

# Phylogenetic relationships of human-pathogenic *Cladosporium* (*Xylohypha*) species inferred from partial LS rRNA sequences

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The controversy about the appropriate taxonomic placement of agents of subcutaneous and systemic mycoses in either *Cladosporium* or *Xylohypha*, both genera characterized by conidia being produced in dry, acropetal chains, was addressed with partial sequencing of LS ribosomal RNA. Observation of catenate anamorphs in species of *Capronia* (Ascomycotina, Herpotrichiellaceae), a genus which also has anamorphs in *Exophiala*, suggested the possibility of a close interrelationship of all human-associated taxa. To test this hypothesis, partial sequences of 43 strains of *Cladosporium*/*Xylohypha* were analysed. Human-pathogenic and saprophytic *Cladosporium* species were found to be phylogenetically distinct from each other and, on the basis of known teleomorph relationships, were considered to be anamorphs of Herpotrichiellaceae and Mycosphaerellaceae, respectively. They should therefore be classified in different anamorph-genera; *Cladosporium* being restricted to plant-associated species. A relatively large proportion of the Herpotrichiellaceae is presumed to be animal-associated. The black yeast genus *Exophiala* was also confirmed to be of herpotrichiellaceous relationship. The genus *Xylohypha* is unrelated.

## Introduction

Species comprising the hyphomycete genus *Cladosporium* Link:Fr. have more or less differentiated conidiophores bearing branched, acropetally elongating chains of dry conidia. Conidia are usually one-celled; ramo-conidia sometimes have one or a few septa. Conidia are ornamented or appear smooth-walled with light-microscopy. Yeast cells are absent.

Since the first revision of the genus by de Vries [1] approximately 40 species have been recognized in the literature [2,3]. Von Arx [4], in his study on the cladosporeid anamorphs of the genus *Mycosphaerella* Johanson recognized four ecological groups. The generic type species, *Cladosporium herbarum* (Pers.:Fr.) Link, is a member of a small group (1) of very common ubiquitous saprophytes. A few species (group 2) are found as mycoparasites. Numerous species (group 3) are plant-

pathogens, usually with a restricted host range. Group 4 contains human-pathogens.

The fourth group can be distinguished morphologically from the saprophytes in the *C. herbarum* group because they lack differentiated conidiophores and have unthickened, pale pigmented conidial scars. Von Arx [4] suggested that they were related to the genus *Fonsecaea* Negroni, which contains aetiologic agents of human chromoblastomycosis, but in the absence of teleomorphs no firm conclusion could be drawn.

The teleomorph of the generic type species of *Cladosporium*, *C. herbarum*, is *Mycosphaerella tassiana* (De Not.) Johanson (Mycosphaerellaceae [5]). Von Arx [6] segregated the genus *Hormoconis* v. Arx & de Vries, based on *Cladosporium resiniae* (Lindau) de Vries, from *Cladosporium* because its teleomorph, *Amorphotheca resiniae* Parbery, has amorphous ascoma walls and is classified in the family Amorphothecaceae. The *Hormoconis* anamorph differs from typical *Cladosporium* species by, for example, the absence of darkened conidial scars.

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Müller *et al.* [7] described a *Cladosporium*-like anamorph with undifferentiated conidiophores in *Capronia pilosella* (P. Karst.) E. Müller *et al.* (Herpotrichiellaceae).

The present article assesses the affinities of the human-pathogenic *Cladosporium* species to either Mycosphaerellaceae or Herpotrichiellaceae. Relationships are established by phylogenetic analysis based on partial sequencing of LS rRNA. Where currently accepted species have identical sequences in this gene region, their identity is confirmed by means of DNA/DNA reassociation for entire genomic comparison.

## Materials and methods

### Strains and culture conditions

The strains studied (Table 1) were grown at 28 °C in 100 ml of a liquid medium (1% glucose, 0.5% peptone, 0.3% malt extract and 0.3% yeast extract) on a rotary shaker at 150 rev min<sup>-1</sup> for 48–96 h, depending on the species. Mycelial pellets were harvested by filtration, washed twice with sterile distilled water and stored at –20 °C after lyophilization.

### Extraction of ribosomal RNA and comparison of sequences

Bulk rRNA was extracted from lyophilized pellets using the micromethod described by Guadet *et al.* [8]. rRNA was purified by a final precipitation in 4 M LiCl to eliminate contaminating double-stranded DNAs. For species from which DNA is isolated with difficulty, such as saprophytic cladosporia, an alternative method of purification was used [9]. One per cent (w:v) Macaloid was added to the lysis solution and purification was carried out with deproteinization four times using Tris-saturated phenol containing 0.1% hydroxyquinoline. Purity of rRNA samples was estimated from spectrophotometric absorbance ratios 260/280 = 2.00–2.15 and 230/260 ≤ 0.5, and their integrity verified by non-denaturing agarose gel electrophoresis. Sequences of 577 nucleotides were obtained by the dideoxynucleotide chain-termination method [10], using bulk rRNA as a template with reverse transcriptase and subsequently modified by incorporation of [<sup>35</sup>S] labelled-dATPaS in a short preliminary elongation step. Sequences were extended by chaining up partial sequences targeted in the bulk rRNA by three oligonucleotide primers complementary to evolutionary conserved regions, 5'-GCATTCCCAAACAACACTCGACTC-3' (266), 5'-TCCCTTTCAACAATTTACAG-3' (401) and 5'-GGTCCGTGTTTCAAGACGG-3' (636). Numbers between brackets indicate the first base position copied with reference to the LS rRNA primary structure of *Saccharomyces cerevisiae* [11].

