The enigmatic *Squamanita odorata* (Agaricales, Basidiomycota) is parasitic on *Hebeloma mesophaeum*

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**Abstract**

*Squamanita odorata* is an agaric which is parasitic on unrecognizable, previously unidentifiable sporophores of a fungal host that is transformed into galls at the bases of the parasite’s sporophore. Amplification and sequencing portions of the nuclear (ITS) and mt rDNA from three samplings originating from two sites (from France and from Switzerland) demonstrate that the galls produce sequences that are identical to that of co-occurring *Hebeloma mesophaeum*. This demonstrates that *S. odorata* is a biotrophic parasite on *Hebeloma mesophaeum*.

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**Keywords:**
Agaricomycetes
Fungicolous fungi
Galls
Mycoparasites

**Introduction**

*Squamanita* is an enigmatic genus in the Agaricales whose species exhibit an unusual parasitism on sporophores of other basidiomycetes (Redhead et al. 1994). *Squamanita* spp. sporophores arise from enlarged bases, that have been labelled ‘sclerotial bodies’ or ‘protocarpic tubers’, and which sometimes give rise to multiple sporophores (Fig 1). This swollen basal structure displays highly variable microscopic features from one *Squamanita* species to another. These features fallaciously suggest affiliation of the genus to the families Agaricaceae, Tricholomataceae or Amanitaceae, depending upon the *Squamanita* sp. investigated. However, the sclerotial bodies were discovered to be deformed sporophores of other agaric species, i.e. they were galls in which *Squamanita* hyphae are growing (Redhead et al. 1994). Whereas previously suggested affiliations of the genus were based incorrectly on host (gall) features, recent molecular approaches have failed to place the genus *Squamanita* close to any other genus, although its monophyly was supported (Moncalvo et al. 2002). Parasitized hosts vary from one *Squamanita* sp. to another, and can sometimes be identified thanks to microscopy and rare parasitized specimens in which the host is still recognizable (Redhead et al. 1994).

Strikingly, the first *Squamanita* species historically described, *S. odorata* (Cool 1918, under the name *Lepiota odorata*), has a highly deformed and thus unknown host even at the generic level (Redhead et al. 1994). *S. odorata* sporophores have a strong, fragrant smell of grapes (amy1-acetate; Guéry & Chiaffi 1994), but their galls have a distinct raphanoid smell and a taste reminiscent of *Hebeloma* species (Vesterholt 1991). Indeed, *H. mesophaeum* sporophores coincidentally fruit around *S. odorata* sporophores (Vesterholt 1991; Guéry & Chiaffi 1994), suggesting that the host could be *Hebeloma*. This paper is dedicated to Camille Divet, in memory of his passion for studying and teaching mycology.

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DNA amplification, cloning, and sequencing

DNA extraction and PCR amplification of the ITS using primers ITS1F and ITS4 were performed as described by Selosse et al. (2002). Swiss samples, and 1993 and 1995 Ruudin samples were handled separately to avoid cross-contamination. Single PCR products were directly sequenced with the same primers on an ABI 3130xl sequencer (Applied Biosystems, Courtaboeuf) using the Big Dye Terminator kit. Whenever direct sequencing failed, PCR products were cloned using a pGEM-T easy vector systems kit (Promega, Charbonnières), followed by transformation into super-competent cells XL1-Blue (Stratagene, Amsterdam). Positive clones were sequenced using ITS1F, as above, to obtain a minimum of ten sequences; a reverse sequence using primer ITS4 was obtained from a single, randomly chosen clone for each different sequence found. For site A samples, the mitochondrial gene for LSU rDNA was amplified and sequenced using primers ML5 (5'-CTCGGCAAA TTATCCTCAATG-3') and ML6 (5'-CATGTAAGGCCTGATAGG GTC-3') with PCR conditions identical to these used for ITS (Selosse et al. 2002). Sequences were edited and aligned using Sequencer 4.5 for MacOsX from Genes Codes (Ann Arbor) and a consensus was generated for cloned sequences. Data were deposited in GenBank (EF091825-EF091828). Searches for similar sequences allowing taxonomic identification were conducted using (1) the BLASTN algorithm available through the NCBI (http://www.ncbi.nlm.nih.gov/BLAST/index.html) and the ectomycorrhizal fungal sequence database UNITE (Köljalg et al. 2005; http://unite.zbi.ee/) as well as (2) the FASTA3 algorithm against the EMBL database (http://www.ebi.ac.uk/ fasta33/nucleotide.html). Only BLAST analyses are reported below for Hebeloma mesophaeum sequences, as identical results were obtained by FASTA3 analysis.

