Diversity and Host Specificity in the Genus *Sarea* Fr. (Ascomycota)

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I. Introduction

First published by Fries in 1825, the genus *Sarea* today comprises two accepted species of resinicolous discomycetes, *Sarea difformis* and *Sarea resinae*. Both species have a very broad geographic range, with *S. difformis* reported from North America, Europe, and northwestern Africa, and *S. resinae* reported from North America, Europe, northern and central Africa, and central and eastern Asia. Both species have also been reported in southern hemisphere locations, such as New Zealand, on non-native trees. *Sarea* species have a broad range of hosts in the Pinaceae, with *S. difformis* reported on *Cedrus atlantica* and both *Sarea* species reported on species of *Pinus*, *Picea*, *Larix*, *Pseudotsuga*, *Abies* and *Tsuga*. In addition, *S. resinae* has been reported on species in the Cupressaceae, including members of the genera *Cupressus*, *Chamaecyparis*, *Juniperus* and *Taxodium*. Though often described as ”rare” in older texts, the number of literature and herbarium records of these fungi, together with the general reliability of finding them when one checks their substrate, suggest that they are actually quite common, but often overlooked. Despite their unusual substrate, commonness, and breadth of geographic and host range, little molecular work has been done in this genus. The most recent taxonomic assessment of these fungi is based entirely on morphological characters, and is now 37 years old [Hawksworth & Sherwood 1981].

II. Materials and Methods

Specimens were mostly collected fresh on resinous exudates of conifers in North America and Europe, with some herbarium specimens from unusual or exotic localities additionally requested and included. DNA was extracted from samples with a Qiagen DNEasy Plant Mini Kit or a Qiagen QIAamp DNA Micro Kit (Qiagen, Hilden, DE). The nuclear internal transcribed spacer (ITS) region, nuclear ribosomal large subunit (LSU) and mitochondrial ribosomal small subunit (mtSSU) regions were amplified by polymerase chain reaction (PCR) with Lucigen EconoTaq DNA Polymerase (Lucigen, Middleton, WI) or REDExtract-N-Amp PCR ReadyMix (MilliporeSigma, Burlington, MA) using primarily the primer pairs ITS1F-ITS4, LR0R-LR5, and mrSSU1-mrSSU3R. Sanger sequencing of PCR products was performed by Genewiz (South Plainfield, NJ) using the same primers.
Morphological examination was performed by rehydrating dried specimens in tap water. Rehydrated apothecia were then sectioned in gum arabic on a freezing-stage microtome at 15 µm. Sections were mounted in a dilute solution of eosin in glycerol, and mounted using a modified “double cover-glass method” [Kohlmeyer & Kohlmeyer 1972].