### Sequence alignment and phylogenetic analysis

Alignment was performed on 43 LS rRNA partial sequences of 577 bases, starting at position 57 of the 5' end, up to position 624, with reference to *Saccharomyces cerevisiae*. Editing, alignment and storage of sequences were carried out using the VSM package version (program developed for PC-compatible computer by R.C.). Ambiguously aligned positions were excluded from analyses. Phylogenetic trees were constructed using three different algorithms. For example, for the neighbour-joining method [12] the algorithm of the VSM package version 2.0 was used. Trees were also obtained for the same data using maximum parsimony analysis with the heuristic search option (PAUP version 3.0) [13]. All trees were inferred using the 'branch and bound' options in attempting to find the most parsimonious tree. When several most parsimonious trees were found, the 100% consensus tree was used as the most parsimonious tree. A bootstrap analysis was performed (heuristic option) to check each topology for robustness [14]. One hundred bootstrap replications were compared. Results were also obtained with the maximum likelihood program rewritten by G. J. Olsen (University of Urbana, IL). Only topologies found to be similar by all three methods were retained.

### DNA analysis

Nuclear DNA was extracted from wet cells harvested after 4 days of growth at 28 °C in 11 shaken broth described above or Sabouraud's broth. DNA was purified by the method of Guého *et al.* [15]. Guanine plus cytosine (GC) content of the DNAs in 0.1 × SSC was determined by thermodenaturation using a Gilford Response II spectrophotometer and the formula %GC = 2.44 (T<sub>m</sub> – 69.4) [16]. The DNA of *Escherichia coli* K-12 with T<sub>m</sub> at 90.5 °C in 1 × SSC was used as a control. DNA/DNA reassociation experiments were carried out by means of the same equipment with the optical technique. The low values of DNA/DNA reassociations were estimated with the formula:

$$D = 100 \times \frac{4vm - (vA + vB)}{2\sqrt{vAvB}}$$

in which  $D$  = per cent reassociation value,  $v$  = value of the slope,  $m$  = mixture of strains  $A$  and  $B$  [16].

## Results

Alignment of sequences of the strains studied is presented in Fig. 1. In the phylogenetic tree two distinct groups (I and II) could be recognized (Fig. 2). Group I contained all human-pathogenic *Cladosporium* species and species of the black yeast genus *Exophiala* Carmichael. Group II

**Table 1** List of strains studied with their sources of isolation

Species	Number	Source
<i>Aureobasidium pullulans</i> (De Bary) Arnaud	CBS 105-22 <sup>T</sup>	M. B. Church, unprecised
<i>Capronia mansonii</i> (Schol-Schwarz) E. Müller <i>et al.</i>	CBS 101-67	wood of <i>Populus tremula</i>
<i>Capronia pilosella</i> (Karsten) E. Müller <i>et al.</i>	CBS 125-88	decaying wood
<i>Cladophialophora ajelloi</i> Borelli	CBS 260-83 <sup>T</sup>	human skin lesion
<i>Cladophialophora ajelloi</i> Borelli	CBS 259-83	human skin lesion
<i>Cladophialophora arxii</i> Tintelnot	CBS 306-94	human tracheal tumour
<i>Cladosporium carrionii</i> Trejos	CBS 160-54 <sup>AUT</sup>	human chromoblastomycosis
<i>Cladosporium carrionii</i> Trejos	CBS 164-54	human chromoblastomycosis
<i>Cladosporium carrionii</i> Trejos	CBS 362-70	human chromoblastomycosis
<i>Cladosporium carrionii</i> Trejos	CBS 454-82	contaminant
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	CBS 170-54 <sup>NT</sup>	leaf of <i>Arundo</i> sp.
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	RV 68106	human skin lesion
<i>Cladosporium devriesii</i> Padhye & Ajello	CBS 147-84 <sup>T</sup>	human mycotic granuloma
<i>Cladosporium elatum</i> (Harz) Nannf.	CBS 146-33 <sup>T</sup>	wood pulp
<i>Cladosporium herbarum</i> (Pers.:Fr.) Link	CBS 399-80	human skin lesion
<i>Cladosporium macrocarpum</i> Preuss	CBS 175-62	grain of <i>Hordeum vulgare</i>
<i>Cladosporium sphaerospermum</i> Penzig	CBS 122-47	decaying stem of <i>Begonia</i>
<i>Cladosporium trichoides</i> Emmons	CBS 173-52 <sup>T</sup>	human brain abscess
<i>Cladosporium trichoides</i> Emmons	CBS 328-65	dog liver abscess
<i>Cladosporium trichoides</i> Emmons	CBS 364-80	cat brain abscess
<i>Cladosporium trichoides</i> Emmons	IP 2246-94	human brain abscess
<i>Cladosporium trichoides</i> var. <i>chlamydosporum</i> Kwon-Chung	CBS 564-82	human brain abscess
<i>Exophiala dermatitidis</i> (Kano) de Hoog	CBS 207-35 <sup>T</sup>	human chromoblastomycosis
<i>Exophiala dermatitidis</i> (Kano) de Hoog	CBS 153-94	human liver abscess
<i>Exophiala jeanselmei</i> (Langeron) McGinnis & Padhye	CBS 528-76	human skin lesion
<i>Exophiala jeanselmei</i> var. <i>lecanii-corni</i> (Benedek & Specht) de Hoog <i>et al.</i>	CBS 232-39	human skin lesion
<i>Exophiala pisciphila</i> McGinnis & Ajello	CBS 537-73 <sup>T</sup>	<i>Ictalurus punctatus</i>
<i>Fonsecaea pedrosoi</i> (Brumpt) Negrone	CBS 271-37 <sup>NT</sup>	human
<i>Fonsecaea pedrosoi</i> (Brumpt) Negrone	IP 2247-94	human chromoblastomycosis
<i>Hormoconis resiniae</i> (Lindau) v. Arx & de Vries	CBS 406-68 <sup>T</sup>	soil
<i>Hortaea werneckii</i> (Horta) Nishimura & Miyaji	CBS 107-67 <sup>NT</sup>	human tinea nigra
<i>Mycosphaerella tassiana</i> (De Not.) Johanson	CBS 111-82	<i>Arctostaphylos uva-ursi</i>
<i>Ochronis gallopavum</i> (W. B. Cooke) de Hoog	CBS 437-64 <sup>T</sup>	<i>Meleagris gallopavo</i> , brain lesion
<i>Phialophora parasitica</i> Ajello <i>et al.</i>	CBS 860-73 <sup>T</sup>	human phaeohyphomycosis
<i>Phialophora verrucosa</i> Medlar	CBS 286-47	human
<i>Ramichloridium mackenziei</i> Campbell & Al-Hedaithy	CBS 650-93	human brain abscess
<i>Rhinoctadiella atrovirens</i> Nannf.	CBS 317-33 <sup>AUT</sup>	pine wood
<i>Taeniolella alta</i> (Ehrenb.:Fr.) Hughes	CBS 488-80	wood of <i>Carpinus betulus</i>
<i>Taeniolella boppii</i> Borelli	CBS 126-86 <sup>T</sup>	human skin lesion
<i>Taeniolella exilis</i> (Karst.) Hughes	IP 2199-93	human skin lesion
<i>Xylohypha emmonsii</i> Padhye <i>et al.</i>	CBS 678-79 <sup>T</sup>	cat skin lesion
<i>Xylohypha ferruginosa</i> (Corda) Hughes	CBS 619-73	decaying wood
<i>Xylohypha nigrescens</i> (Pers.:Fr.) Mason	CBS 909-70	wood of <i>Fraxinus</i> sp.

