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Molecular analyses confirm the relationship between *Stephanospora caroticolor* and *Lindtneria trachyspora*

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Abstract—The relationship between *Lindtneria trachyspora*, with resupinoid basidiomes, and *Stephanospora caroticolor*, with sequestrate basidiomes, is discussed. Analyses of the ITS1, ITS2 and 5.8 S ribosomal DNA sequences confirm the morphological and chemical affinities established by early authors.

Key words—*Stereales, Russulales, Stephanosporaceae*, *Lindtneriaceae*, rDNA

Introduction

The taxa included in the family *Stephanosporaceae* Oberw. & E. Horak show sequestrate habit. According to Kirk et al. (2001) and following Hibbett & Thorn (2001), this family is one of the 11 considered in the order *Russulales* Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David. The only species of this family in the Northern hemisphere, *Stephanospora caroticolor* (Berk.) Pat. (Pegler et al. 1993), is very easy to recognize by the bright orange basidiome, the evanescent peridium and the characteristic spores with a peri-appendicular corona.

In the family *Corticiaceae* Herter, all the taxa have a resupinate basidiome and are included in the order *Polyporales*. In agreement with Hjortstam (1987), Kirk et al. (2001) include the species of the genus *Lindtneria* Pilát in this family. During the last year, we have

studied different collections of *Lindtneria trachyspora* (Bourdot & Galzin) Pilát and *Stephanospora caroticolor*. Both taxa show the same basidiome colour and the spores have the same morphology. Based on microscopic and ultrastructural characters of both taxa (e.g. strongly sculptured spores), Oberwinkler & Horak (1979) proposed to include *Lindtneria* Pilát and *Stephanospora* Pat. in the same family (*Stephanosporaceae* Oberwinkler & Horak). The close relationship between both genera was also accepted in Jülich (1981), but he proposed the monogeneric family *Lindtneriaceae* Jülich.

The main purpose of this study was to know if molecular data support the hypothesis that *L. trachyspora* and *S. caroticolor* are closely related species.

Material and Methods

MATERIAL EXAMINED—All material is deposited in the herbarium of the Real Jardín Botánico (Madrid, Spain). *Lindtneria trachyspora*.- Germany, Bavaria, Oberbayern, district Bad Tölz-Wolfratshausen, in the valley of the river Isar between Vorderriß and Wallgau, on decayed wood lying on the riverbank, 47 33´north and 11 24´west, 795 m elev., 1/X/1997, leg. et det. S. Raidl (SR413, MA-Fungi 47773); *idem,* 19/X/1998 (SR702, MA-Fungi 47774); *idem*, 22/X/1999 (SR907, MA-Fungi 47775). *Stephanospora caroticolor:* Germany, Bavaria, Oberbayern, district Bad Tölz-Wolfratshausen mixed forest near the Pupplinger Au near Wolfratshausen, 10/X/1999, leg. Prof. W. Steglich (MA-Fungi 47684).

MOLECULAR METHODS—A small quantity (less that 10 mg) of each collection was subjected to molecular analysis of the internal transcribed spacer regions of rDNA (ITS1 and ITS2), including the 5.8S. Total DNA was isolated using E.Z.N.A. Fungal MiniPrep Kit (Omega-Biotech, Doraville, USA) as described in Martín & García-Figueres (1999). Primer pair ITS1F and ITS4 was used to obtain amplifications of both ITS regions, including the 5.8S of the ribosomal RNA gene cluster and small flanking parts of the SSU and LSU genes; primers were described in White et al. (1990). Amplifications were done using Ready-to-Go® PCR Beads (Amersham-Biosciences, UK) as mentioned in Winka et al. (1998). Results of amplifications were assayed from 5µl aliquots by gel electrophoresis of 2% Pronadisa D-1 Agarose (Lab. Conda, Spain). Amplification products were cleaned using the E.Z.N.A. Clean kit (Omega Biotech, USA) and both strands were sequenced separately using primers ITS1F and ITS4 at the Authomatic Sequencing Service (CIB-CSIC, Madrid). Sequence Navigator ™ Sequence Comparison software (Perkin Elmer, USA) was used to identify the consensus sequence from the two strands of each ITS region. The new sequences have been logged in the EMBL database with

