

# *Muribasidiospora: Microstromatales or Exobasidiales?*<sup>1</sup>

Dominik BEGEROW, Robert BAUER and Franz OBERWINKLER

Lehrstuhl Spezielle Botanik und Mykologie, Botanisches Institut, Universität Tübingen, Auf der Morgenstelle 1, D-72076 Tübingen, Germany.  
E-mail: dominik.begerow@uni-tuebingen.de

Received 16 September 2000; accepted 11 January 2001.

Characteristics of hyphal septation, cellular interaction, sporulation, cultures and/or nucleotide sequences from the 5' terminal domain of the nuclear large subunit rRNA gene of some species of *Exobasidium*, *Microstroma* and *Muribasidiospora* were examined and compared. Our analyses show that the order *Microstromatales* comprises *Microstroma*, *Muribasidiospora triumfetticola* and the anamorphic species *Rhodotorula bacarum*, *R. phylloplana* and *Sympodiomyopsis paphiopedili*, whereas *Muribasidiospora hesperidium* and *Muribasidiospora indica* are members of the *Exobasidiales*. Because of the high degree of morphological divergence between *Muribasidiospora triumfetticola* and *Microstroma*, a new genus, *Volvocisporium*, and a new family, *Volvocisporiaceae*, of the *Microstromatales* are proposed. Apart from the phylogenetic implications, the most interesting observations in the present work are the unusual development of the basidia, especially of the basidiospores, and the nutrient transport system from the host attacking hyphae to the basidia in *Muribasidiospora triumfetticola*.

## INTRODUCTION

In the new system of *Ustilaginomycetes* (Bauer, Oberwinkler & Vánky 1997), all species lacking teliospores belong either to the *Microstromatales* or *Exobasidiales*. The order *Microstromatales* with the single family *Microstromataceae* was erected for species having simple-septate hyphae and local interaction zones without the formation of interaction apparatus. Haustoria or other intracellular fungal organs are lacking. The species of *Microstroma*, the single genus currently placed in the *Microstromatales*, are parasitic on hamamelids. The basidia protrude through stomata and sporulate in the gastroid mode of spore release on the leaf surface (Oberwinkler 1978, 1982). In contrast to the *Microstromatales*, the *Exobasidiales* possess a high degree of morphological divergence (Bauer, Begerow & Oberwinkler 1998). However, they are united in having highly characteristic local host-parasite interaction zones with the formation of complex interaction apparatus containing an interaction ring (Bauer *et al.* 1997). Molecular analyses confirmed this group (Begerow, Bauer & Oberwinkler 1997). On the basis of sporulation Bauer *et al.* (1998) distinguished the families *Brachybasidiaceae*, *Cryptobasidiaceae*, *Exobasidiaceae*, and *Graphiolaceae*. The brachybasidiaceous and graphiolaceous species occur on monocots, whereas the cryptobasidiaceous and exobasidiaceous species are parasitic on dicots (Bauer *et al.* 1998).

The new system of *Ustilaginomycetes* also revealed that parallel evolution with their hosts was apparently a widespread principle in the ustilaginomycetous phylogeny (Bauer *et al.* 1997). Thus, genera with species parasitic on hosts of distant plant groups are possibly polyphyletic. One of these genera is the non-teliosporic tropical genus *Muribasidiospora* (Rajendren 1968). *Muribasidiospora indica*, the type of the genus, and *M. hesperidium* live on *Rhus* spp. (*Anacardiaceae*), *M. celtidis* is known from *Celtis tetrandra* (*Ulmaceae*), whereas *M. triumfetticola* parasitizes *Triumfetta rhomboidea* (*Tiliaceae*). Initially, *Muribasidiospora* was considered to be a taxon of the *Exobasidiaceae* (Rajendren 1968). Subsequently, a new family, *Muribasidiosporaceae*, placed in the *Hymenomycetes*, was erected by Rajendren (1969). In the present study, we compare morphological, ultrastructural and molecular characters of *Muribasidiospora hesperidium*, *M. indica* and *M. triumfetticola* with those of some typical species of *Microstromatales* and *Exobasidiales* in order to estimate the phylogenetic position of these fungi. From *Muribasidiospora celtidis* no material was available.

## MATERIALS AND METHODS

The specimens, respective characters studied, and the origin of the sequences, are listed in Table 1.

For the study of sori, hand sections through infected areas of leaves were mounted in 3% KOH and examined with light microscopy using phase contrast optics.

