

Ustilaginomycetes on *Selaginella*¹

Robert Bauer²
Kálmán Vánky
Dominik Begerow
Franz Oberwinkler

Universität Tübingen, Botanisches Institut, Lehrstuhl
Spezielle Botanik und Mykologie, Auf der Morgenstelle
1, D-72076 Tübingen, Germany

Abstract: The lycophytes represent the most primitive host group for Ustilaginomycetes. Morphology and ultrastructure of the two known smut species on *Selaginella*, *Melanotaenium oreophilum* and *M. selaginellae*, as well as teliospore germination and the 5' end of the nuclear large subunit rRNA gene of the first species were studied and compared. The results of molecular analyses suggest that these two smut fungi belong either to the Doassansiales or Georgeriales. The presence of complex interaction apparatus including cytoplasmic compartments favors the placement in the Doassansiales. *M. oreophilum* and *M. selaginellae* share the type of septal pore apparatus and host-parasite interaction with the Doassansiales, but not with the Georgeriales. They differ from the other members of the Doassansiales in the pigmentation of the teliospores and, at least in *M. oreophilum*, in the basidial type. To accommodate these two species in the Doassansiales, a new genus, *Melaniella*, and a new family, Melaniellaceae, are proposed. Descriptions and illustrations are given for *Melaniella oreophila* and *M. selaginellae*.

Key Words: Basidia, Doassansiales, Exobasidiomycetidae, Lycophyta, *Melaniella*, Melaniellaceae, molecular systematics, morphology, rRNA, smut fungi, ultrastructure

INTRODUCTION

Two smut fungi are known to infect species of *Selaginella* P. B., *Melanotaenium selaginellae* Henn. & E. Nyman on *Selaginella* sp. in Java (Hennings 1900), and *M. oreophilum* Syd. on *Selaginella chrysocaula* Spring and *Selaginella chrysorrhiza* Spring in India

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² Email: robert.bauer@uni-tuebingen.de

(Sydow 1935). The systematic position of these two fungi was uncertain, partly because no teliospore germination was known, and partly because of the host plant family, which is unique for Ustilaginomycetes. However, ultrastructural studies revealed that they are members of the Ustilaginomycetes, belonging to the Doassansiales (Bauer et al 1997). Within the Doassansiales, they were tentatively placed in the Doassansiaceae by Bauer et al (1997). In this paper, morphological, ultrastructural and molecular characteristics of these two smut fungi are described in order to determine their systematic position.

MATERIALS AND METHODS

For light microscopy (LM), dried spores were placed in lactophenol by gently heating to the boiling point. For scanning electron microscopy (SEM), dried spores were fixed on double-sided adhesive tape, mounted on specimen stub, sputter-coated with gold-palladium, ca. 20 nm, and examined in a scanning electron microscope at 10 kV.

For the study of teliospore germination, small pieces of soaked sori filled with teliospores were squashed between two microscope-slides. The spores were spread on water agar (WA) in Petri dishes, kept at room temperature (ca 22 C) for one wk, at 29 C for an additional wk, and again for 2 wk at room temperature. As soon as the first basidia were present, a suitable piece of WA (about 10 mm square) was cut out, transferred to a slide, and observed by LM initially without and subsequently with a coverglass.

For transmission electron microscopy (TEM), rehydrated specimens were fixed with 2% glutaraldehyde. After six transfers in 0.1 M Na-cacodylate buffer, the material was postfixed in 1% OsO₄ in the same buffer for 1 h in the dark, washed in distilled water, and stained in 1% aqueous uranyl acetate for 1 h in the dark. After five washes in distilled water, the material was dehydrated in an acetone series, embedded in Spurr's plastic and sectioned with a diamond knife. Serial sections were mounted on copper-slot-grids, post-stained with lead citrate for 5 min, and examined in a transmission electron microscope at 80 kV.

For sequence analysis, DNA of *Melanotaenium oreophilum* was isolated from herbarium specimen HUV 17501 using the SDS method as described by Begerow et al (1997). The 5' region of the nuclear large subunit of the ribosomal RNA gene was amplified using the polymerase chain reaction and the primers NL1 and NL4 (O'Donnell 1993). The PCR product was purified using the QIAquick[®] protocol (QIAGEN). This dsDNA was sequenced directly using the ABI PRISM[®] Dye-Termination Cycle Sequencing Kit (Applied