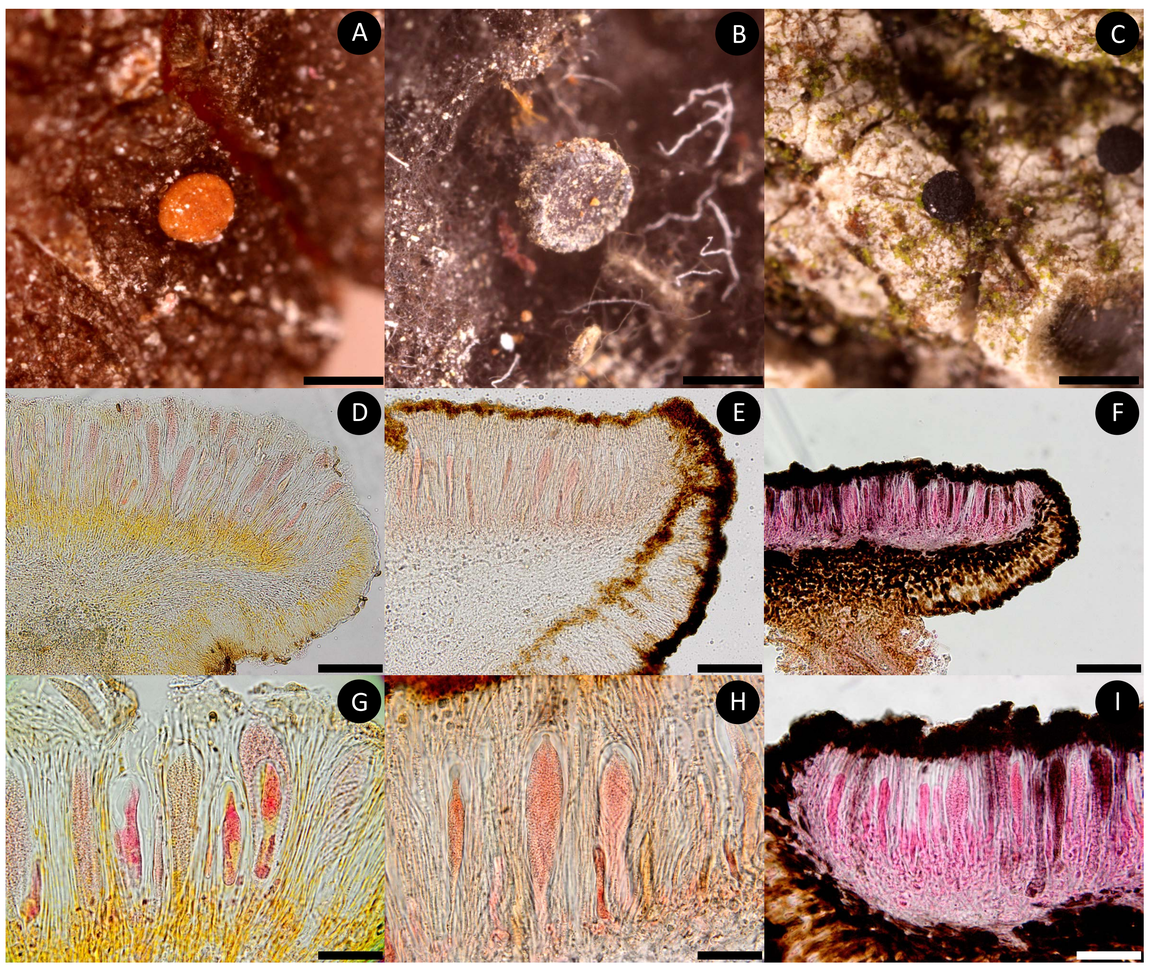

Figure 1. Macro- and microphotographs of *Sarea* specimens.
A-C) macro photographs of representative apothecia.
D-I) microphotographs of 15 µm thick sections mounted in a dilute solution of eosin in glycerol.
A,D,G) A specimen of *Sarea resinae* collected on *Picea glauca* at the Eagle Hill Institute in Maine, number JM0006.
B,E,H) A specimen of a possible undescribed *Sarea* species, collected on *Chamaecyparis lawsoniana* in Klamath National Forest in northern California, number JM0068.1.
C,F,I) A specimen of *Sarea difformis* collected on *Pinus tabuliformis* planted at the Arnold Arboretum in Massachusetts, number JM0011.
Scale bar: A-C) 500 µm, D-F) 75 µm, G-I) 30 µm.

III. Results

Molecular analyses have shown a high degree of genetic diversity in the genus *Sarea*. *Sarea difformis* was not recovered as monophyletic in the 1-marker ML analysis, but specimens of *S. difformis* in both analyses fall into three clades. Specimens of *S. difformis* sequenced were collected on hosts from three genera in the Pinaceae, but do not sort in a manner consistent with a hypothesis of host specificity at the genus level. *Sarea resinae*, on the other hand, exhibits less structure. Sequenced specimens fall into many clades, with 8-13 specimens as singletons. Although most clades exhibit host specificity at the family level, one well supported clade is composed of three specimens collected on resin of *Picea spp.*, and one on resin of *Cupressus forbesii*.
Geography exhibits an erratic influence on clade composition in both species. Clades range from composed of specimens collected in a single city (Boston, MA) to specimens collected over 9000 kilometers apart. Additionally, specimens collected in close geographic proximity are often distributed among multiple clades. For example, specimens of *S. resinae* from New England are distributed among five clades.
Morphological examination of specimens reveals little obvious intraspecific variation that aligns with the molecular analysis. One clear exception is a specimen collected in Klamath National Forest on the resinous exudates of *Chamaecyparis lawsoniana*. The black color and pigment distribution resemble *S. difformis*, but molecular analysis suggests it is more closely related to *S. resinae*. The pruinose exterior, the presence of hyaline granules in the hymenium, and the absence of an amyloid reaction in the hymenium differentiate it from either *Sarea* species, and suggest that it may be a new species, the first in the genus in 120 years.

IV. Discussion

Although most clades show support for a hypothesis of different lineages exhibiting host specificity at the host family level, there is at least one exception. One possible explanation for this is that resin composition, and not genetic relatedness, is preferred by different *Sarea* lineages. While in most cases, these coincide [Lambert et al. 2005], it is possible that convergent evolution in resin composition has occurred between species of *Picea* and *Cupressus forbesii* that results in a similar habitat. Resin composition of different hosts can be assessed to test this hypothesis.
Geography will be more difficult to separate as a factor. Many conifers have been introduced worldwide, either as ornamentals or for commercial purposes. It is clear that, in some areas, *Sarea* species must have been introduced. For instance, the Cape Verde Islands host no native conifers, but specimens of *Sarea* were collected there on pines introduced from the Canary Islands and Europe. It seems reasonable that these *Sarea* lineages were likewise introduced from Europe, but in both cases molecular data shows them to be most related to specimens collected in North America. Additional specimens must be collected and analyzed to clarify the origin of these different lineages.
Finally, it is possible that these lineages in both *S. difformis* and *S. resinae*, with their intraspecific variation in ITS of up to 7%, may more truly represent novel species. Biometrical analysis of sequenced specimens and additional analysis of new specimens must be performed to verify whether these lineages are distinct at a specific or infraspecific level.

