other rice-producing regions of the world. Shortly after Rice is planted, the paddies are often inoculated with *Azolla*, which multiplies rapidly to cover the water, serving to suppress weeds. More importantly, as the Mosquito Ferns die and disintegrate, the nitrogen that their bacterial partners have fixed is released to the Rice plants, contributing greatly to growth of the crops. Rice and Wheat are the world's

most important food crops, but Rice is a staple food for more people than any other crop. In China alone, over 1.2 million ha (3 million acres) of Rice are grown with *Azolla*, which is thought to more than double productivity. Harvested *Azolla* is also used to some extent as a mulch and as a fertiliser for terrestrially-grown crops. Various systems and cultural practices have been devised to maximise the benefits of *Azolla* use given situations, goals and constraints of the farm system.

Integrated farming systems

'Polyculture' has been described as a 'species-diversified cropping system'; it is the simultaneous culture of economically useful plants and possibly also animals. 'Aquaponics' is a special kind of polyculture: the combined cultivation of plants and animals in water to produce food (and sometimes other useful products). In Asia, *Azolla* is commonly grown in rice fields not just to increase rice yield but also simultaneously to feed aquatic animal species such as fish (notably Tilapia, Loach, and Grass Carp), shrimp, ducks, and geese, which simply consume the Mosquito Ferns. In such systems, *Azolla* serves as a nitrogen source for the plants and a food source for the animals. Excreta from the animals reinforce the fertilisation of the plants. The animals also tend to feed upon and destroy weeds and insect pests that harm the planted crop. As a small, floating plant, *Azolla* grows below the leaves of aquatic plants such as Rice, and so does not compete for light and space with the crop. As the rice crop approaches maturity, its shade kills the *Azolla*, which decomposes very rapidly, providing nitrogen and other elements just as the Rice increases its demand for nutrients for grain maturation. With such complex systems, considerable knowledge is necessary in order to ensure that the elements work together; in the case of the Rice–*Azolla*–livestock system, as much as 2000 years of practical experience in Asia have provided the required knowledge.

Eco-farming

'Eco-farming' and 'agro-ecology' are phrases that have recently been applied to the use of sustainable agricultural techniques that are relatively friendly to the environment and biodiversity. In contrast to 'factory farming' that is based on monocultured crops doused with chemical fertilisers and pesticides, resulting in degradation of soils and fuelling climate change, eco-farming and agro-ecology tend to be small-scale, based on a diverse mix of crops, especially those that contribute nitrogen to the soil. The integrated farming



Figure 7. A pony enjoying a meal of Mosquito Fern. Photo courtesy of E. Sjödin.

system described in the preceding is an excellent example of this philosophy. A recent United Nations report very strongly recommends this approach as a means of simultaneously addressing the growing threat of hunger in much of the Developing World as well as the growing threats to biodiversity and the environment (see: http://www.srfood.org/images/stories/pdf/press_releases/20110308_agroecology-report-pr_en.pdf).

Livestock feed

Mosquito Ferns are also agriculturally important as fodder and forage for livestock, including cattle, pigs, goats, ducks, geese and chickens. Harvested *Azolla* is simply added as a nutritional supplement to the regular feed of mammals and poultry. In some areas of Africa, natural, wild growths of Mosquito Ferns have been harvested from lakes and ponds. In Asia, however, techniques for growing Mosquito Ferns in small artificial ponds as well as natural water bodies are very well developed.

Human food potential

Azolla is thought to be suitable for human consumption, although to date only experimental preparation of food products has been attempted. Nitrogen is essential in proteins, and plant species that are associated with nitrogen-fixing bacteria are usually rich in protein. Indeed, Mosquito Ferns are very rich in protein, as much as a quarter of their dry weight. The amino acid profile (a measure of protein quality) is good, although there are minor deficiencies of methionine, cysteine, and lysine.

Erik Sjödin of Sweden has recently conducted experimental preparation of foods based on Mosquito



Figure 8. Photos showing experimental culinary preparations of Mosquito Fern. Photos courtesy of E. Sjödin.

Ferns (<http://www.eriksjoedin.net>). He has kindly allowed us to reproduce his recipe for 'Azollaburgers':

Ingredients:

100 g of fresh Azolla (or 50 g of fried Azolla)
Breadcrumbs (a required binder)
Salt, pepper, fresh herbs

- (1) Fry fresh Azolla in a pan for 1–2 minutes.
- (2) Chop the fried Azolla, and mix it with spices and herbs.
- (3) Mix in breadcrumbs until the mixture can be formed into burgers.
- (4) Fry the burgers in vegetable oil.

Mosquito control

Another use of Mosquito Ferns is as a destroyer of mosquito larvae. Mosquito larvae are aquatic organisms, but must come to the water surface to obtain oxygen. A thick carpet of *Azolla* reduces the ability of the larvae to reach the surface to breathe. It has also been shown that a thick floating carpet of the plants reduces deposition of eggs by mosquitoes. Mosquito-borne diseases, especially malaria, may be reduced by increasing the cultivation of *Azolla*. Although Mosquito

Ferns have been demonstrated to be capable of reducing Mosquito populations to some extent, doubt has been expressed that the overall effect is significant.