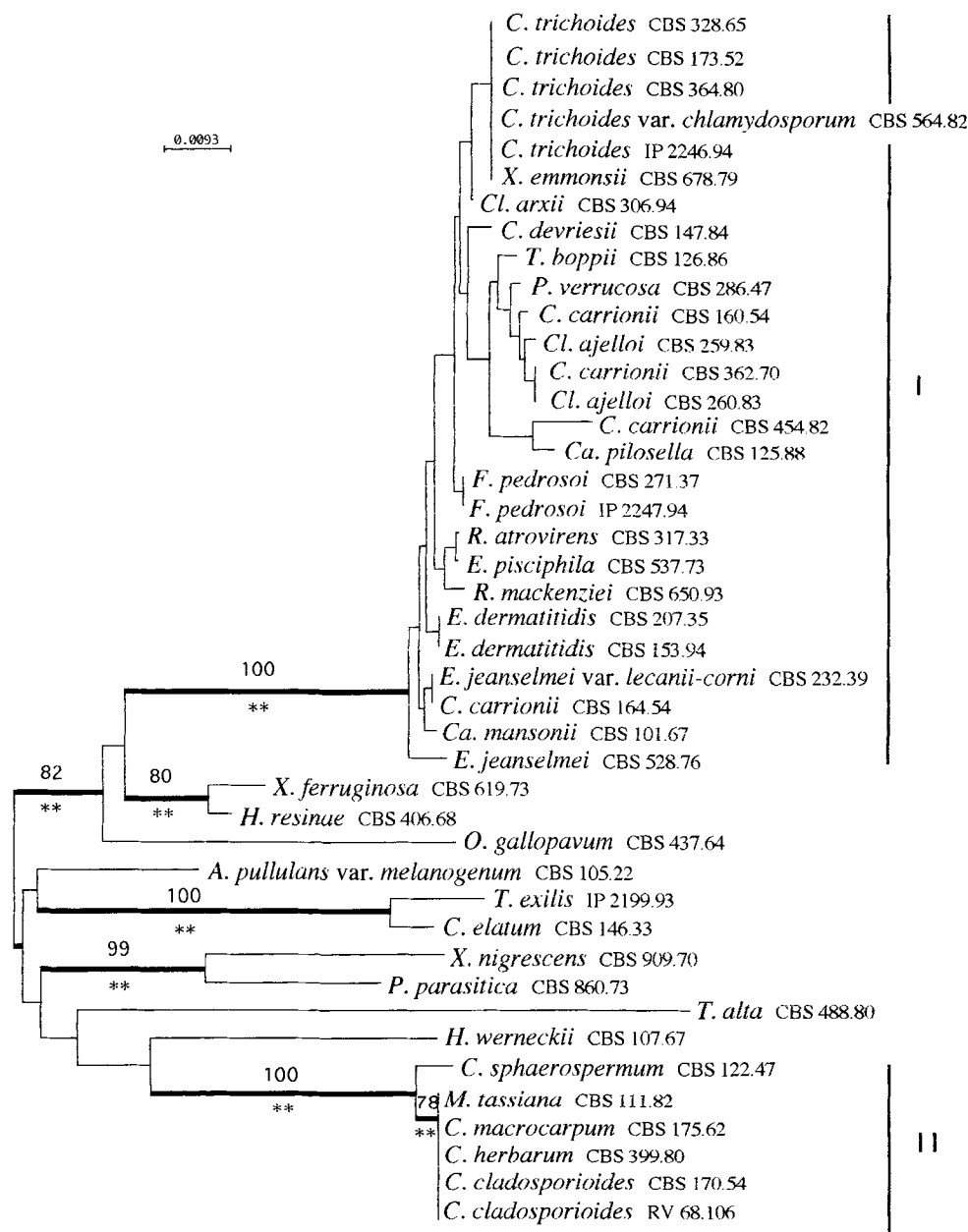
CBS = Centraalbureau voor Schimmelcultures, Baarn, the Netherlands; IP = Pasteur Institute, Paris, France; RV = Institut of Tropical Medecine, Antwerp, Belgium; T = type culture; NT = neotype culture; AUT = authentic culture.