the Accession Numbers AJ419224 (*Stephanospora caroticolor* MA-Fungi 47686) and AJ419225 (*Lindtneria trachyspora* MA-Fungi 47774). SEQAPP software for multiple sequences was used to compare these sequences. Alignment gaps were marked.

Results and Discussion

MORPHOLOGY—As shown in Fig. 1, the spores in both taxa are globose, spinulose with peri-appendicular corona. *Lindtneria trachyspora* is the only species of this genus in Europe with globose and spinulose spores. Spores of the other two species mentioned in Ryvarden & Gilbertson (1993), *L. leucobryophila* (Henn.) Jülich and *L. flava* Parm., are ellipsoid and warted. Hjortstam (1987) included in his key, a species, *L. pterospora* Reid, with globose spores, but prominent wing-like crests. Oberwinkler & Horak (1979) consider that from the microscopic and ultrastructural point of view *Lindtneria trachyspora* and *Stephanospora caroticolor* are practically indistinguishable.

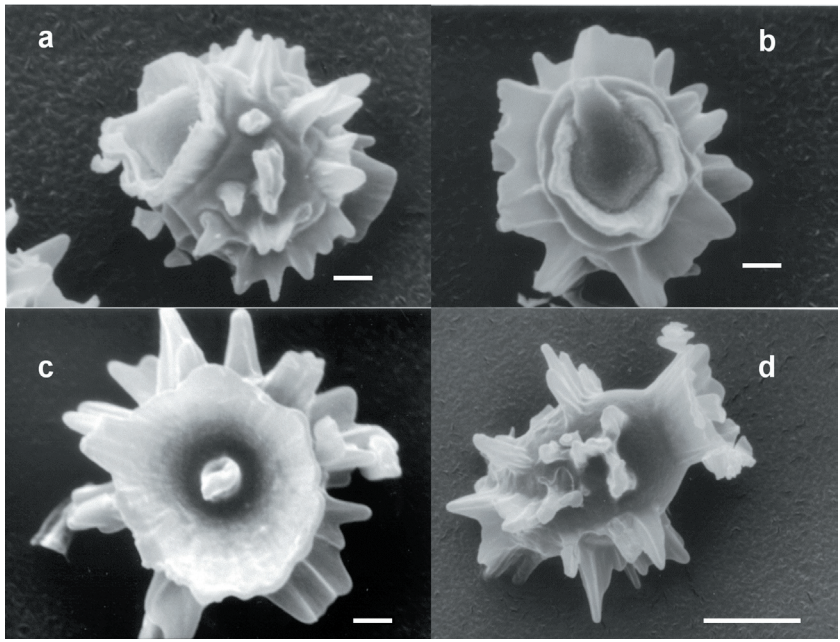

Fig. 1 Spores: a) *Lindtneria trachyspora* MA-Fungi 47773; b) *L. trachyspora* MA-Fungi 47774; c) *L. trachyspora* MA-Fungi 47775; d) *Stephanospora caroticolor* MA-Fungi 47686. (Bar figs 1-3= 1 μm; Bar fig. 4= 5 μm).

MOLECULAR ANALYSIS—The total aligned sequences ITS1-1 and ITS-2, including the 5.8S nrDNA, were 739 base pairs (bp) long, without ambiguous areas (Fig.2). The nucleotide differences among all pairwise comparisons was 37 bp in ITS1 and 31 in ITS2; there were not differences in the 5.8S rDNA gene. In ITS1 15 differences are due to deletions, 16 to transitions and 6 to transversions. In ITS2 the differences are quite similar: 16 deletions, 10 transitions and 5 transversions.

