Doassinga, a new genus of Doassansiaceae

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Abstract: In order to examine the systematic position of Entyloma callitrichis Liro, morphological, ultrastructural and molecular characters of E. callitrichis and some species of the Entylomataceae, occupying a basal position within this family. In contrast with the other species of Doassansiaceae, the teliospores of E. callitrichis are not arranged in spore balls. Therefore, the new genus Doassinga is proposed to accommodate E. callitrichis.

Key Words: Entyloma, molecular phylogeny, morphology, rDNA, Rhamphosphora, smut fungi, taxonomy, ultrastructure, Ustilaginomycetes

INTRODUCTION

A new phylogenetic system of smut fungi was published by Bauer et al. (1997), based on ultrastructural characters of host-parasite interaction and hyphal septation. The results were also confirmed by molecular analyses (Begerow et al., 1997). In the new system, the order Doassansiaceae R. Bauer & Oberw. has two families Rhamphosphoraceae R. Bauer & Oberw., with one monotypic genus, Rhamphosphora D. Cunn., and Doassansiaceae (Azbukina & Karatygin) R. T. Moore, emend. R. Bauer & Oberw., containing 36 species in 7 genera. These are Burrellia Setchell (7 spp.), Doassansia Cornu (19 spp.), Heterodoassansia K. Vánky (4 spp.), Nannfeldtiomyces K. Vánky (2 spp.), Narassinhania Thirum. & Pavgi, emend. K. Vánky (1 sp.), Pseudodoassansia (Setchell) K. Vánky (1 sp.), and Traicya H. & P. Sydow (2 spp.). Additionally, Entyloma callitrichis Liro was placed into the Doassansiaceae by Bauer et al. (1997).

This is hard to understand because the morphology of teliospores and sori of E. callitrichis is very similar to that of typical species of Entyloma de Bary (see Vánky, 1994). Therefore, we compare here morphological, ultrastructural and molecular characters of E. callitrichis and some species of Entylomataea and Doassansiaceae in order to examine the systematic position of this fungus.

MATERIALS AND METHODS

The abbreviations of herbaria and culture collections are: F.O. = Herb. F. Oberwinkler, HUV = Herb. Ustil. Vánky, R.B. = Herb. R. Bauer, B.B.A. = culture collection of the “Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin”. Material used for morphological and ultrastructural studies is indicated in the figure captions. Sequences were obtained from cultures of Doassansia epilobi Farlow (F.O. 38252), D. hygrophilae Thirum. (HUV 15474), Entyloma callitrichis Liro (R.B. 1079), E. gaillardianum K. Vánky (R.B. 2055), E. microsporum (Unger) Schröter (F.O. 37329), E. polysporum (Peck) Farlow (HUV 2960), Nannfeldtiomyces spargani (Lagerh.) K. Vánky (BBA 68271), and Rhamphosphora nymphaeae D. Cunn. (R.B. 862). The sequence of E. ficariae Thümen ex Fischer v. Waldh. was taken from literature (Boeckhout et al., 1995).

For light microscopic (LM) studies of the spores, dried spores were rehydrated in lactophenol by gently heating to the boiling point under a cover glass. For scanning electron microscopic (SEM) studies, host tissues with spores were picked up on double-sided adhesive tape, mounted on a specimen stub, sputter-coated with ca. 20 nm gold-palladium, and examined in a SEM at 10 kV.

For the study of germination, spores were spread thinly on water agar (WA) and on malt-yeast-peptone agar (MYP) in Petri dishes kept at room temperature (ca. 22 C). As soon as basidiospores were produced, a suitable piece of medium (about 10 mm square) was cut out, transferred to a slide, and covered with a cover glass. A small droplet of lactophenol with cotton blue, added to the side of the square of medium, fixed and stained the basidia and basidiospores.