Biosystems) on an automated sequencer (ABI 373A, Applied Biosystems). Sequence of *M. oreophilum* is deposited in GenBank as AF 073288. Sequence of *Ustilago hordei* (Pers.) Lagerh. was published by Berres et al (1995) and is deposited in GenBank as L 20286. Sequences of *Tilletaria anomala* Bandoni & Johri and *Tilletia caries* (DC.) Tul. were published by Boekhout et al (1995) and are deposited in GenBank as AJ 235284 and AJ 235307, respectively. The sequences of the other species (Begerow et al 1997) are deposited in GenBank as follows: *Conidiosporomyces ayresii* (Berk.) Vánky, AF 009848; *Doassansia epilobii* Farlow, AF 007523; *Doassansia hygrophilae* Thirum., AF 007524; *Doassinga callitrichis* Vánky, R. Bauer & Begerow, AF 007525; *Entorrhiza aschersoniana* (Magnus) Lagerh., AF 009851; *Etyloma dactylidis* (Pass.) Cif., AF 009853; *Etyloma gaillardianum* Vánky, AF 007530; *Etyloma holwayi* Syd. & P. Syd., AF 009854; *Etyloma microsporum* (Unger) Schröt., AF 007530; *Etyloma polysporum* (Peck) Farlow, AF 007529; *Eratomyces patelii* (Pavgi & Thirum.) M. Piepenbr. & R. Bauer, AF 009855; *Exobasidium rhododendri* (Fuckel) Cram., AF 009856; *Exobasidium rostrupii* Nannf., AF 009857; *Exobasidium vaccinii* (Fuckel) Woronin, AF 009858; *Georgefischeria riveae* Thirum. & Naras., AF 009861; *Graphiola phoenicis* (Moug.) Poiteau, AF 009862; *Melanotaenium brachiariae* Viegas, AF 009864; *Microstroma juglandis* (Bereng.) Sacc., AF 009867; *Nannfeldtiomyces sparganii* (Lagerh.) Vánky, AF 007527; *Rhamphospora nymphaeae* D. Cunn., AF 007526.

An alignment of 516 bp was created using MEGALIGN of the Lasergene-package (DNASTAR, Inc. 1997). The PHYLIP package, version 3.572 (Felsenstein 1995), was used to perform the following analyses: neighbor joining and maximum likelihood with standard parameters, maximum parsimony (heuristic) with the jumble option turned on 10 replicates, bootstrap analyses with 1000 replicates for neighbor joining and maximum parsimony, and with 100 replicates for maximum likelihood.

RESULTS

Sporulation.—*Melanotaenium oreophilum* sporulated in the leaves, whereas *Melanotaenium selaginellae* sporulated in young stems (FIG. 1). Teliospores were smooth in *M. oreophilum* (FIGS. 2, 4, 6) and irregularly tuberculate in *M. selaginellae* (FIGS. 3, 5, 7). The teliospores of both species were scattered in intercellular spaces of the host tissue (FIG. 8) without rupturing it. Teliospores often exhibited two, more rarely just one appendage formed by remnant of the wall of the sporogenous hyphae, indicating a mainly intercalary position of the teliospore initial (not illustrated).

Hyphae.—*Melanotaenium oreophilum* and *M. selaginellae* possessed only intercellular hyphae with electron-opaque, fibrillate walls and clamps (FIG. 9). The septa showed the typical tripartite basidiomycetous profile (FIGS. 9–11). Septal pores were simple with

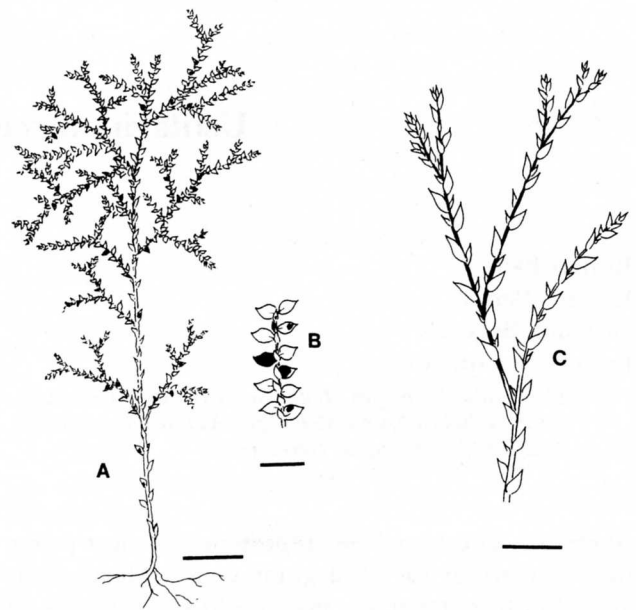


FIG. 1. Sori of *Melanotaenium oreophilum* = *Melaniella oreophila* and *Melanotaenium selaginellae* = *Melaniella selaginellae*. A, B. *Melaniella oreophila* (HUV 15689, NEOTYPE) on leaves of *Selaginella delicatula*. C. Sori of *Melaniella selaginellae* (BPI 195193, TYPE) in young stems of *Selaginella* sp. Scale bars: A, C = 1 cm, B = 2 cm.

more or less rounded pore lips and they were enclosed on both sides by caps (FIGS. 10, 11).

The hyphae had many contact areas with host cells where complex interaction apparatus containing cytoplasmic compartments were formed (FIGS. 12, 13; for a detailed description of this type of interaction apparatus see Bauer et al 1997). Host response to infection was often visible opposite the interaction apparatus (FIGS. 12, 13).

Basidia.—Teliospores of *M. oreophilum* germinated with aerial holobasidia. On the top of the basidia, (3–)4(–5) aseptate ballistosporic basidiospores with abaxially oriented hilar appendices were produced on sterigmata. Discharged basidiospores became two-celled by a transverse septum. On WA, the cells of the basidiospores germinated apically and laterally with cylindrical, hyaline and thin-walled yeast-like cells. Basidiospores that came in contact with the WA during development usually became septate and germinated on the basidia. Basidiospores that developed on basidia under the coverglass in contact with the WA tended to a more symmetrical form (FIG. 14).

Sequence analyses.—Sequence of *Melanotaenium oreophilum* was aligned with those of *Entorrhiza aschersoniana* (Entorrhizomycetidae), *U. hordei* (Ustilaginomycetidae) and 20 representative species of the

