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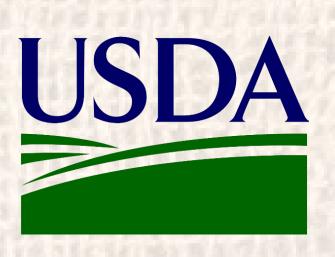
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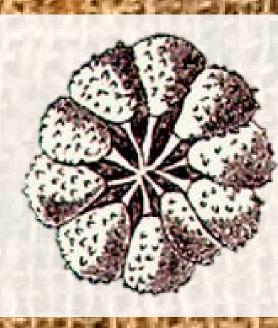
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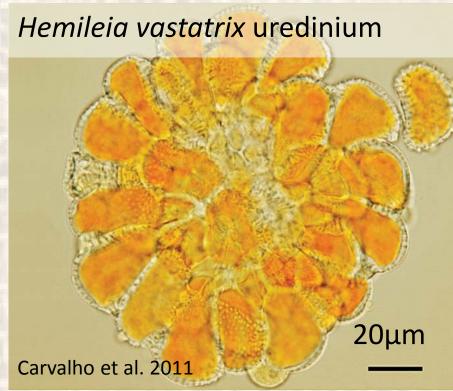


Jordan Bailey¹, M. Catherine Aime², Mehrdad Abbasi² & Lisa Castlebury¹

Phylogenetics for the genus *Hemileia* - using herbarium specimens

1869 - The genus *Hemileia* Berk. and Br. was established with the arrival of the 'coffee leaf rust' disease in Sri Lanka, caused by the fungus *Hemileia vastatrix*.





H. vastatrix urediniospore

Species of *Hemileia* produce unique urediniospores with a 'hunchback' shape, and a smooth ventral side. Hemileia means half smooth. These emerge through the stomata, rather than rupturing the epidermis.



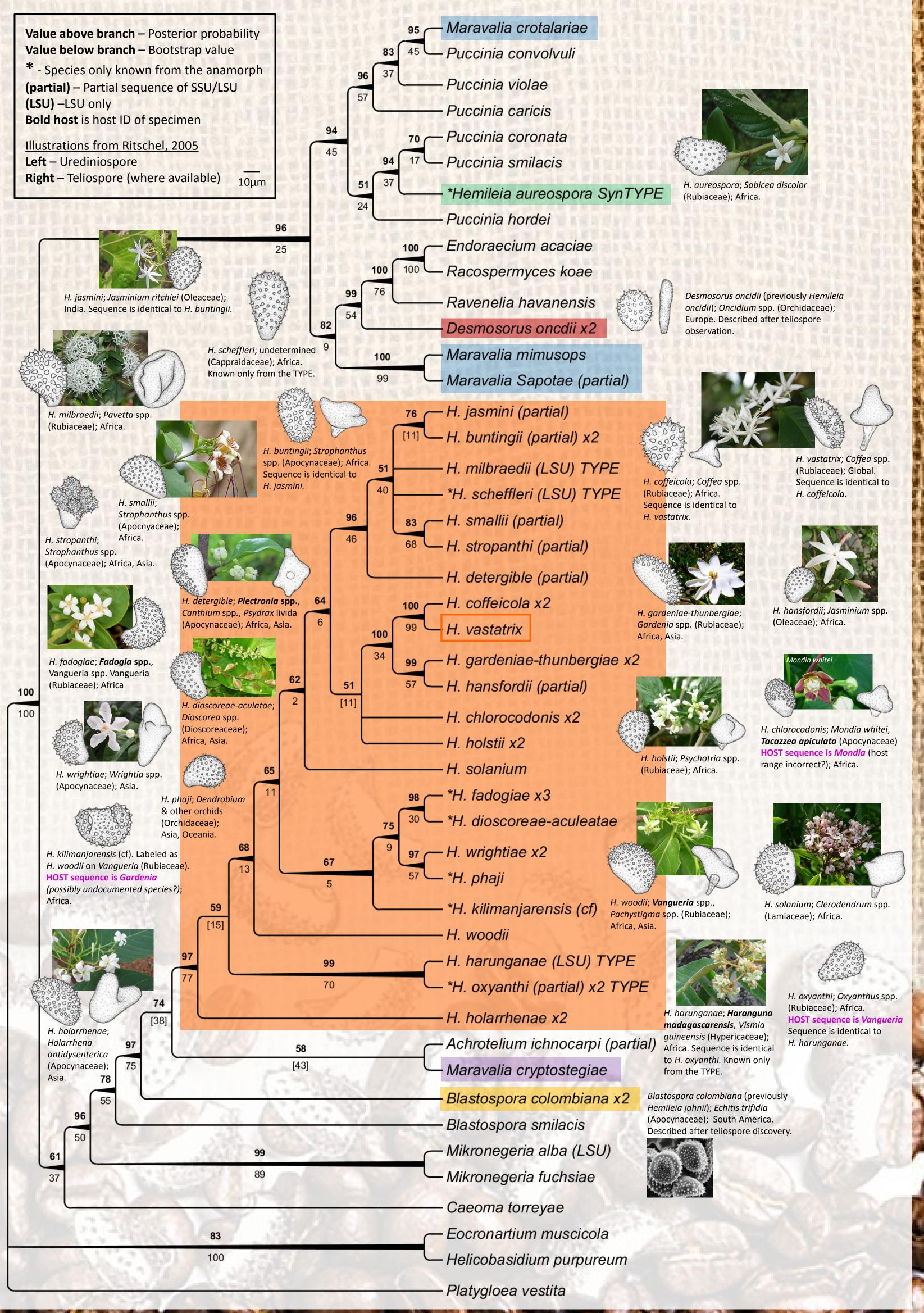
Teliospore morphology is an important characteristic in rust demarcation. In *Hemileia*, teliospores are elusive and for more than a dozen species this stage remains unobserved.

