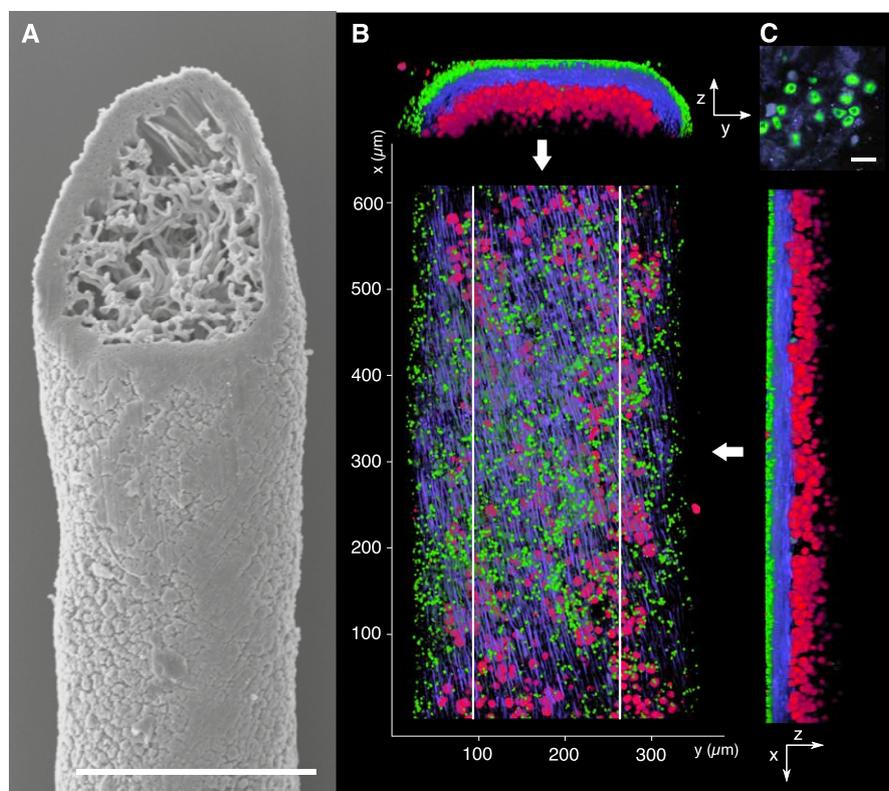


# Transcriptomics leads to discovery of basidiomycete yeasts on lichen cortices – and speculation as to function

Molecular phylogenetic studies in *Bryoria* have led to some traditionally separated hair lichens being regarded as conspecific. One such case is the dark brown *B. fremontii* with vulpinic acid only in the apothecia and soralia (when present) and the yellowish *B. tortuosa* with the same compound throughout. In order to investigate the basis of the phenotypic differences between these two synonymised “species”, Spribille *et al.* (2016) embarked upon a transcriptome analysis to investigate whether gene expression might account for the increased production of vulpinic acid. It was an investigation which led to surprising results and fascinating speculations, even making the cover of *Science*.

Quite unexpectedly, the phylogenetic analyses revealed the presence of basidiomycete sequences of the recently described gall-forming lichenicolous genus *Cyphobasidium* (Millanes *et al.* 2016). FISH-hybridization probes were then designed to determine where these fungi occurred, and, unexpectedly, they were abundant as yeast morphs on the cortex of *B. tortuosa* where they were imbedded in vulpinic acid residues, but almost entirely absent in *B. fremontii*. Six thalli of *B. fremontii* and nine of *B. tortuosa* were used. The group then went on to search for the occurrence of these yeasts in a range of other macrolichen genera from the same family as *Bryoria*, *Parmeliaceae*, which fell into different species in a single clade, and also from a representative of *Cladoniaceae* which was allied to some other yeast families in the same larger clade which were not associated with lichens; the new order *Cyphobasidiales* was introduced to embrace these fungi. Failure to detect these fungi previously, other than when forming easily visible galls, is attributed to multi-template PCR bias; a 595-base pair group I intron downstream of the primer binding site ITS1F, doubling the length of the ITS.