<sup>1</sup> Part 188 in the series *Studies in Heterobasidiomycetes* from the Botanical Institute, University of Tübingen.

**Table 1.** Specimens and characters studied.

Specimens	Hosts	Characters studied <sup>1</sup>	Sequences <sup>2</sup>	Source <sup>3</sup>
<i>Conidiosporomyces ayresii</i>	<i>Panicum maximum</i>	S	AF 009848 <sup>B</sup>	HUV 17460
<i>Doassansia epilobii</i>	<i>Epilobium montianum</i>	S	AF 007523 <sup>B</sup>	F.O. 38252
<i>Entyloma dactylidis</i>	<i>Agrostis stolonifera</i>	S	AF 009853 <sup>B</sup>	R.B. 915
<i>Entyloma ficariae</i>	<i>Ficaria verna</i>	S	AJ 235295 <sup>BO</sup>	CBS 480.91
<i>Entyloma holwayi</i>	<i>Cosmos caudatus</i>	S	AF 009854 <sup>B</sup>	M.P. 1769
<i>Erratomyces patelii</i>	<i>Phaseolus vulgaris</i>	S	AF 009855 <sup>B</sup>	M.P. 1991
<i>Exobasidium arescens</i>	<i>Vaccinium myrtillus</i>	H, Sp		R.B. 992
<i>Exobasidium arescens</i>	<i>Vaccinium myrtillus</i>	C, S, Sp	AF 352057*	R.B. 2047
<i>Exobasidium oxycocci</i>	<i>Vaccinium oxycoccos</i>	C, H, Sp		F.O. 18925
<i>Exobasidium pachysporum</i>	<i>Vaccinium uliginosum</i>	C, H, Sp		R.B. 947
<i>Exobasidium vaccinii</i>	<i>Vaccinium vitis-idaea</i>	C, H, S, Sp	AF 009858 <sup>B</sup>	R.B. 945
<i>Exobasidium rhododendri</i>	<i>Rhododendron ferrugineum</i>	C, H, S, Sp	AF 009856 <sup>B</sup>	R.B. 2050
<i>Georgefischeria riveae</i>	<i>Rivea hypocrateriformis</i>	S	AF 009861 <sup>B</sup>	HUV 15614
<i>Graphiola phoenicis</i>	<i>Phoenix canariensis</i>	C, S	AF 009862 <sup>B</sup>	F.O. 29350
<i>Malassezia furfur</i>	Yeast	S	AF 063214 <sup>G</sup>	CBS 1878
<i>Malassezia sympodialis</i>	Yeast	S	AJ 249953 <sup>G</sup>	CBS 7222
<i>Microstroma album</i>	<i>Quercus robur</i>	C, H, S, Sp	AF 352052*	R.B. 2072
<i>Microstroma juglandis</i>	<i>Juglans regia</i>	H, Sp		F.O. 17500
<i>Microstroma juglandis</i>	<i>Juglans regia</i>	H, Sp		F.O. 37098
<i>Microstroma juglandis</i>	<i>Juglans regia</i>	C, H, S, Sp	AF 009867 <sup>B</sup>	F.O. 39211
<i>Muribasidiospora hesperidium</i>	<i>Rhus glaucescens</i>	H, Sp		IMI 5074
<i>Muribasidiospora indica</i>	<i>Rhus mysorensis</i>	H, Sp		IMI 114750
<i>Muribasidiospora indica</i>	<i>Rhus mysorensis</i>	H, Sp		Type
<i>Muribasidiospora indica</i>	<i>Rhus lancea</i>	C, H, S, Sp	AF 352058*	AMH 390
<i>Muribasidiospora triumfetticola</i>	<i>Triumfetta rhomboidea</i>	H, Sp		F.O. 47397
(as <i>Muribasidiospora triumfettaeicola</i> )				Type <sup>4</sup>
<i>Muribasidiospora triumfetticola</i>	<i>Triumfetta rhomboidea</i>	C, H, S, Sp	AF 352053*	R.B. 2070 <sup>4</sup>
(as <i>Muribasidiospora triumfettaeicola</i> )				Topotype
<i>Rhizophora nymphaeae</i>	<i>Nymphaea alba</i>	S	AF 007526 <sup>B</sup>	R.B. 862
<i>Rhodotorula bacarum</i>	Yeast	C, S	AF 352055*	IGC 4391
<i>Rhodotorula phylloplana</i>	Yeast	C, S	AF 352056*	Ex-type strain
				IGC 4246
<i>Symptodiomyces paphiopedili</i>	Yeast	C, S	AF 352054*	Ex-type strain
				CBS 7429
<i>Ustilago hordei</i>	Not cited	S	L 20286 <sup>BE</sup>	Type strain
				D.M.11.2C

<sup>1</sup> C = Culture; H = Hyphal septation and cellular host-parasite interaction, S = Sequence; Sp = Sporulation and basidia.