2016 — Extensive research has been conducted on coffee leaf rust and with *H. vastatrix*. Beyond the 2005 monograph by Ritschel, however, little has been done with the genus. Here we present the first phylogenetic study of the genus *Hemileia*.

METHODS

- Sequenced all ≈1750 bp of the SSU (fragmented into four parts) & the first ≈350 bp of the LSU. Attempted for 91 Herbarium specimens.
- Specimens with all fragments, and select specimens with partial fragments, were aligned (MAFFT, Geneious®9.0.4).
- In total, 33 specimens covering 23 Hemileia species were included, as well as 4 specimens of 2 species previously considered Hemileia and specimens of the sister taxon Maravalia.
- 28 additional rust species were included for context.
- Maximum likelihood (RaxML) and Bayesian (MrBayes) inference were used.

RESULTS - Phylogram obtained from Bayesian analysis (thickened branches indicate a node also recovered in ML). Additional rust species have been reduced for presentation.



CONCLUSIONS

Almost all *Hemileia* species sampled here are placed in the same clade within the Mikronegeriaceae.

- Hemileia topology does not reflect host or geographic associations; species infecting Rubiaceae, Apocynaceae and other hosts, from Africa and Asia, are mixed throughout.
- Six *Hemileia* species for which teliospores have not been observed (marked with an asterisk) are confirmed to be *Hemileia*.
- H. aureospora places with Puccinia and needs to be removed from Hemileia.
- The placement of both B. colombiana (previously H. jahnii)
 and D. oncidii (H. oncidii) supports their removal from
 Hemileia.

M. cryptostegiae is the only species of *Maravalia* sampled here to place in the Mikronegeriaceae, all other *Maravalia* are placed elsewhere in the Pucciniales. This genus, especially the species *M. cryptostegiae*, require further study.

DIFFICULTIES

Using herbarium material restricts us to small fragments and is inconsistent in amplification due to varying quality of material. Fragmenting large gene regions is problematic. Host identification is a major crux in rust taxonomy as rust species are often strongly associated with their host. Here we had several misidentified hosts, including the *H. oxyanthi* type. These misidentified hosts lead to three misidentified specimens, and has raised various questions.

FUTURE WORK

Future work on *Hemileia* requires fresh material, with accurate host identification. This would greatly improve the success rate for sequencing and allow for easier exploration of additional genes.

It is apparent from this first investigation that an in-depth investigation, using type material, especially of the less common *Hemileia* species, is needed.

The primers designed here can be applied to additional *Hemileia* specimens, and can also be used in other rust studies using herbarium specimens, such as *Maravalia*.

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