	1 (57)	11	21	31	41	51	61	71	81	91
C. trichoi	AGGAAAAGAA	ACCAACAGGG	AUUGCCUCAG	UAACGGCGAG	UGAAGCGGCA	ACAGCUCAAA	UUUGAAAUCU	GGCCUCUU**	GGGGUCCGAG	CCGUAAUUUG
Cl. arxii	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C. devries	-----	-----	-----	-----	-----	-----	-----	-----	-----	UN-----
T. boppii	-----	-----	-----	-----	-----	-----	-----	-----	-----	NN-----
P. verruco	-----	-----	-----	-----	-----	-----	-----	-----	-----	UU-----
C. carrion	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Cl. ajello	-----	-----	-----	-----	-----	-----	-----	-----	-----	N-----
Cl. ajello	-----	-----	-----	-----	-----	-----	-----	-----	-----	N-----
C. carrion	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Ca. pilose	-----	-----	-----	-----	-----	-----	-----	-----	-----	NN-----
F. pedroso	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
R. atrovir	-----	-----	-----	-----	-----	-----	-----	-----	-----	UN-----
E. pisciph	-----	-----	-----	-----	-----	-----	-----	-----	-----	N-----
R. mackenz	-----	-----	-----	-----	-----	-----	-----	-----	-----	N-----
E. dermati	-----	-----	-----	-----	-----	-----	-----	-----	-----	NN-----
E. lecanii	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Ca. manson	-----	-----	-----	-----	-----	-----	-----	-----	-----	NN-----
E. jeansel	-----	-----	-----	-----	-----	-----	-----	-----	-----	UN-----
X. ferrugl	-----	-----	-----	-----	-----	-----	-----	-----	-----	UU-----
H. resiniae	-----	-----	-----	-----	-----	-----	-----	-----	-----	UU-----
O. gallopa	-----	-----	-----	-----	-----	-----	-----	-----	-----	UU-----
A. pullula	-----	-----	-----	-----	-----	-----	-----	-----	-----	UU-----
T. exilis	-----	-----	-----	-----	-----	-----	-----	-----	-----	UU-----
C. elatum	-----	-----	-----	-----	-----	-----	-----	-----	-----	UU-----
X. nigresc	-----	-----	-----	-----	-----	-----	-----	-----	-----	UU-----
P. parasit	-----	-----	-----	-----	-----	-----	-----	-----	-----	UU-----
T. alta	-----	-----	-----	-----	-----	-----	-----	-----	-----	UU-----
H. werneck	-----	-----	-----	-----	-----	-----	-----	-----	-----	UU-----
C. sphaero	-----	-----	-----	-----	-----	-----	-----	-----	-----	UU-----
C. herbaru	-----	-----	-----	-----	-----	-----	-----	-----	-----	UU-----
C. trichoi	UAGAGGAUGU	UUCGGG*UAC	CGCCUCGGUU	UAAAUUUUU	GGAAACAGAAU	GUCAGAGAGG	GUGAGAAUCC	CGUCUUGAAC	CGGGUG*UAG	GGCCUAUGUC
Cl. arxii	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C. devries	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
T. boppii	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
P. verruco	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C. carrion	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Cl. ajello	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Cl. ajello	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C. carrion	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Ca. pilose	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
F. pedroso	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
R. atrovir	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
E. pisciph	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
R. mackenz	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
E. dermati	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
E. lecanii	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Ca. manson	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
E. jeansel	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
X. ferrugl	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
H. resiniae	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
O. gallopa	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
A. pullula	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
T. exilis	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C. elatum	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
X. nigresc	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
P. parasit	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
T. alta	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
H. werneck	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C. sphaero	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C. herbaru	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C. trichoi	AAACUCCUUC	GACGAGUCGA	GUUGUUUGGG	AAUGCAGCUC	CAAAUGGGUG	GUAAAUUUCA	UCUAAAGCUA	AAUAUUGGCC	AGAGACCGAU	AGCGCACAAAG
Cl. arxii	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C. devries	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
T. boppii	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
P. verruco	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C. carrion	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Cl. ajello	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Cl. ajello	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C. carrion	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Ca. pilose	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
F. pedroso	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
R. atrovir	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
E. pisciph	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
R. mackenz	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
E. dermati	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
E. lecanii	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Ca. manson	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
E. jeansel	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
X. ferrugl	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
H. resiniae	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
O. gallopa	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
A. pullula	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
T. exilis	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C. elatum	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
X. nigresc	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
P. parasit	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
T. alta	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
H. werneck	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C. sphaero	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
C. herbaru	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