Stunning illustrations show the abundant occurrence of these yeast morphs on the cortex of *B. capillaris*, a species not with vulpinic acid but well-known for other depsidone compounds it produces; in this case they seem to be embedded in surface polysaccharides. The authors suggest that



Flourescent imaging of *Bryoria capillaris*. A. Scanning electron micrograph of cortex surface. B. Fish hybridizations showing the *Cyphobasidium* yeasts (green), lichen fungal partner (blue), and algal partner (red); chlorophyll A autofluorescence. Note the almost continuous yeast-containing layer. See Spribille *et al.* (2016) for a fuller explanation. Figure courtesy of Toby Spribille.

these fungi may be an integral part of the lichen symbiosis in view of the intimate physical integration, and correlations with the chemical products in particular, but the physiological basis of that remains obscure. They speculate that the *Cyphobasidium* yeasts may be involved in the production of vulpinic acid in the *B. fremontii*/*B. tortuosa* case, and that compounds such as this found in lichens may not be produced by the fungal partner alone as now generally accepted. However, that is not in doubt where the isolated fungal partners are grown in pure culture and characteristic compounds are still produced. While transcriptomics is unlikely to resolve the question with respect to vulpinic acid, at least until the enzyme pathways leading to it have been elucidated, their proposition merits exploration with cultures of the isolated fungal partner of *B. fremontii*/*B.*

*tortuosa*. This clearly is a direction future research might take to test this novel hypothesis. It could also be pertinent to determine the populations of these yeasts in the vulpinic acid containing apothecia and soralia of the dark *B. fremontii*, and in other lichens which can have the compound in just parts of their thalli, such as the *Sulcaria sulcata* f. *vulpinoides* described from China (Prov. Yunnan).

Contrary to some of the press announcements, there is no need to change the concept of “lichen” in the light of these exciting findings. The term “symbiosis” was first coined by Albert B Frank in 1877 (not Anton de Bary in 1879 as commonly cited) especially for the algal/fungal associations in lichens. It refers to organisms living together with no overtone of mutualism – and all organisms in any symbiosis each have independent names.

Who could have even dared to dream that lichenicolous fungi, one of the areas of interest that I have strived to encourage since the 1970s, could ever get top-billing in *Science*! The authors, especially Tony Spribille, are to be congratulated on their originality, lateral thinking, energy, and the way they have pursued and examined their

hypotheses using the latest technologies. Their discoveries also promises to open up new and exciting avenues for research into the lichen symbiosis and its chemistry.

Millanes AM, Diederich P, Wedin M (2016) *Cyphobasidium* gen. nov., a new lichen-inhabiting lineage in the *Cystobasidiomycetes*

(*Pucciniomycetes*, *Basidiomycetes*, *Fungi*). *Fungal Biology* 120: 1468–1477.

Spribille T, Tuovinen V, Resl P, Vanderpool D, Wolinska H, *et al.* (2016) Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* 353: 488–492.

## A 35–55 Mya gilled mushroom from Baltic amber

Agarics are poorly represented in the fossil record, but occasionally preserved in amber. A minute gilled mushroom, with the cap and stipe just 1.8 mm tall, has now been discovered in Eocene Baltic amber for the first time and described by Poinar (2016) as a monotypic new genus: *Gerontomyces lepidotus*<sup>1</sup>. The deposits in which it was found are dated at 35–55 Mya. The cap is convex and densely scaly, and a mere 1 mm diam. The gills are few in number (12–14) but no spores were found. The features suggest a placement in *Tricholomataceae*, and the fungus recalls the modern *Squamanita* but lacks the trace of any veil on the stipe.

The fungus recalls *Archaeomarasmium leggettii*, described from Cretaceous amber

in the USA by Hibbett *et al.* (1997), in the sparse gills, but that fungus has a proportionally narrower stipe and lacks scales. That species and other reports of gilled basidiomes preserved in amber are discussed further by Taylor *et al.* (2015) who also referred *Archaeomarasmium* to *Tricholomataceae*.

Hibbett DS, Grimaldi D, Donoghue MJ (1997) Fossil mushrooms from Miocene and Cretaceous ambers and the evolution of *Homobasidiomycetes*. *American Journal of Botany* 84: 981–991.

Poinar G jr (2016) A gilled mushroom, *Gerontomyces lepidotus* gen. et sp. nov. (*Basidiomycota*: *Agaricales*), in Baltic amber. *Fungal Biology* 120: 1090–1093.

Taylor TN, Krings M, Taylor E (2015) *Fossil Fungi*. Amsterdam: Academic Press.