Recently, Steglich and collaborators from the Department Chemie der Universität München (Munich, Germany) have identified and isolated two pigments that give the bright orange colour to the peridium and gleba of *Stephanospora caroticolor* (Hellwig 1999, Lang et al. 2001): stephanosporin (young basidiomes) and 2-chloro-4-nitrophenol (mature basidiomes). From the methanol extractions other compounds were identified: 4-hydroxy-acetanilide (paracetamol), 4-amino-2-chlorophenol and 4-nitrophenol. These authors have found 4-nitrophenol and traces of 2-chloro-4-nitrophenol in the resupinate basidiomes of *Lindtneria trachyspora*. The oxidative transformation of stephanosporin into 2-chloro-4-nitrophenol (fungicide) is a complex enzymatic process. In agreement with Lang et al. (2001), the presence of this compound in *L. trachyspora* and *S. caroticolor* supports the inclusion of both taxa into the same family by Oberwinkler & Horak (1979). The ITS rDNA is highly variable in fungi, even among closely related taxa. Kretzer & Bruns (1997) investigated the secotioid genus *Gastrosuillus* Thiers to clarify its relationships with *Suillus* Mich. ex Gray. The phylogenetic analysis of the unambiguous sites of the alignment allowed them to transfer all known *Gastrosuillus* species to *Suillus*. Ko, Hong & Jung (1997) found that the sequences obtained from eight species of *Trichaptum* Murrill were very variable (e.g. from the 460 ITS1 positions, only 101 were optimally aligned). The alignment among our isolates has no unambiguous parts, and the sequences dissimilarities are not very high (ITS1: 37 positions: ITS2: 31 positions). However, more studies including chemical and molecular methods should be done in the other species of *Lindtneria* and *Stephanospora* to conclude that both genera belong to the same family.

The linkages between sequestrate fungi and epigeal basidiomycetes have been reported many times in the literature (Malençon 1931, Singer 1975). Molecular analyses, mainly from rDNA, give additional evidence of relationship between agarioid/boletoid and sequestrate fungi

(Hibbett et al. 1997). In particular, in the order *Boletales* (Bruns et al. 1989, Baura et al. 1992, Kretzer et al. 1996, Kretzer & Bruns 1997, Johanesson & Martín 1999), *Cortinariales* (Martín & Rocabruna 1999, Martín & Moreno 2001) and *Russulales* (Martín et al. 1999, Calonge & Martín 2000, Miller et al. 2001) these affinities have been established.

In our previous studies in *Cortinariales* (Martín & Rocabruna 1999, Martín & Moreno, 2001) based on morphological, ecological and molecular data, we conclude that the agaricoid basidiomes of *Setchelliogaster rheophyllus* (Bertault & Malençon) Malençon & Moreno (= *Naucoria rheophylla* Bertault & Malençon) and the gasteroid basidiomes of *Setchelliogaster tenuipes* (Setchell) Pouzar (=*Secotium tenuipes* Setchell) are two morphotypes of the same species. Köljalg et al. (1998) in their database of complete ITS and part of the LSU DNA included more than 40 tomentelloid and thelephoric species: parsimony and distance analyses confirmed that a *Tomentella radiosa* (P. Karst.) Rick with a resupinoid basidiome is the same species as *Thelephora terrestris* Ehrh.: Fr.