Fig. 1

	301	311	321	331	341	351	361	371	381	391
<i>C. trichoi</i>	UAGAGUGAUC	GAAAGAUGAA	AAGCACUUUG	AAAAGAGAGU	UAAACAGAUU	GUGAAAUUGU	UGAAACGGAA	GCGCUUACAA	CCAGACUUGA	CCCGCGGGU
<i>Cl. arxil</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>C. devries</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>T. boppi</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>P. verruco</i>	-----	-----	-----A	-----	-----	-----	-----	-----	-----	-----A
<i>C. carrion</i>	-----	-----	-----A	-----	-----	-----	-----	-----	-----	-----A
<i>Cl. ajello</i>	-----	-----	-----A	-----	-----	-----	-----	-----	-----	-----A
<i>Cl. ajello</i>	-----	-----	-----A	-----	-----	-----	-----	-----	-----	-----A
<i>C. carrion</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----C	-----
<i>Ca. pilose</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----U	-----A-U
<i>F. pedroso</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>R. atrovir</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>E. pisciph</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----C
<i>R. mackenz</i>	-----	-----	-----	-----A	-----	-----	-----GG	-----	-----	-----AU
<i>E. dermati</i>	-----	-----	-----	-----	-----C	-----	-----	-----	-----	-----C
<i>E. lecanii</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----A
<i>Ca. manson</i>	-----	-----	-----	-----	-----C	-----	-----	-----	-----	-----C
<i>E. jeansel</i>	-----	-----	-----A	-----	-----	-----	-----	-----GG	-----	-----
<i>X. ferrugi</i>	-----	-----	-----	G-----	-----N	-----	-----	-----	-----	C A--C-UC-A
<i>H. resiniae</i>	-----	-----	-----	G-----	-----C	-----	-----	-----	-----	-----UC-A
<i>O. gallopa</i>	-----	-----	-----	G-----	C-----C	-----	-----	-----DA-GUGG	G-----	A--U-GAU
<i>A. pullula</i>	-----	-----	-----	G-----	A-----C	-----	-----	-----	-----U	UUUAAA-U
<i>T. exillis</i>	-----	-----	-----	G-----	C-----C	-----	-----	-----CG--*--G	-----	UU--A-UU-C
<i>C. elatum</i>	-----	-----	-----	G-----	C-U-C-C	-----	-----	-----	-----	CU-UA-UU-C
<i>X. nigresc</i>	-----	-----	-----	G-----	U-C-C	-----	-----	-----U--U--G	-----	CU-UA-UU-A
<i>P. parasit</i>	-----	-----	-----C	-----G	C-C-C	-----	-----	-----G--CC-UG	-----	U--NN--A
<i>T. alta</i>	-----	-----G	-----	-----	A-C-C	-----	-----	-----U--G--G	-----	C U--UU
<i>H. werneck</i>	-----	-----	-----	G-----	A-C-C	-----C	-----	-----U-ACG-G	-----	U--UU
<i>C. sphaero</i>	-----	-----	-----	G-----	A-C-C	-----	-----	-----G-A-G	-----	C U--UU
<i>C. herbaru</i>	-----	-----	-----	G-----	A-C-C	-----	-----A	-----G-A-G	-----	C U--UU
<i>C. trichoi</i>	UCCCCUUGC	UUCUGCUUG	GUUACUCCGC	CGUGUCCAGG	CCAACAUCGG	UUCUGGGGGU	CGGUCAAAGG	CCCUGGGAUU	GUAUCUACC	AC*****GGG
<i>Cl. arxil</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>C. devries</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>T. boppi</i>	-----AGC	-----U-GC	-----C-----U	-----	-----	-----A-----	-----U-----	-----	-----	-----C
<i>P. verruco</i>	-----UAGC	-----U-GC-A	-----U-----	-----	-----	-----A-----	-----C-----	-----C-----	-----	-----
<i>C. carrion</i>	-----UAGC	-----U-GC-A	-----U-----	-----	-----	-----A-----	-----U-----	-----C-----	-----	-----
<i>Cl. ajello</i>	-----*GAGC	-----U-GC-C	-----U-----	-----	-----	-----A-----	-----U-----	-----C-----	-----	-----
<i>Cl. ajello</i>	-----UAGC	-----U-GC-A	-----U-----	-----	-----	-----A-----	-----U-----	-----C-----	-----	-----
<i>C. carrion</i>	-----U-GCGU	-----GCGC	-----G-----U	-----	-----	-----C-----	-----A-U-----	-----G-----	-----C-----	-----C
<i>Ca. pilose</i>	-----UCCG	-----U-GC-A	-----G-----C	-----	-----	-----U-----	-----A-U-----	-----U-----	-----C-----	-----
<i>F. pedroso</i>	-----	-----	-----	-----	-----	-----	-----	-----U-----	-----	-----C
<i>R. atrovir</i>	-----C-----	-----G-----	-----C-----	-----C-----	-----	-----	-----U-----	-----UG-----	-----	CGG-----C
<i>E. pisciph</i>	-----C-----	-----U-G-----	-----C-----	-----U-----	-----	-----	-----U-----	-----A-----	-----	CGG-----C
<i>R. mackenz</i>	-----C-----	-----G-----	-----U-----	-----U-----	-----	-----	-----U-----	-----N-G-----	-----U-----	CGG-----C
<i>E. dermati</i>	-----C-----	-----U-G-----	-----U-----	-----U-----	-----	-----	-----U-----	-----UG-----	-----	CGG-----C
<i>E. lecanii</i>	-----C-----	-----U-G-----	-----C-----	-----U-----	-----	-----	-----U-----	-----G-C-----	-----	CGG-----C
<i>Ca. manson</i>	-----NN-----	-----C-----	-----U-----	-----N-----	-----	-----	-----U-----	-----G-----	-----	CGG-----C
<i>E. jeansel</i>	-----C-----	-----U-G-----	-----U-----	-----U-----	-----	-----	-----U-----	-----G-----	-----	CGG-----C
<i>X. ferrugi</i>	-----AU-UCA-G	-----C-G-----	-----NGC-----G-----	-----G-N-----U-----	-----G-----	-----UC-----U-----	-----U-----AU-----A-----	-----U-----	-----	CGG-----C