<sup>1</sup>The scientific names in this paper were not validly published by Poinar (2016) as no MycoBank or other repository accession numbers were provided. These names are therefore validated by George Poinar here:

***Gerontomyces lepidotus* Poinar, gen. sp. nov.**

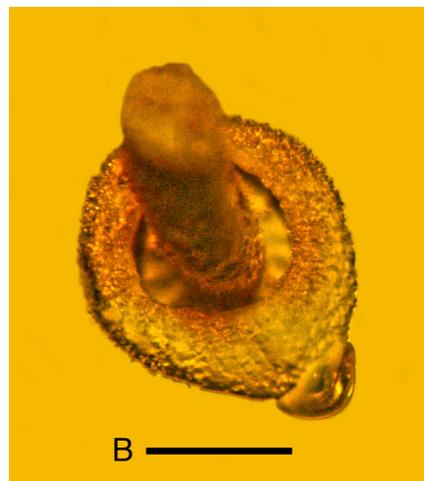
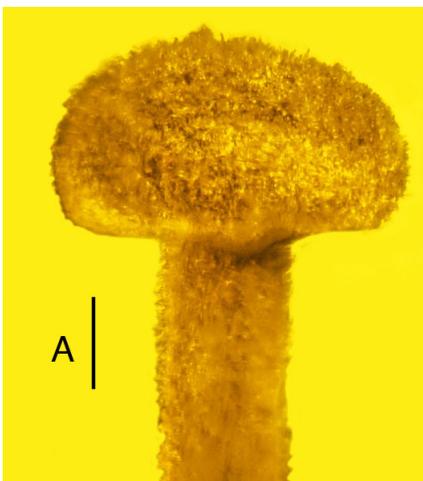
MycoBank MB818741 (*Gerontomyces*)

MycoBank M818992 (*Gerontomyces lepidotus*)

*Description:* Poinar (*Fungal Biology* 120: 1091–1092, 2016).

*Type:* **Russia: Kalinin District:** Samland Peninsula of the Baltic Sea, in amber, *Myc-12*

(Bone Room, 1573 Solano Ave., Berkeley, CA 94707 – holotype).



*Gerontomyces lepidotus* (holotype). A. Detail of pileus and stipe. B. Ventral view showing the lamellae. Bars A = 0.2 mm, B = 0.5 mm. Photos: G Poinar.

## Unravelling relationships between wood-inhabiting fungi and their hosts using a citizen science generated database

The importance of dead timber as a habitat for fungi has long been recognised, but, apart from charismatic polypore species of particular conservation importance,

the information on host specificity is often limited to impressions from casual field observations. These authors have analysed data in the Danish Fungal Atlas

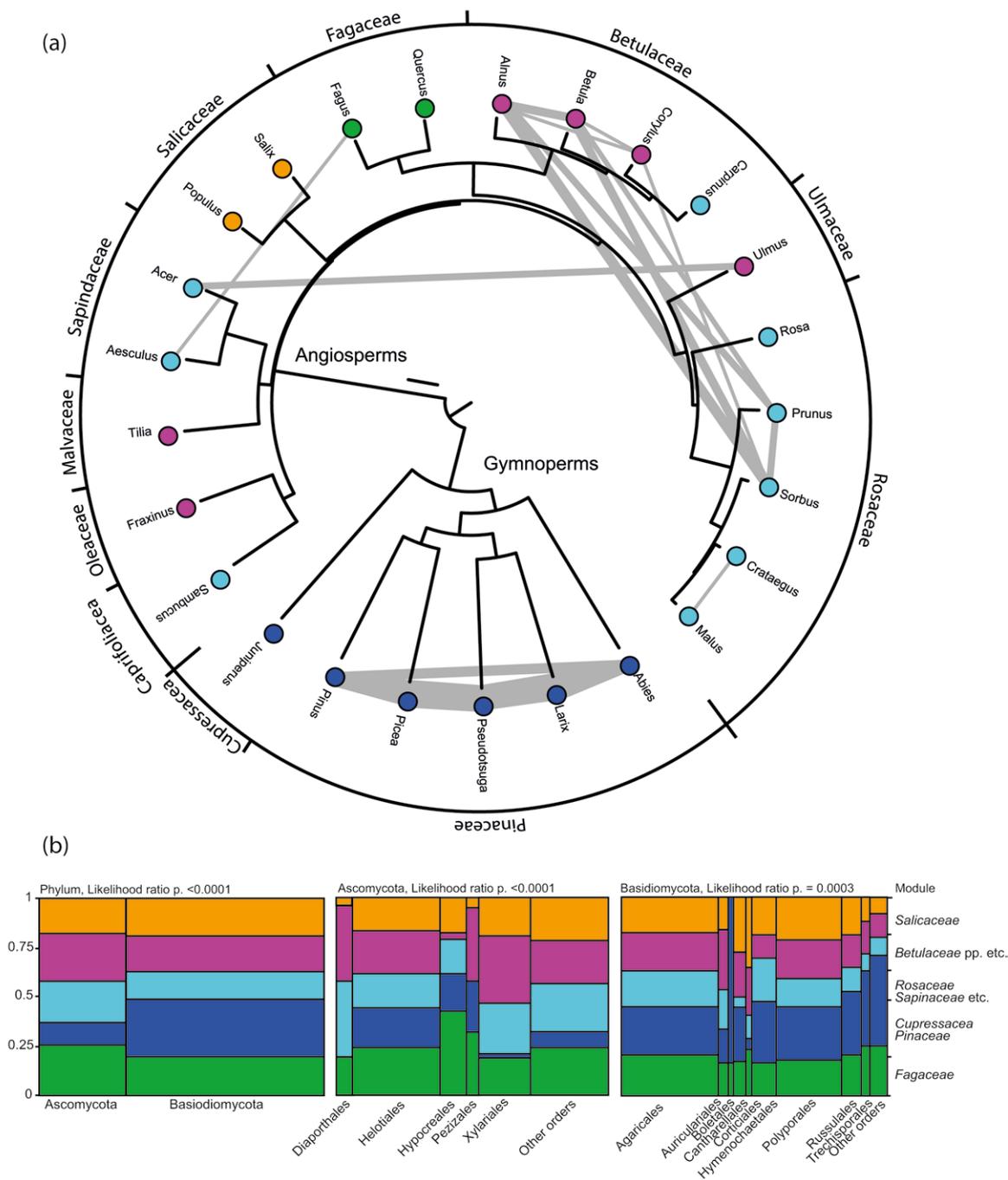
macromycete database which had been contributed by a citizen-scientist project that ran for the five years 2009–2013. There were over 400 contributors and a rigorous

