Hebeloma, Pioneer Genus in Forensic Mycology

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A few years ago, it must have been 2009, I was part of the Netherlands Forensic Institute, in particular of the Line Department Microtraces (Non Human Biological Traces). This experience has had great impact on my evolution as a scientist. Likewise, I've become more than moderately interested in forensics – the study of evidence discovered at a crime scene and used in a court of law. Forensic science is a multidisciplinary field, considering the full range of botanical, zoological, and inorganic evidence (Mildenhall et al., 2006). This article deals with some aspects of forensic mycology, a very specific sub-discipline of forensics, involving the genus Hebeloma *and its possible applications.*

Introduction

Hebeloma (Fr.) Kumm. is a genus of ectomycorrhizal fungi in the Strophariaceae (Basidiomycota: Agaricales) consisting of, mostly, pioneer species – they occur as the first of sequential successive steps – while a few other species decompose animal wastes. Species delineation is traditionally based upon morphological characters. The different species in *Hebeloma*, however, are neither distinctive nor colorful (Arora, 1986). David Arora, in his *Mushrooms demystified* (1986), refers to *Hebeloma* as "[…] yet another faceless and featureless collection of brownish mushrooms." Species identification, even for specialists in the field, is difficult and the different species concepts and infrageneric classifications are controversial. And pretty confusing, too. Depending on the author, sections, subsections, or even *stirps* exist to "solve" the genus (Aanen et al., 2000). Attempts have been made to clarify phylogenetic relationships within the genus, although these approaches were restricted to specific sections (Eberhardt et al., 2013) or contained many unresolved or unsupported branches (Aanen et al., 2000). Yet, species are still being described and the interest in *Hebeloma* remains, as the fungus may provide unbiased scientific evidence for use in the court of law, and in criminal investigation and trial.

Findings on Animal Remains

Hebeloma vinosophyllum Hongo was described as an agaric species without any specific ecological requirements, but, indeed, it does have a preference for dead (mammalian) carcasses. In 1968, a bunch of fruiting bodies of *H. vinosophyllum* was found in Kyoto (Sagara, 1976). Close examination of the soil showed that the remains of a dog constituted the source of nitrogen. In 1975, again, a single fruiting body of the same species was found near the Kyoto

The Flaw of Reiterating

It's been stated that many authors are likely reiterating the claims of previous authors, not exactly knowing who actually was the one seeing it firsthand. This makes me think of Andre Vltchek's (2013) article *Harare: Is It Really the Worst City on Earth?* which reveals similar findings at the journalistic level.

Surveys by The Economist *and others relentlessly portrayed Zimbabwe's capital city Harare as the 4th worst city in the world. Most of us wouldn't even think of questioning this quote. But Vltchek did. He decided to visit Harare and found that it is a great, literary city to live in, unexpectedly falling in love with this so-called worst city on Earth. Facts and data about Zimbabwe are developed "somewhere in the cloud" to serve Western media. Afterwards it is recycled over and over again, never updated, often with colorful words thrown in to reinforce the statements. "It's those bloody journalists again," I hear you, but why then do we see suggestions in scientific papers that are only partially or not at all true?*

Hebeloma crustuliniforme courtesy M. Wood.

University campus, upon soil in which cat bones were found in the top 20 cm layer (Sagara, 1976). The year after, at the same spot, more fruiting bodies appeared.

Maybe the forest tree type was more influential than the animal remains? Well, no. The first collection of *H. vinosophyllum* was found in a stand of angiosperm *Castanopsis cuspidata* (Fagaceae); the second in an area dominated by gymnosperm *Pinus densiflora*.

Also in 1998 fruiting bodies of *H. vinosophyllum* were observed in close association with a skull and bones, this time originating from a jungle crow (*Corvus macrorhynchos*) in Urawa (Saitama City, Japan) (Fukiharu et al., 2000) – the first and so far only case with birds.

So far it is unclear whether or not the closely related *Hebeloma sarcophyllum* Peck, occurring in Europe, North Africa, and North America, has the same ecological tendencies. Also other *Hebeloma* species, like the eastern North American and European *H. syrjense*, Japanese and European *H. radicosum*, and Australian *H. animophilum* are known to grow in association with rotting / decomposing animal remains (Carter and Tibbett, 2003). *Hebeloma syrjense* is often referred to as the "corpse finder," yet may turn out to be synonymous with *H. radicosum* (Bunyard, 2004).

Cleaning Symbiosis

The carrion-associated fungi that fruit at later stages of the succession process after decomposition include many ectomycorrhizal species. A number of *Hebeloma* species and *Laccaria bicolor* have been demonstrated to produce fruiting bodies in animal waste sites by forming ectomycorrhizae; specimens originated in plots that allowed plant root development while plots with blocked colonization by roots did not result in fruiting bodies. (*Laccaria* species do not seem to be specific to animal remains as they originate in any "disturbed" site.)

We know some other tripartite relationships between fungi, plants and animals. The difference is that the associated animals in this case cannot disperse fungal spores. Then why do these associations exist?

An animal consists for the greater part of carbohydrates, lipids and proteins. When it dies, these high-quality nutrients become available for other organisms. The remains offer a temporary living for many invertebrates. The remains of an animal undergo quick and considerable changes. This "rotting" takes place in a few stages – fresh stage, bloat stage, early decomposition, late decomposition, and dry stage. The bacterial decomposition would be blocked after a while if the decomposition products were not removed. Here's where the fungi become important in this storyline (Sagara, 1995); both the associated fungi and the plants (in the form of ectomycorrhizae) can absorb these products of decomposition. This results in the translocation of the absorbed waste material above the ground – by the fruiting of the fungi as well as in the growth of the plants. This tripartite association is referred to as a *cleaning symbiosis*.

Thus?

Is there a forensic potential for *Hebeloma* species? Sure. Then can we start using it in crime investigation? Not yet. We don't know how trustworthy the findings are as to forensic cases. Different authors have stated that more research is needed to develop fungi into suitable forensic tools (Carter and Tibbett, 2003; Bunyard, 2004).

For example, it is important to understand that fungi do not grow upon buried cadavers, but rather on the subsequent release of nitrogen during the cadaver's decomposition (in the microclimatic patch, which is called the cadaver decomposition island, cDI) (carter and Tibbett, 2003; Carter et al., 2007). How this relates to carcass decomposition is unclear, though. How much nitrogen is released? Under what form is nitrogen released (simple organic nitrogen, ammonium, nitrate)? These approximations are needed in order to make accurate estimations of the post-burial interval (PBI).

As a final point of discussion, experimental settings should be taken into account – temperature, humidity, oxygen availability, and soil characteristics – as these can influence the rate of decomposition. Also the carcass species (body composition, fat ratio, muscle mass) and size will have effects on decomposition rate as well as carcass-associated species composition and succession (van Wielink, 2004).

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