<i>H. resiniae</i>	-----AU-GG--*	-----CACC-----	-----NGC-----NG-----	-----NNN-----CU-----	-----G-----	-----CUG-----U-----	-----U-----AU-----	-----U-----	-----	CGG-----C
<i>O. gallopa</i>	-----C-AGG-GCN	-----GCG-----	-----UG-----UUC-----	-----A-C-GA-----	-----G-----	-----A-----C-----	-----A-----A-----	-----GAA-----	-----	CGG-----C
<i>A. pullula</i>	-----GG-GG-CU	-----CUGA-CG-----	-----U-----A-U-----	-----U*-----GA-----	-----G-----A-----	-----UC-----C-----	-----A-----U-----	-----U-----	-----	CGG-----C
<i>T. exillis</i>	-----AGU-GC*	-----U-----CC-----	-----UGC-----UU-----	-----U-CAGG-----	-----G-----A-----	-----UG-----C-----	-----A-----AU-----	-----U-----UCU-UC-C	-----	CGG-----C
<i>C. elatum</i>	-----AU-GGGC*	-----U-----CC-----	-----UGC-----UU-----	-----U-C-GG-----	-----G-----A-----	-----UG-----C-----	-----U-----AU-----	-----U-----UCUAUC-C	-----	CGG-----C
<i>X. nigresc</i>	-----AU-GGUG	-----CA-C-----	-----UGC-----U-----	-----UA-UU-----	-----G-----	-----U-C-UA-A-----	-----G-AU-----A-----	-----UUG-----	-----	CGG-----C
<i>P. parasit</i>	-----A-AGC*	-----CCG-----	-----NNC-----	-----NN-----	-----G-----	-----C-CC-G-----	-----G-AU-----	-----U-----	-----	CGG-----C
<i>T. alta</i>	-----AU-GG-C	-----ACC-----	-----UC-----U-UU-----	-----U-G-C-UC-----	-----G-----A-----	-----C*-N-CC-C-----	-----A-U-----	-----G-C-----	-----	CGG-----C
<i>H. werneck</i>	-----G-GG-C*	-----ACC-----	-----UC-----U-GC-----	-----GCC*-G-----	-----G-----AC-----	-----GG-A-C-C-----	-----A-U-----	-----GUGC-----	-----	CGG-----C
<i>C. sphaero</i>	-----G-GG-C*	-----ACC-----	-----U-C-----GC-----	-----GCCU-G-----	-----G-----U-----	-----C-GG-U-CC-C-----	-----U-----AU*-A-----	-----UGGA-----	-----	CGG-----C
<i>C. herbaru</i>	-----G-GG-C*	-----ACC-----	-----UC-----G-C-----	-----GCCU-G-----	-----G-----U-----	-----C-GG-U-CC-C-----	-----U-----AU*-A-----	-----UUGA-----	-----	CGG-----C
<i>C. trichoi</i>	UAGACUUADA	GACCAGGGUG	UCAUGC GGCC	UCCCGGGACC	GAGGAACGGC	CU*CCGGCUC	GGAUGUUGGC	CUAAUGG		
<i>Cl. arxil</i>	-----	-----	-----A-----	-----	-----	-----	-----	-----		
<i>C. devries</i>	-----	-----	-----U-A-----	-----	-----	-----	-----	-----		
<i>T. boppi</i>	-----	-----	-----U-----	-----	-----	-----	-----	-----		
<i>P. verruco</i>	-----	-----G-N-----	-----	-----	-----	-----	-----	-----		
<i>C. carrion</i>	-----	-----	-----A-----	-----	-----	-----	-----	-----		
<i>Cl. ajello</i>	-----	-----	-----	-----	-----	-----	-----	-----		
<i>Cl. ajello</i>	-----	-----	-----A-----	-----	-----	-----	-----	-----		
<i>C. carrion</i>	-----	-----C-----	-----U-----	-----	-----	-----	-----	-----		
<i>Ca. pilose</i>	-----	-----	-----C-----U-A-----	-----	-----	-----	-----	-----		
<i>F. pedroso</i>	-----	-----	-----A-----	-----	-----	-----	-----	-----		
<i>R. atrovir</i>	-----C-----	-----	-----A-----	-----	-----	-----	-----	-----		
<i>E. pisciph</i>	-----C-----	-----	-----	-----	-----	-----	-----	-----		
<i>R. mackenz</i>	-----C-----	-----	-----	-----	-----	-----	-----	-----		
<i>E. dermati</i>	-----C-----	-----	-----A-----	-----	-----	-----	-----	-----		
<i>E. lecanii</i>	-----C-----	-----G-N-----	-----	-----	-----	-----	-----	-----		
<i>Ca. manson</i>	-----C-----	-----N-----	-----A-----	-----N-----	-----	-----	-----	-----		
<i>E. jeansel</i>	-----C-----	-----	-----A-----	-----A-----	-----	-----	-----	-----		
<i>X. ferrugi</i>	G--UG-----	-----C-UC-----	-----CA-----A-----	-----A-----	-----	-----	-----U-----A-----	-----C-----		
<i>H. resiniae</i>	G--UG-----	-----C-NA-----	-----CA-----A-----	-----NN--U-----N-----	-----	-----	-----N-----	-----A-----		
<i>O. gallopa</i>	G--UG-----	-----C--G-C-----	-----CA-----	-----A--NN-----	-----	-----	-----AUUU--AA-----	-----C-----		
<i>A. pullula</i>	AG-UG-----	-----C-----	-----A--A-----	-----AG-----U-----	-----	-----	-----U-----	-----		
<i>T. exillis</i>	AG-C-----	-----GGG--*--AC-	-----A--A-UA-----	-----AG--U-----U-----	-----	-----	-----A-U-----A-----	-----C-----		
<i>C. elatum</i>	AG-C-----	-----GGG--*--A-	-----A--AA-----	-----AG--U-----U-----	-----	-----	-----A-U-----A-----	-----C-----		
<i>X. nigresc</i>	G--UG-----	-----C--CUU--A	-----A--A-CUU*	-----AUGA-----	-----	-----	-----*--U--AA-----	-----C-----		
<i>P. parasit</i>	G--UG-----	-----C--UCU-C-	-----A--A-U--**	-----*G--G-----	-----	-----	-----**--U--AA-----	-----C-----		
<i>T. alta</i>	GU-UG-----C-	-----C--GC--C-	-----GA-----	-----GG-C--U-----	-----	-----	-----U--CA-----	-----C-----		
<i>H. werneck</i>	G*-CA-----	-----C--GC--C-CA	-----CA--A-----G	-----C-U-CC--GU-----	-----	-----	-----U--A-----	-----C-----		
<i>C. sphaero</i>	G--UG-----	-----C--UCCA-----	-----AUGCAGC-AG	-----CG-----**	-----	-----	-----U--A-----	-----C-----		
<i>C. herbaru</i>	G--UG-----	-----C--UCUU-----	-----AUGCAGC-AG	-----CG-----**	-----	-----	-----U--A-----	-----C-----		