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         *       *   *** *
TRALIN_2  CATTATCGAATCGTTGAAAC TTGGCTGTCGCTGGCTTTCT CCTTCCTGGGGATCGCATGT
CARSTE_1  CATTATCGAATCGTTAAAAC TTGGCTGTCGCTGGCTCTCT CCTTCTCGAG-ATTGCATGT

               ** !!               *                                !
TRALIN_2  GCACGCCCATGTTCAAACTT TTCATTTTAACCTCCTGTGC ACTTTTGTGGATCGTGGATG
CARSTE_1  GCACGCTTAGTTTCAAACTT TTCATTCTAACCTCCTGTGC ACTTTTGTGGATCGTGGAAG

                                                   !                !
TRALIN_2  GATAACACTT-GTCAAAGTA ACATTTGGTTTTGGGGATCG AGAGTCTGCTTTCCCCTACT
CARSTE_1  GATAA-ACTTTGTCAAAGTA ACATTTGGTTTTGGGGATCG C-AGTCTGCTTTCCCCTACA

                    * *                                  *          *
TRALIN_2  ATTTGTCCA-CGGTTCATGT CTTTTTA-CTA--CAAACCA TTAAACAAAAA----CCTAG
CARSTE_1  ATTTGTCCAATGATTCATGT CTTTTTAACTATACAAACCA TTAAATAAAAAATAACTTAG

              *  !  *                                  !*
TRALIN_2  AATGTTTAACGAGTTGGGGA CTTTAGTGACCCTCTCTTAA AAATTTATACAACTTTCAAC
CARSTE_1  AATGCTTTACAAGTTGGG-A CTTTAGTGACCCT-TCTTAA AATCTTATACAACTTTCAAC


TRALIN_2  AACGGATCTCTTGGCTCTCG CATCGATGAAGAACGCAGCG AAATGCGATAAGTAATGTGA
CARSTE_1  AACGGATCTCTTGGCTCTCG CATCGATGAAGAACGCAGCG AAATGCGATAAGTAATGTGA

TRALIN_2  ATTGCAGATTTCAGTGAATC ATCGAATCTTTGAACGCACC TTGCACCCTGTGGTATTCCA
CARSTE_1  ATTGCAGATTTCAGTGAATC ATCGAATCTTTGAACGCACC TTGCACCCTGTGGTATTCCA

                                       !
TRALIN_2  CAGGGTATGCCCGTTTGAGT ATCATTAATTCATCAACTCC AAAACCTTTGTGTTCTTGGT
CARSTE_1  CAGGGTATGCCCGTTTGAGT ATCATTAAGTCATCAACTCC AAAACCTTTGTGTTCTTGGT

           *                                * *   *
TRALIN_2  GTTTGGTCT--GAGGGTCAT TGCAGGCTCTTTGTAAAGCC -GGCTCCCTTTAAATCCATT
CARSTE_1  GCTTGGTCTTTGAGGGTCAT TGCAGGCTCTTTATGA-GTC AGGCTCCCTTTAAATCCATT

                 *                 !      *                 *        !
TRALIN_2  AGCGAGATTTCCCTGTGCTG AACTAGTCTCTCGACGTGAT AGTCTATCTGCGTCAAATGT
CARSTE_1  AGCGAGACTTCCCTGTGCTG AACTTGTCTCTTGACGTGAT AGTCTATCTACGTCAAATTT

          !                        !                          !
TRALIN_2  AGAGTTCTCGTTGGGCTTGC TTCAAATCCGTCCTCTTTGG ACAATCGGAATAAGAGTTGC
CARSTE_1  TGAGTTCTCGTTGGGCTTGC TTCTAATC-GTCCTCTTTGG ACAATCGGAATTAGAGT-GC

           *         *               * !
TRALIN_2  ACATATCTGTGTGCATCCCT CTTTCCAGAACCATTTTGAT CTCAAATCGGGTAGGACTAC
CARSTE_1  ATATAT----GCG---CCCT -TTTC-AAATCCATTTTGAT CTCAAATCGGGTAGGACTAC

TRALIN_2  CCGCTGAACTTAAGCATAT
CARSTE_1  CCGCTGAACTTAAGCATAT
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Fig. 2 Alignment of the ITS1 and ITS2 sequences, including the 5.8 S rDNA of *Lindtneria trachyspora* (TRALIN-2, AJ419225) and *Stephanospora caroticolor* (CARSTE-1, AJ419224) (*: transitions; !: transversions).

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