Searching for New Species of *Amanita* From the Remote Neo-Tropics of Guyana

A Report By
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Introduction
The species found within the genus *Amanita* (Family Amanitaceae, Phylum Basidiomycota, Kingdom Fungi) are a group of fungi with over 600 described species. They are primarily terrestrial, forest-inhabiting, and forming what are known as mycorrhizal associations with their symbiotic plant partners (Bas 1969 & Hawksworth et al. 1995). Genus *Amanita* members have the common characteristics of white spores, free gills, and the presence of both partial and universal veils. *Amanita* is divided between two subgenera. Subgenus *Amanita* has spores that are in-amyloid when exposed to iodine whereas subgenus *Lepidella* has amyloid spores when exposed to iodine (amyloid reaction). Subgenus *Amanita* contains sections *Amanita* and *Vaginatae*. Subgenus *Lepidella* contains sections *Amidella*, *Lepidella*, *Phalloideae* and *Validae*. Sections are *sensu* (Corner and Bas 1962), and are further distinguished by macroscopic and microscopic characteristics of the universal and partial veil (Corner and Bas 1962 & Jenkins 1977).

Though intensively studied in the temperate regions of the world, little is known about their biogeographic distributions within the tropics (Corner and Bas 1962, Bas 1969, Jenkins 1977, Wieland 1986, Reid and Eicker 1991, Simmons, Henkel & Bas 2002). This lack of understanding prompted research by Henkel et al. to conduct field-based research in Northeastern South America, primarily in the country of Guyana. This investigation has resulted in numerous new species of macro-fungi being discovered, including those within genus *Amanita* (Henkel 1999, Henkel, Aime & Miller 2000, Miller, Henkel, James & Miller 2001). However, further sampling has consistently provided increases to the species inventory of Henkel et al. (personal dialogue) and merited the need for increased sampling with focus on specific target genera, specific to this report, *Amanita*.

**Objectives**

Using funds provided for in part by the Explorers Club in the Summer of 2008, I traveled with Dr. Henkel to his permanent study area within the remote jungle of the Pakaraima Mountains of Guyana. While there I participated in field studies as a part of his research, with my personal investigation being centered on the collection of genus *Amanita* fruiting bodies (i.e. “mushrooms”) for taxonomic description within the field and creation of voucher specimens to analyze in his laboratory at Humboldt State University. The intent of the field and laboratory based analysis and description was to identify geographic range extensions of previously described species of *Amanita* and if no suitable matches were found, to bring them to the realm of scientific knowledge by doing morphology-based descriptions and publish my findings for the benefit of the larger scientific community.

**Background**

This isolated region of the country is unique in that it contains multiple hectares dominated by a single leguminous tree species of the genus *Dicymbe* (Henkel 2003). This type of forest stratification is of great interest to tropical ecologists due to the typical high-diversity structure commonly thought of and associated with neo-tropical forests. Trees able to establish and eventually dominate forest environments tend to have certain traits that enable them to eventually exclude other tree species from these areas, leading to what is known as *mono-dominance*. One such trait that is of significant interest to me is the capacity of the mono-dominant Guyanese tree species (*Dicymbe corymbosa* Spruce ex Benth.) to establish mutually symbiotic relationships with ectomycorrhizal fungal species and thus greatly enhance its capacity to take in nutrients from the environment and give it a competitive advantage over other species, making this symbiosis the most important establishment trait held by this tree species (Henkel et al. 2002).

The Pakaraima Mountains form the eastern extension of the Guyana Highlands Phytogeographic
Province (“Pantepuia”), a portion of the Guiana Shield geological region with a distinct flora and numerous endemic species (Figure 1; Maguire 1970; Funk et al. 2007). Additionally, this remote sandstone mountain region is unique for the neo-tropics in having significant areas of forest dominated by leguminous trees in the genus Dicymbe (Henkel 2003). Dominance of tropical rainforests by a single tree species (e.g. “mono-dominance”) is a phenomenon of great interest to tropical ecologists due to the nearly universal high tree diversity otherwise found in tropical forests (Richards 1996; Leigh et al. 2004). Mono-dominant tropical tree species appear to have unique combinations of life history traits, compared to “typical” rainforest tree species, which allow them, over time, to grow in high density associations and competitively exclude other tree species, leading to mono-dominance. Research has investigated the ecological attributes of Dicymbe corymbosa Spruce ex Benth., the most important mono-dominant tree species in Guyana, and demonstrated an extraordinary suite of traits which promote mono-dominance, including mast fruiting and resulting high seedling establishment (Henkel et al. 2005a), vegetative regeneration leading to increased life span of individual reproductive trees (Woolley et al. 2007), and, most importantly, the ectomycorrhizal habit and its ability to enhance the nutrient recycling capabilities of D. corymbosa (Henkel et al. 2002; Mayor and Henkel 2005). The ectomycorrhizal habit of D. corymbosa also fosters a wealth of symbiotic ectomycorrhizal fungi, a fungal group which is poorly documented in neo-tropical forests and a focus of this proposal.