system of validating records involved noting descriptive features, and preparation of dried voucher specimens sent to specialists for validation or re-identification. 110 712 records marked as from 'bark' or 'wood' were extracted from the database to form the basis of this study. Records were first scrutinised, and those that were of ectomycorrhizal species or were deficient in

some way were rejected; 83 637 remained and were analysed by host and host traits, including physical and chemical properties of wood compiled from various sources. There were 1044 species of fungi represented in the cleaned data set, of which 399–429 species were used in the modularity analysis. Data were standardised for sampling effort by comparing similar numbers of records

from each host genus by rarefaction and extrapolation. Host genera with less than 100 fungal records were omitted, leaving 25 woody genera for analysis.

A sophisticated network approach was also used to detect modules in the host/fungus data. Analyses for species richness, host information, and host wood traits were conducted. Species richness was positively



(a) Phylogeny of the woody hosts and indications (in grey bars with the extent reflected by their thickness) of the wood-inhabiting fungi they share; and (b) a schematic breakdown of the proportions of the phyla and orders of fungi on different host families. See Heilmann-Clausen *et al.* (2016) for full explanation. Figure courtesy of Jacob Heilmann-Clausen.

correlated with host size, wood pH, and number of species in the host genus, but negatively with lignin percentages. However, time since establishment emerged as of limited importance. That lack of correlation suggests, surprisingly, that any new tree plantings can be expected to contribute to species richness of wood-inhabiting fungi.

The phylogenetic relationships of the hosts were compared with the phyla and orders to which the fungi belonged. There were strong modular connections between the fungi in some plant groups, most strongly in the gymnosperms (*Cupressaceae* and *Pinaceae*), but also between *Betulaceae*, *Rosaceae*, and *Ulmaceae*. In the analysis of the orders of fungi, the extremely

low representation of *Xylariales* on gymnosperms, and the high representation of *Hypocreales* on *Fagaceae* were especially marked. By comparison, the variation of basidiomycete orders between woody plant families was much less marked. It would be interesting to see if similar results were obtained if comparable data sets could be obtained in regions with extensive old-growth forests and analysed in a similar way. Old-growth forests are so restricted in Denmark, that this might explain at least in part the little correlation with the time since establishment of the trees.

This study is not only of interest for its scientific results, however, but for demonstrating how citizen scientists can

be involved in making observations and recording that information in databases suited for sophisticated analysis by specialists. The citizen scientists involved in this case will also have benefitted from having their identifications checked, enhancing their personal mycological skills.

Heilmann-Clausen J, Maruyama PK, Bruun HH, Dimitrov D, Læssø T, *et al.* (2016) Citizen science data reveal ecological, historical and evolutionary factors shaping interactions between woody hosts and wood-inhabiting fungi. *New Phytologist*: DOI: 10.1111/nph.14194.