For studies of the soral and ultrastructural characters, fresh material was fixed with 2% glutaralde-
hyde in 0.1 M Na-cacodylate buffer at pH 7.2 for several days. 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. Semithin sections were stained with new fuchsin and crystal violet, mounted in “Entellan” low viscosity resins and studied in a light microscope (LM). Ultrathin sections were mounted on copper slot-grids, poststained with lead citrate for 5 min, and examined in a transmission electron microscope (TEM) at 80 kV.

For molecular studies, DNA was isolated from cultures obtained from germinated spores, using the SDS method as described previously (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 NLI and NLA (Boekhout et al., 1995). The PCR product was purified using the QIAquickTM 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). An alignment of 551 basepairs 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. The alignment of rDNA sequences is available from the third author upon request. Our sequences are also deposited in GenBank (acc. No. AF 007523–AF 007530).

TAXONOMY

Interestingly, no “Entyloma” spp. described from aquatic or paludal plants belong to the genus Entyloma. “Entyloma” nymphaeae (D. Cunn.) Setchell on Nuphar and Nymphaea spp. (Nymphaeaceae) has been transferred to Rhampshospora nymphaeae. “Entyloma” sparganii (Lagerh.) Lagerh. has been transferred to Nannfeldtiumyces sparganii (Lagerh.) K. Vánky (1981: 171); it forms loose spore balls on Sparganium spp. (Sparganiaceae). “Entyloma” fluviatus Liro on Glycera spp. (Poaceae) has been moved to Ustilentyloma fluviatus (Liro) K. Vánky (1970: 328), which has phragmobasidia and belongs to the Ustilacylomataceae R. Bauer & Oberw. of the Microbotryales R. Bauer & Oberw. “Entyloma” ulei Speg. on Callitrichica verna L. is Sorosphaera ulei (Schröter) Liro in the Plasmodiophoraceae (Liro, 1935, p. 14). Entyloma callitrichis Liro (on Callitrichica spp., Callitrichaceae) was the sole remainder Entyloma spp. living on aquatic plants.

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Sori on aquatic or paludal plants producing spots. Spores solitary, weakly pigmented, embedded in the host tissues. Spore germination of Tillettia-type, resulting in holobasidia. The septal pore is simple with a membraneous cap. Host-parasite interaction by complex interaction apparatus containing cytoplasmic compartments. Haustoria are lacking.

Doassinga callitrichis (Liro) K. Vánky, R. Bauer & D. Begerow, comb. nov. Figs. 1–8


FIG. 1. Sori of Doassinga callitrichis form indistinct spots on the leaves of Callitrichica palustris. Bar = 1 cm.
Figs. 2–7. *Doassinga callitrichis* on *Callitriche stagnalis* (R.B. 923 = HUV 12657 = Vánky, Ustilaginales exs. No. 560). 2. Transversal section of a sorus with scattered spores between the parenchyma cells of leaf tissue. Bar = 25 μm. 3–5. Spores and intercellular hyphae in LM, in SEM and in TEM. In TEM the spore wall has a thin, uniform, electron opaque internal layer, and an irregular, electron-inhomogeneous external layer. Bars = 5 μm. 6. Host-parasite interaction of *Doassinga callitrichis*. Intercellular hypha (ih) of *D. callitrichis* attached to a host cell wall (hcw) showing a complex interaction apparatus within the fungal cell (but outside the fungal cytoplasm) with portions of a highly branched, cisternal net containing electron-opaque material (cia). Opposite to complex interaction apparatus electron-opaque deposits (d) can be seen adjacent to the host plasma membrane. Bar = 0.35 μm. 7. Septal wall and pore in TEM. The pore has a rounded lip, and is enclosed on both sides by a membranous cap (arrows). Bar = 0.1 μm.
Sori (Fig. 1) in leaves and stems as indistinct, greenish-yellow to pale yellow-brown spots with indefinite margins measuring 0.5–1.5 mm diam, or larger by confluence. Spores embedded in host tissue (Fig. 2), scattered between parenchyma cells. Spores (Figs. 3, 4) globose, ovoid to broadly ellipsoidal, (8–) 9–13.5 × 10–16 μm, hyaline to pale yellow; wall in LM apparently smooth to verrucose, ca. 1 μm thick, in SEM provided with low, irregular, anastomosing warts. Spore germination (Fig. 8) results in asceptate basidia apically bearing 4–8 long, fusiform or sigmoid, gastroid (not forcibly released) basidiospores, 1–1.5 × 15–60 μm. Parasitic hyphae only intercellular (Figs. 5, 6). Haustoria lacking. Host-parasite interaction (Fig. 6) characterized by complex interaction apparatus containing cytoplasmic compartments. Septa have simple pores with membranous caps (Fig. 7).