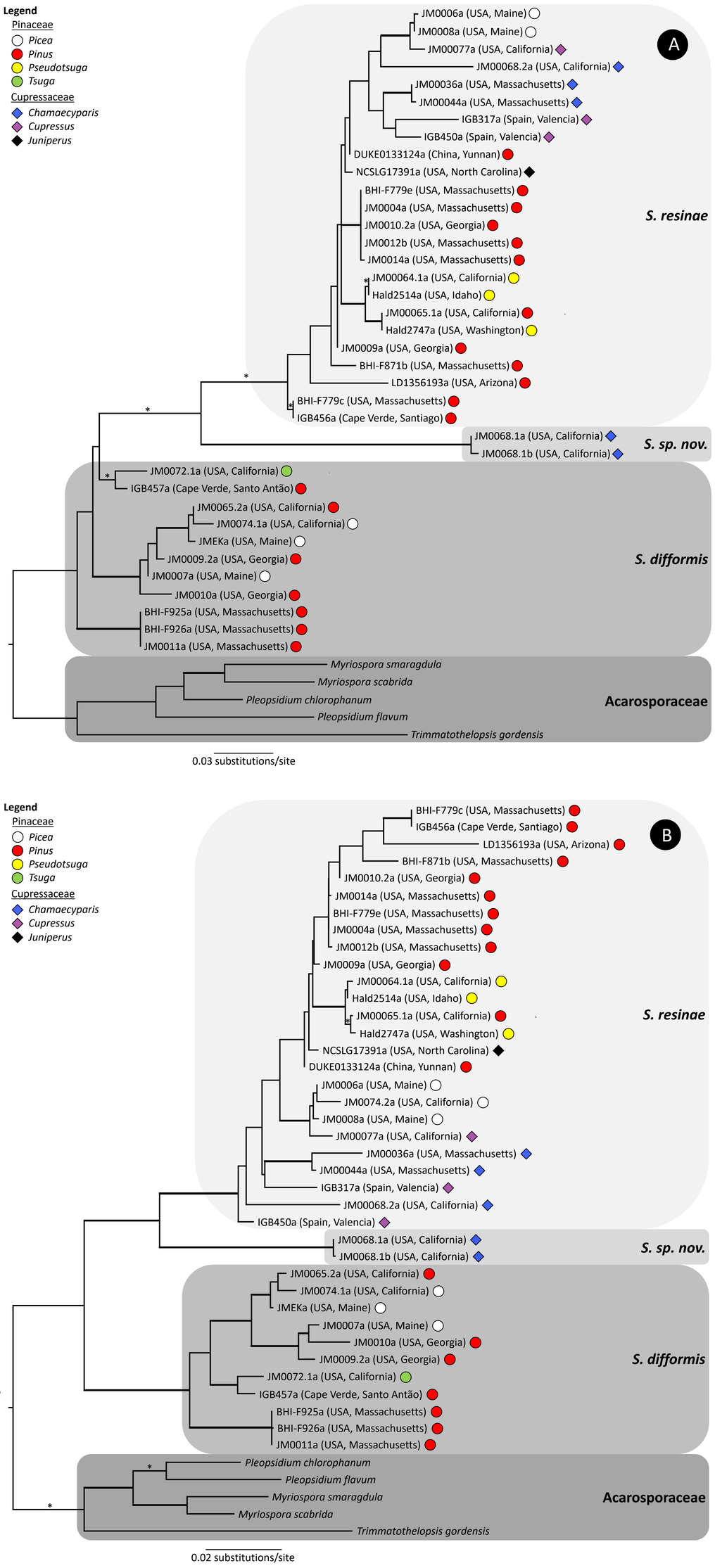

Figure 2. The maximum likelihood (ML) trees estimated from A) a dataset of one ribosomal marker (ITS) and B) a concatenated dataset of two ribosomal (ITS, LSU) and one mitochondrial (mtSSU) markers representing *Sarea* species. Thickened branches represent Bayesian posterior probabilities ≥ 0.95 from the BEAST analysis and ML bootstrap values ≥ 70% from the PAUP analysis, and asterisks on a branch indicate that only one of these thresholds was exceeded. Colored blocks represent current species concepts. JM0068.1 is excluded from the species concept of “*S. resinae*” due to its pigmentation and some differences in morphology.

V. Acknowledgements

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VI. Bibliography

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