<sup>2</sup> Origin of sequences: B = Begerow, Bauer & Oberwinkler (1997), BE = Berres, Szabo & McLaughlin (1995), BO = Boekhout, Fell & O'Donnell (1995), G = Guillot & Gueho (1995), \* = new sequences.

<sup>3</sup> AMH = Herbarium of the Agarkar Institute, Pune, India; CBS = Culture collection of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; D.M. = D. Hills, Corvallis, USA; HUV = Herbarium Ustilaginales Vánky, Tübingen, Germany; F.O. = Herbarium F. Oberwinkler, Tübingen, Germany; IGC = Portuguese Yeast Culture Collection, Portugal; IMI = CABI Bioscience UK Centre, Egham, UK; M.P. = Herbarium M. Piepenbring, Tübingen, Germany; R.B. = Herbarium R. Bauer, Tübingen, Germany.

<sup>4</sup> Material obtained from M. S. Patil, India.

For the study of basidiospore germination and cultures, spores were spread thinly on malt-yeast-peptone agar (Bandoni 1972) in Petri dishes kept at room temperature.

For TEM, fresh material or rehydrated herbarium specimens (indicated in the figure legends) were fixed with 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at room temperature overnight. Following six transfers in 0.1 M sodium cacodylate buffer, samples were postfixed in 1% osmium tetroxide 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, samples were dehydrated in acetone, using 10 min changes at 25%, 50%, 70%, 95%, and 3 times in 100% acetone. Samples were embedded in Spurr's plastic and sectioned with a diamond knife. Semi-thin sections were stained with new fuchsin and crystal violet, mounted in Entellan and studied by

light microscopy (LM). Ultrathin, serial sections were mounted on formvar-coated, single-slot copper grids, stained with lead citrate at room temperature for 5 min, and washed with distilled water. They were examined using a transmission electron microscope operating at 80 kV.

For molecular studies, DNA was isolated from cultures or herbarium specimens using the SDS method as described previously (Begerow *et al.* 1997). The 5' region of the nuclear large subunit of the rRNA 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<sup>TM</sup> protocol (QIAGEN). This dsDNA was sequenced directly using the ABI PRISM<sup>TM</sup> Dye-Termination Cycle Sequencing Kit (Applied Biosystems) on an automated sequencer (ABI 373A, Applied Biosystems). An alignment of 534 basepairs was created using MEGALIGN of the Lasergene-

package (DNASTAR, 1997). PAUP (Swofford 1998) was used to calculate all phylogenetic analyses. Neighbour joining analyses were calculated with several substitution models and maximum parsimony analysis was performed in two steps to achieve highest reliability. In the first step the heuristic search was performed with 10000 replicates for stepwise random addition but without swapping. The 26 best trees were used in the second step to calculate an intensive swapping procedure. Bootstrap values were calculated for 10000 replicates.