Methodology-Field

A combination of charter aircraft, canoe and foot travel allowed access to the research site in the remote Upper Potaro River Basin in western Guyana established by Dr. Henkel (5° 18' 04.8" N; 59° 54' 40.4" W) (Fig. 1). All fugal specimens were collected in an area of approximately 10 km² coinciding with an established research site base camp (Henkel 2002). The study area consisted of an array of six 1-hectare plots, three in D. corymbosa-dominated ectomycorrhizal forest, and three in non-ectomycorrhizal, tree-diverse mixed forest (Henkel 2002). Collections were made periodically during both structured sampling sets within plots and opportunistically whenever suitable specimens were encountered in the field.

Fruiting bodies were examined in the field for fresh characteristics. Color characteristics were coded according to Kommerup & Wanscher (1981) and described subjectively. Spore deposits when obtained were also examined for fresh colour characteristics. Collection and description of Amanita species involved digital photographs of fresh-collected specimens and hand-lens facilitated microscopic examinations taking place at the research camp. Numbered collections of fungal morphospecies were field dried using silica gel, and stored in air-tight containers for transport back to the laboratory (Miller et al. 2002).

I spent six weeks in the bush being guided by indigenous peoples of the area whose knowledge of the flora is crucial in correctly locating fruiting bodies of my target fungi. It was essential to be in the field for the main fungal fruiting season of May-July to obtain the widest diversity of Amanita species possible in fresh condition to aide in the description of new species. Also, certain characteristics of fruiting body tissue structure can be seriously damaged or lost if not examined fresh in the field.

Methodology-Laboratory

Once the key macro- and microscopic taxonomic field features had been recorded, specimens
were preserved via dehydration using silica gel and packaged for transport back to Dr. Henkel’s laboratory at Humboldt State University. Following protocols developed by Dr. Henkel I compared detailed taxonomic descriptions of collections to published worldwide literature and keys. Temporary slides were created of tramal tissue from dried specimens of both pileus (cap) and stipe (stem) along with squash mounts of hymenium (gills) to examine spore characteristics. Tissue samples were rehydrated for comparison using a solution of 5% KOH. Chemical tests included reactant tests against Melzers solution and Iodine tests. Permanent herbarium specimens of all fungal collections are deposited at the University of Guyana and Humboldt State University in conjunction with permitting agreements with the Guyana EPA and Dr. Henkel’s ongoing research.

Results

Thus far no new species have been identified and no range extensions have been confirmed. Further investigation is necessary to continue comparisons to previously described species.

Summary

The Neo-Tropics of the Pakaraima Mountains of Guyana are a valuable location for continued ecological research focused around the dynamics associated with mono-dominant *Dicymbe corymbosa* forests. Each new year of research not only expands on information gained from previous years but provides new insights that may spur new directions of research associated with this eco-type. Species of the genus *Amanita* have a unique possibility for continued research due to their mycorrhzal association with *D. corymbosa*. The portion of Guyana that our study site was located in is also unique with respect to exploration because while the area is inhabited by indigenous peoples, very little of it has been documented and explored from a western perspective.

Acknowledgments

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Literature Cited


**Figure 1.** Guyana's location in South America and location of study sites in the Upper Potaro Basin of western Guyana.
Figure 2. Photograph of myself (center, in red) along with the rest of our research team including out indigenous field assistants.

Figure 3. Mysect area and arguab largest within our study

Figure 4. An ex...
meristematically active cellular Cambium tissue, providing more evidence for how it achieves mono-dominant establishment.

**Figure 5.** Rainfall activity can raise and lower the level of the river within hours, resulting in unexpected challenges coming back from field plots.

**Figure 6.** A parasite wasp had paralyzed a tarantula and was dragging it back to its burrow, where it would lay eggs inside and the hatching larvae would feed on the still-living host.

**Figure 7.** The Bush Master is an incredibly poisonous snake that can kill without medical attention. Not only were we located too far to reach aid in time, but it was not uncommon for hospitals to be unsupplied with anti-venom. This snake was found in camp after we had been awake and milling about for over two hours.
Figure 8. Our study site was a pristine rainforest, which made all sorts of wildlife viewing possible. This infant sloth fell out of a tree less than 50m from where we were collecting.