Water purification

Azolla has been found to have potential value as a 'nutrient mop' for treating waste water. Mosquito Ferns are especially efficient in removing phosphates from water, a chief contributor to algal blooms. Unlike almost all other aquatic plants, Mosquito Ferns are not limited by lack of nitrogen, and in nitrogen-deficient waters they are especially useful for removing unwanted chemicals. The most promising remedial application of *Azolla* is the removal from contaminated water of heavy metals, such as chromium, cadmium, and nickel. Because of the ease of harvesting, it may be possible to efficiently recover the accumulated metals once they have been absorbed by the plants.

Experimental fuels

In the 1970s, it was discovered that *Anabaena azolla* uses light energy to release hydrogen from water.

Under natural conditions, this hydrogen is immediately combined with the nitrogen that is fixed by the bacterium to produce ammonia, which is subsequently provided to the host fern as its nitrogen source. It has been shown that when *Azolla* and its symbiotic partner are grown under certain conditions (for example, in an atmosphere that excludes nitrogen), the cyanobacterium produces stable hydrogen gas from water. Hydrogen gas can be used as a non-polluting, high-energy fuel. Indeed, hydrogen can produce more energy than any other non-nuclear fuel, and if produced on a large scale, could be extremely important in addressing the problem of dwindling petroleum reserves. This phenomenon has not yet resulted in a practical application, but the possibility of this is intriguing.

Another interesting experimental finding is that *Azolla* can be fermented anaerobically (in the absence of oxygen) to produce methane gas, which might also be used as fuel. Mosquito Ferns may be more productive than most current terrestrial biofuel crops.

Prospects

Mosquito Ferns are extremely important as efficiently generated feed for production of meat, poultry, fish, and shrimp, and they have potential for directly generating human food, and also producing biofuels. Their value as biofertilisers that substitute for synthetic fertilisers and thereby reduce the use of fossil fuels is immense. As the human population continues to outpace increases in food production, *Azolla* is likely to become increasingly significant.



Figure 9. A young girl being introduced to Mosquito Fern. Photo courtesy of E. Sjödin.

Believe it or not

- Particular pigments in photosynthetic cells capture a small range of photosynthetically active light. One of the secrets of the very high productivity of *Azolla* is the fact that the light-capturing pigments in the plants (chlorophyll a and b, and carotenoids) complement the light-capturing pigments in the bacterial partner (chlorophyll a and phycobins) to capture most of the visible light in the 500 to 700 nm spectrum. This allows the partners to capture a wider range of the sunlight's wavelengths than either could alone.
- Since Mosquito Ferns are aquatic plants, it is not surprising that the water content of the plants is high: 85–95%.
- According to a Vietnamese legend, 300 years ago a Vietnamese woman named Ba Heng discovered that the presence of Mosquito Ferns in rice paddies increased production. In her honour, an annual festival in the fall is still celebrated.
- 'Water fern cake' or beo cake (*bánh bèo*) is a Vietnamese speciality, which is not made with Mosquito Ferns, despite the name. It is, however, named for its resemblance to Mosquito Ferns floating on water. Water fern cake is a thin, steamed rice cake, the top stuffed with minced shrimp, scallions, green bean paste, and fried shallots.
- The 'Azolla Event' is a spectacular climate change thought to have been triggered by *Azolla* in ancient times, when several dozen now extinct species of Mosquito Ferns occurred. Following the elimination of the dinosaurs about 65 million years ago, the climate of Earth became very different from that of today. Fifty-five million years ago, Earth had a hot (up to 12°C or 22°F warmer than today), tropical climate, with palm trees growing at both poles. This is believed to have resulted from increased carbon dioxide in the atmosphere, caused in part by extensive volcanism, and consequent heating by the greenhouse effect. It is hypothesised that extensive, dense patches of *Azolla* developed in freshwater lakes in the Arctic area, and perhaps also in the Arctic Ocean (which at the time was almost completely enclosed by land, and consequently was less saline than today because of the inflow of water from rivers). Because of the heat and the very high levels of carbon dioxide, incredible growth of

Mosquito Ferns took place. It has been estimated that as the plants died, layers of dead plants up to 8 m (26 feet) accumulated on the ocean floor (cold temperatures at the bottom of deep waters is thought to have prevented decay of the Mosquito Ferns). This removal ('sequestration') of carbon dioxide by the Mosquito Ferns reduced the greenhouse effect and so cooled the planet. Carbon dioxide levels in the atmosphere are thought to have dropped from 3500 parts per million to about 650 ppm, the world turned cold, ice caps formed at both poles, and sea level dropped. This cold phase has been maintained until today, but now global warming is threatening to reverse the situation.

- In the flowering or seed plants, non-swimming sperm are delivered to the egg by a tube that develops from the pollen grain, penetrates the stigma, and grows directly to the egg. By contrast, animals, including humans, have sperm which swim to reach and fertilise eggs. Swimming sperm are also present in the more primitive classes of plants: algae, mosses, cycads, Ginkgo, and ferns (including Mosquito Ferns).
- *Azolla* is one of the fastest-growing plants on Earth, capable of producing 9 tonnes of protein per hectare (4 tonnes per acre). It has been suggested that growing plants like *Azolla* for human food could require only about 200 square metres (239 square yards or about half an acre) of land to feed an average human—less than one hundredth of the area now required for the average American. Such remarkable growth has made *Azolla* one of the principal plant species that is being considered for food production during space voyages and for colonising the universe.

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