## RESULTS

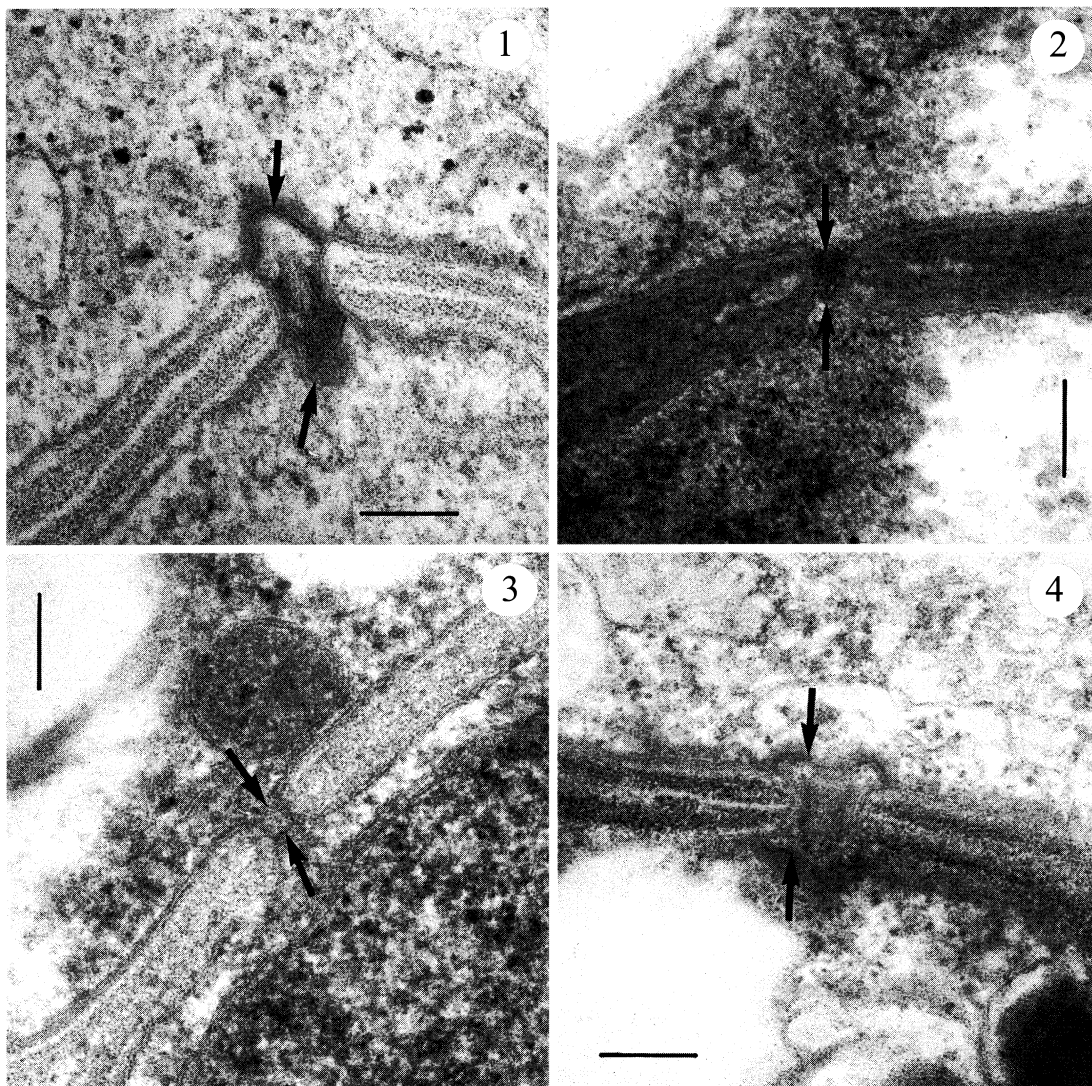
### Septal pores

Septal pore apparatus in the species studied of *Exobasidium*, *Microstroma* and *Muribasidiospora* are simple with more or less rounded pore lips and they are enclosed on both sides by membrane caps (Figs 1–4). In *Exobasidium*, *Muribasidiospora*

*hesperidium*, and *M. indica* the pores and caps are larger than those observed in *Microstroma* and *Muribasidiospora triumfetticola* (compare Figs 1 and 4 with Figs 2 and 3).

### Cellular interactions

Cellular interactions were discussed in detail by Bauer *et al.* (1997) and are therefore only briefly summarized here. All species studied interact with their respective hosts by the formation of local interaction zones (Figs 5–8). In *Microstroma*, *Muribasidiospora*, *Exobasidium arescens* and *Exobasidium pachysporum* these interaction sites are located in intercellular hyphae attached with host cells, whereas in *E. oxycocci*, *E. rhododendri* and *E. vaccinii* they are located in haustoria (see also Bauer *et al.* 1997). Species of *Microstroma* and *Muribasidiospora triumfetticola* on the one hand, and species of *Exobasidium*, *Muribasidiospora hesperidium* and *M. indica* on the other, share similar interaction sites. *Microstroma* spp. and



**Figs 1–4.** Septal pore apparatus, each with nonswollen margin and two membrane caps (arrows). Note that the pores and the respective membrane caps illustrated in Figs 1 and 4 are larger than those illustrated in Figs 2–3. Bars = 0.1  $\mu$ m. **Fig. 1.** *Exobasidium pachysporum*. **Fig. 2.** *Microstroma juglandis* (F.O. 37098). **Fig. 3.** *Muribasidiospora indica* (F.O. 47397). **Fig. 4.** *Volvocisporium triumfetticola* (syn. *Muribasidiospora triumfetticola*; R.B. 2070).