*Doassinga callitrichis* is known hitherto only from a few places in Europe, on *Callitriche palustris* L. (*C. verna* L.) and *C. stagnalis* Scop. (Callitrichaceae).

The described anamorph, *Entylomella callitrichis* Liro (1938: 118) represents the basidiospores of spores germinated in situ.

**Etymology.** Doass from Doassania, showing the close relationship to this group of smut fungi; singa from singularis = solitary, alone, referring to the single spores, not agglutinated in spore balls.

**RESULTS**

*Doassinga callitrichis* (Fig. 1), *E. microsporum*, the type of *Entyloma* de Bary, and *R. nymphaeae* as an example of Doassansiiales without spore balls were studied morphologically and ultrastructurally. These three fungi form hyaline, single teliospores in the leaves of their hosts (Figs. 2–4, 9–10). The teliospores are scattered in the host tissue (Figs. 2, 3). Teliospore germination results in holobasidia (Figs. 8, 15, 16). *Doassinga callitrichis* and *R. nymphaeae* have long, narrow, fusiform or sigmoid basidiospores (Figs. 8, 15). The basidiospores germinate by producing similar yeast cells perpendicularly to the long axis of the basidiospore (Figs. 8, 15). Conjugation of basidiospores on the basidia has not been observed in these two fungi, and ballistocondia were not produced. In *E. microsporum*, however, the basidiospores are shorter, thicker and usually produce ballistocondia after conjugation (Fig. 16).

The septal pore apparatus in the three species is composed of a simple pore surrounded by a membrane cap at either side of the pore (illustrated for *D. callitrichis* and *R. nymphaeae* in Figs. 7, 14).

*Doassinga callitrichis* and *E. microsporum* grow only intercellularly (Figs. 6, 12), whereas *R. nymphaeae* forms haustoria (Fig. 13). On the other hand, *E. microsporum* interacts with its host by the formation of simple interaction apparatus at contact areas (Fig. 12, for a detailed description see Bauer et al., 1997), whereas *D. callitrichis* and *R. nymphaeae* form complex interaction apparatus with cytoplasmic compartments (Figs. 6, 11, 13; for a detailed description see Bauer et al., 1997). Interaction apparatus in *Rhamphospora* are located in both intercellular hyphae in contact with host cells (Fig. 11) and in haustoria (Fig. 13).

The molecular analyses resulted in three different phyllogenetic hypotheses which are shown in Fig. 17. The neighbor joining analysis resulted in a topology with *D. callitrichis* at the base of the Doassansiiales (Fig. 17a). Maximum likelihood analysis resulted in a similar topology and the differences are not supported by bootstrap values (Fig. 17b). Maximum parsimony analysis resulted in three most parsimonious trees and the consensus tree is well supported within the Doassansiiales but results in a polytomy within the Entylomatales (Fig. 17c).
Figs. 9–14. TEM of spores, host-parasite interactions and septal pore of *Rhamphospora nymphaeae* on *Nymphea alba* and *Entyloma microsporum* on *Ranunculus repens*. 9. Transverse section of a spore of *R. nymphaeae*. The spore wall has a more or less regular, electron-light endospore and an exospore with an electron-dense, irregular internal layer and an interrupted, electron-light external layer (R.B. 826). Bar = 5 μm. 10. Transverse section of a spore of *E. microsporum*. The spore wall has a regular, medium electron-dense layer of endospore and a thicker, irregular, electron-light exospore (R.B. 1067). Bar = 5 μm. 11. Host-parasite interaction of *R. nymphaeae*. Intercellular hypha (ih) attached to a host cell wall (hcw). Within the fungal cell a complex interaction apparatus (arrow) can be seen. Note the electron-opaque deposits (d) opposite the interaction apparatus (R.B. 826). Bar = 1 μm. 12. Host-parasite interaction of *E. microsporum*. Intercellular hypha (ih) attached to a host cell wall (hcw) showing the simple interaction apparatus (arrow) within the fungal cell (R.B. 1080). Bar = 0.2 μm. 13. A haustorium (H) of *R. nymphaeae* in a host cell. The haustorium has a complex interaction apparatus (arrow; R.B. 1076). Bar = 2 μm. 14. Septal wall and porus of *R. nymphaeae*, which are very similar to those of *D. callistrichis* shown in Fig. 7 (R.B. 826). Bar = 0.1 μm.
DISCUSSION