**Fig. 1** Alignment of 29 LS rRNA partial sequences starting from position 57 of the 5' end until position 624 with reference to *S. cerevisiae* (Georgiev *et al.*, 1981). *C. trichoides*, CBS 173-52, is used as leading strand. Dashes indicate nucleotides identical to this reference; asterisks represent deletions necessary for alignment; N corresponds to unidentified nucleotides. Only sequences differing by at least one position are listed.



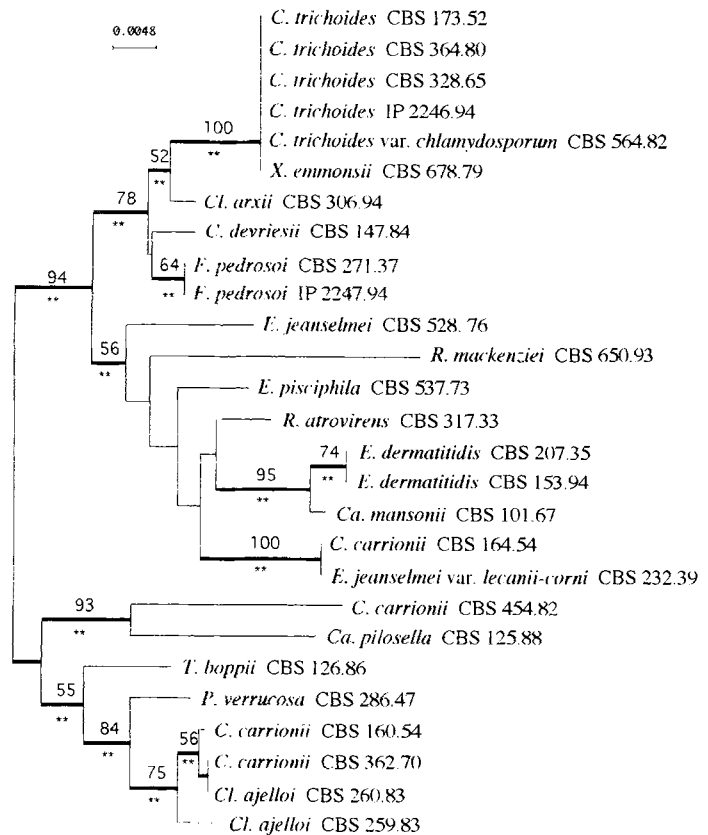
**Fig. 2** Unrooted phylogenetic tree of studied *Cladosporium*-like species generated with the neighbour-joining method on the basis of unambiguously aligned positions (1–71, 83–116, 128–173, 197–330, 438–450, 475–483, 503–514, 518–526, 535–550, 555–577). The robustness of the tree was assessed by maximum parsimony (bold lines connecting taxa indicate common branches found with neighbour-joining and parsimony), maximum likelihood (double asterisk with  $P < 0.01$ ) and bootstrap analysis applied to the maximum parsimony (only values greater than 50% are given above the branches). Scale bar indicates accumulated changes per 100 nucleotides.

contained saprophytic *Cladosporium* species together with *Mycosphaerella tassiana*. The monophyletic branches I and II are very distant from each other.