Results and discussion

Hebeloma mesophaeum sporophores produced slightly different sequences from each site. Sequences A1 (at Ruudin; GB accession number EF091826) and B1 (at Villars-sur-Glane; EF091827) shared 613 out of 619 bp (Table 1). Their closest relatives in GenBank by BLAST analysis were AY748854 (an uncultured ectomycorrhiza of Hebeloma sp.) and AB211272 (from Hebeloma mesophaeum). The latter only had seven differences out of 619 bp with the consensus of A1 and B1, a variation that is usual in Hebeloma spp. (Aanen et al. 2000; Boyle et al. 2006; H. Beker, pers. comm.). No better match was retrieved from UNITE.

All but two attempts to amplify the ITS from Squamanita odorata sporophores were successful (Table 1). Sequencing produced identical sequences from both sites (A2; GB accession number EF091828). The closest GenBank relatives by BLAST analysis matches were two Bolbitiaceae: AF325658 (Descoues sp.) and AF325623 (Seltchelloagaster tenuepis). However, the affinity of S. odorata to the Bolbitiaceae is tenuous because (1) the expected value is quite high (10^-97; this value represents the likelihood of sequence matches expected by random chance), (2) the similarities are restricted to the 5.8S region of the sequence (not shown; note that GenBank contained no Squamanita ITS sequence before) and (3) FASTA3 research

Materials and methods

Investigated samples

Site A is grassy and surrounded by young Picea abies on sandy soil at Ruudin (Département de la Sarthe, East France, elevation 52 m. 47°56'50"N, 00°15'42"E), as described in Guény & Chiaffi (1994). We investigated a specimen from the initial October 1993 sampling provided by M. Chiaffi (sampling 1; voucher number: M. Chiaffi 931109) and five specimens, as well as two nearby Voucher number: M. Chiaffi 931109) and five specimens, as well as two nearby Hebeloma mesophaeum sporophores, collected in October 1995 by C. Divet (voucher for Squamanita odorata sample 2: MAS95SSQ02, M.-A. Selosse at CEF-CNRS).

Site B is situated at Villars-sur-Glane (Canton de Fribourg, 2 km away from Fribourg, Switzerland, elevation 658 m, 46°47'25"N, 07°07'31"E) on molassic soil. Immediately after a disturbance in 1980, trees (Salix caprea, Pinus sylvestris, Picea abies, Betula verrucosa and Sorbus aucuparia) were planted on the site. S. odorata first fruit in 1990 and, in 2000, three specimens (voucher number: F. Ayer 91-012-2540) and a H. mesophaeum sporophore were collected by F. Ayer. All specimens were dried upon DNA extraction.

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provided Collybia cinnrata (AF361318 and AF361316) as closest relatives. Although Squamanita appeared as a sister group to Phaeocollybiia spp. in the phylogeny by Moncalvo et al. (2002), that placement lacked statistical support: the phylogenetic position of the genus Squamanita therefore deserves further work.

All but one investigated gall produced PCR products, and provided a single apparent band after electrophoresis on gel. Unfortunately, this failed to produce any sequence for S. odorata. In contrast to contamination by the other sampled sporophores, and that sequence of the host (sample 2 of site B; Table 1).

By Nagasawa (1990) suggests that these authors collected a different species (tentatively labelled ‘S. phaeolepioticola’, an unpublished name, by Redhead et al. 1994). Indeed, Nagasawa et al. (1990) suspected this due to conidia produced by clampless hyphae, instead of clamped hyphae in true S. odorata.

Our data also demonstrate that some integrity of H. mesophaeum persists in the gall, so that DNA can be successfully recovered, as would be expected if S. odorata is a biotrophic parasite. Although the direction of the parasitism cannot be directly inferred from our observations, we favour the later explanation, rather than a parasitism of H. mesophaeum on S. odorata, as (1) S. odorata sporophores never grow alone, and (2) the biology of the Squamanita genus makes Hebeloma sequences unlikely to be adventitious in galls. Moreover, S. odorata often grows in anthropically disturbed sites (as A and B in this study, or sites described in Læssøe 1985); it could be hypothesized that such environments entail some weakness or maladaptation of H. mesophaeum favouring interaction with S. odorata. Alternatively, it could be that anthropically disturbed sites make S. odorata more obvious to mycologists.