The Doassansiaceae share the following characteristics with the Entylomatales: hyaline or poorly pigmented teliospores, sporulation in vegetative parts of their hosts, holobasidial type of teliospore germination, and simple septal pores with membrane caps (Bauer et al. 1997). In contrast with the Entylomatales, the Doassansiaceae do not form ballistoconidia in the saprobic phase and they live on aquatic plants. Ultrastructurally, they differ from the Entylomatales by the formation of globular, complex interaction apparatus with cytoplasmic compartments (Bauer et al. 1997). *Doassinga callitrichis* shows the characters of Doassansiaceae that differentiate them from the Entylomatales. There are also some microscopical characters that differentiate *D. callitrichis* from the *Entyloma* spp. on terrestrial plants. For example, probably as an adaptation to aquatic propagation (Ingold, 1979), *D. callitrichis* has in common with *R. nymphaeae*, but not with *E. microsporum*, the formation of long, narrow, fusiform or sigmoid basidiospores and yeast cells. In *Entyloma* spp. on terrestrial host plants, but not in *D. callitrichis* and *R. nymphaeae*, the basidiospores on the basidia usually fuse and occasionally form ballistoconidia.

Within the Doassansiaceae, *D. callitrichis* shares the lack of spore balls only with the Rhamphosphoraceae and the lack of haustoria with the Doassansiaceae (Bauer et al. 1997). Therefore, there are two possibilities of systematic interpretation of *D. callitrichis*. Either *D. callitrichis* represents a member of Rhamphosphoraceae without haustoria, or it represents a member of Doassansiaceae without spore balls.

In the molecular analyses, Entylomatales and Doassansiaceae form two clearly separated orders within the Exobasidiomycetidae, as already shown in Begerow et al. (1997). These two groups are well separated in the different analyses of this study as shown in Fig. 17. All three analyses show bootstrap values of 100% for the two orders Entylomatales and Doassansiaceae. Within the Entylomatales the arrangement of the dif-
Fig. 17. Topology resulting from neighbor-joining analysis of a 551 alignment. a. Neighbor joining analysis with bootstrap values of 1000 replicates. b. Maximum likelihood analysis with bootstrap values of 100 replicates. c. consensus tree of the three most parsimonious trees with bootstrap values of 1000 replicates. Distances are in terms of the number of expected changes per site. Values below 50% are not shown.

different species is not well supported statistically and also the distances are very short. Only a closer relationship of the two species on Asteraceae might be concluded. The separation of Rhamphosporaceae and Doassaniiaceae, as defined by Bauer et al. (1997) is also confirmed by the molecular analyses (Fig. 17). Doassina callitrichis is outside the spore ball producing species of Doassaniiaceae in all three analyses and never clustered with R. nymphaeae. Obviously, spore balls in the Doassaniiaceae are not the result of convergent evolution.

In general, the shown characters demonstrate that D. callitrichis is a member of the Doassaniiaceae sensu Bauer and Oberwinkler, occupying a position at the base of this group. However, it differs from the other species of Doassaniiaceae by the lack of spore balls. Therefore, a new genus is necessary to accommodate it.

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LITERATURE CITED