<table>
<thead>
<tr>
<th>Sample Origin</th>
<th>Investigated part of sporophore</th>
<th>ITS (nu-rDNA) sequencea</th>
<th>Mitochondrial rDNA sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site A (Ruaudin, France), 1993, sampling M. Chiaffy Squamanita odorata sample 1</td>
<td>Gall</td>
<td>A1 + A2 (cloned, 9 + 1)</td>
<td>A3</td>
</tr>
<tr>
<td></td>
<td>Sporophore</td>
<td>A2 (d. s.)</td>
<td>No PCR product</td>
</tr>
<tr>
<td>Site A (Ruaudin, France), 1995, sampling C. Divet S. odorata sample 1</td>
<td>Gall</td>
<td>A1 + A2 (cloned, 3 + 7)</td>
<td>A3</td>
</tr>
<tr>
<td></td>
<td>Sporophore</td>
<td>No PCR product</td>
<td>A3</td>
</tr>
<tr>
<td>S. odorata sample 2</td>
<td>Gall</td>
<td>A2 (cloned, 10 + 0)</td>
<td>No PCR product</td>
</tr>
<tr>
<td></td>
<td>Sporophore</td>
<td>A2 (d. s.)</td>
<td>No PCR product</td>
</tr>
<tr>
<td>S. odorata sample 3</td>
<td>Gall</td>
<td>A1 + A2 (cloned, 7 + 3)</td>
<td>No PCR product</td>
</tr>
<tr>
<td></td>
<td>Sporophore</td>
<td>A2 (d. s.)</td>
<td>No PCR product</td>
</tr>
<tr>
<td>S. odorata sample 4</td>
<td>Gall</td>
<td>A1 + A2 (cloned, 4 + 6)b</td>
<td>A3</td>
</tr>
<tr>
<td></td>
<td>Sporophore</td>
<td>A2 (d. s.)</td>
<td>No PCR product</td>
</tr>
<tr>
<td>S. odorata sample 5</td>
<td>Gall</td>
<td>A1 + A2 (cloned, 4 + 6)b</td>
<td>A3</td>
</tr>
<tr>
<td></td>
<td>Sporophore</td>
<td>A2 (d. s.)</td>
<td>No PCR product</td>
</tr>
<tr>
<td>H. mesophaeum sample 1</td>
<td>Gall</td>
<td>A1 + A2 (cloned, 9 + 1)</td>
<td>A3</td>
</tr>
<tr>
<td></td>
<td>Sporophore</td>
<td>A2 (d. s.)</td>
<td>No PCR product</td>
</tr>
<tr>
<td>Site B (Villars-sur-Glaˆne, Switzerland), 2000, sampling F. Ayer</td>
<td>Gall</td>
<td>A2 + B1 (cloned, 8 + 2)b</td>
<td>Not studied</td>
</tr>
<tr>
<td></td>
<td>Sporophore</td>
<td>A2 (d. s.)</td>
<td>Not studied</td>
</tr>
<tr>
<td>S. odorata sample 2</td>
<td>Gall</td>
<td>A2 + B1 (cloned, 8 + 2)b</td>
<td>Not studied</td>
</tr>
<tr>
<td></td>
<td>Sporophore</td>
<td>A2 (d. s.)</td>
<td>Not studied</td>
</tr>
<tr>
<td>S. odorata sample 3</td>
<td>Gall</td>
<td>A2 (cloned, 10 + 0)</td>
<td>Not studied</td>
</tr>
<tr>
<td></td>
<td>Sporophore</td>
<td>A2 (d. s.)</td>
<td>Not studied</td>
</tr>
</tbody>
</table>

a ‘d. s.’, direct sequencing was possible; otherwise, whenever PCR products were cloned, the cloning ratio of each sequence obtained is given (n = 10). GenBank accession numbers: A1, EF091826; A2, EF091828; B1, EF091827; A3, EF091825.

b Additionally, a chimaeric sequence involving the two obtained sequences was found in an 11th clone.
Several features of *S. odorata* and other *Squamanita* spp. remain intriguing, such as the many host jumps in the evolution of the *Squamanita* genus, in spite of the high specificity of each *Squamanita* species. A second question is that of the persistence of the parasite populations over the years, e.g. from 1990 to 2003 at site B (F. Ayer, pers. comm.); do some propagules persist, such as the thick-walled chlamydospores, or do vegetative mycelia persist in soil, perhaps associated with that of *H. mesophaeum*? In the relationship between *Suillus bovinus* and *Gomphidius roseus*, hyphae of the latter associate with *S. bovinus* ectomycorrhizae (Olsson et al. 2000). A similar situation could occur with *H. mesophaeum* as it too is ectomycorrhizal, however, other *Squamanita* hosts are not (Redhead et al. 1994). Alternatively, repeated inoculation of sporophores by persistent propagules, as in *Hypomyces* spp. (Douhan & Rizzo 2003), could be involved. This also questions the exact nature of the interface between the two species, and the way in which *Squamanita* hyphae recover organic matter from their host, a biotrophic parasitism also reported in other *Squamanita* spp. and in *Entoloma abortivum* parasitic on *Armillaria* spp. (Czederpilz et al. 2001). Besides identification of *H. mesophaeum* as a host for *S. odorata*, this exquisite case of fungal parasitism thus deserves further study.

**Acknowledgements**

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**References**