The remaining species are all excluded from the monophyletic groups I and II. Among these were two saprophytic *Xylohypha* species, including the generic type species, *Xylohypha nigrescens* (Pres.:Fr.) Mason, which is at a large phylogenetic distance from the human-

pathogenic *Cladosporia*. *Phialophora parasitica* Ajello et al., which is known primarily as a plant-pathogen but is occasionally reported from cases of phaeohyphomycosis, proved to be unrelated to the type species of *Phialophora* Medlar, *Phialophora verrucosa* Medlar. Two *Taeniolella* species, originally described from rotten wood, were found to be unrelated to each other, and were both found to be distant from *Taeniolella boppii* Borelli, described

**Fig. 3** Unrooted phylogenetic tree of Herpotrichiellaceae and related anamorphs. Positions 1–78, 95–491 and 498–577 were used for analyses performed as for Fig. 1.



from human skin lesions. The black yeasts *Aureobasidium pullulans* (de Bary) G. Arnaud and *Hortaea werneckii* (Horta) Nishimura & Miyaji were distant from the other black yeasts now classified in *Exophiala*. The neurotropic species *Ochroconis gallopavum* (W. B. Cooke) de Hoog was also found in an isolated position among the species sampled here.

The largest monophyletic group in the study (I) contained the following taxa (Fig. 3):

- Two species of the herpotrichiellaceous Ascomycetes *Capronia*, viz. *C. mansonii* (Schol-Schwarz) E. Müller *et al.* and *C. pilosella*, which are known to have *Exophiala*-like and *Cladosporium*-like anamorphs [7] respectively.
- Four black yeasts belonging to the genus *Exophiala*, in addition to *Rhinocladiella atrovirens* Nannf. which also has an *Exophiala* synanamorph.
- The type strain of *Taeniolella boppii*, originating from human skin.
- The type strain of *Ramichloridium mackenziei* Campbell & Al-Hedaithy, originating from human brain.
- All analysed human-pathogenic *Cladosporium* species, among which are agents of systemic, neurotropic and disseminated mycoses.

- All agents of chromoblastomycosis, viz. *Fonsecaea pedrosoi* (Brumpt) Negroni, *Phialophora verrucosa*, *Cladosporium carrionii* Trejos and *Cladophialophora ajelloi* Borelli.

The human-pathogenic *Cladosporium* species, *C. trichoides* C. W. Emmons var. *trichoides*, *C. trichoides* var. *chlamydosporum* Kwon-Chung and the analysed strain of *Xylohypha emmonsii* Padhye *et al.* [17], CBS 678-79, proved to have identical partial sequences. Their identity was confirmed with nDNA/DNA reassociation experiments, resulting in values of 95–100% (Table 2). In contrast, *Cladosporium devriesii* Padhye & Ajello and *Cladophialophora arxii* Tintelnot differed from *Cladosporium trichoides* in 11 and 7 positions, respectively, and, in addition, gave low values with nDNA/DNA reassociation (Table 2). Partial sequences of *C. devriesii* and *Cladophialophora arxii* differed at six positions and were also found to be different by DNA/DNA reassociation (Table 2).

Isolates of *Cladosporium carrionii* were dispersed over three sub-branches. An authentic strain, CBS 160-54, differed in one position from the type strain of *Cladophialophora ajelloi*, CBS 260-83. A maximum nDNA/DNA reassociation value of 85% was found (Table 3). A second

	<i>C. trichoides</i> 173-52	<i>C. trichoides</i> 328-65	<i>X. emmonsii</i> 678-79	<i>C. trichoides</i> 564-82	<i>C. devriesii</i> 147-84
<i>C. trichoides</i> 173-52	X		100/0		
<i>C. trichoides</i> 2246-94		97/0	95/0		
<i>C. trichoides</i> 328-65		X	99/0	100/0	27/11
<i>C. trichoides</i> 364-80	99/0				
<i>Cl. arxii</i> 306-94		24/7	27/7		28/6

**Table 2** nDNA/DNA reassociation percentages and number of LS rRNA base changes between members of *Cladosporium trichoides*, *Xylohypha emmonsii*, *Cladosporium devriesii* and *Cladophialophora arxii*

*C* = *Cladosporium*, *Cl* = *Cladophialophora*.

	<i>Cl. ajelloi</i> 260-83	<i>C. carrionii</i> 160-54	<i>C. carrionii</i> 164-64	<i>C. carrionii</i> 362-70
<i>Cl. ajelloi</i> 260-83	X	85/1	10/32	98/0
<i>Cl. ajelloi</i> 259-83	27/5	35/4		31/5
<i>E. jeanselmei</i> var. <i>lecanii-corni</i> 232-39			99/0	

**Table 3** nDNA/DNA reassociation percentages and number of LS-RNA base changes between members of the *Cladosporium carrionii* complex

*C* = *Cladosporium*, *Cl* = *Cladophialophora*,  
*E* = *Exophiala*.

strain of *Cl. ajelloi*, CBS 259-83, was somewhat more distinctive, and gave DNA/DNA reassociation values of 27–35% with its nearest neighbours (Table 3). In contrast, DNA of *Cladosporium carrionii*, CBS 362-70, and of *Cladophialophora ajelloi*, CBS 260-83, which had identical sequences, reassociated at 98% (Table 3). One *C. carrionii* strain, CBS 164-54, which displayed an *Exophiala* synanamorph, was found identical to *Exophiala jeanselmei* var. *lecanii-corni* (Benedek & Specht) de Hoog, CBS 232-39, and this was confirmed by a DNA/DNA reassociation value of 99%. Another *C. carrionii* strain, CBS 454-82, occupied a relatively isolated position. Its nearest neighbour was *Capronia pilosella*, which has a *Cladosporium*-like anamorph. A strain of *Exophiala dermatitidis* (Kano) de Hoog with a *Cladosporium*-like synanamorph, CBS 153-94 [18], was found to have identical sequences to the type strain, which was confirmed by a DNA/DNA reassociation value of 100% (results not shown). Two strains of *Fonsecaea pedrosoi* also proved to be identical by both methods.

Mole% G + C of DNA are listed in Table 4. Strains found in Group I had rather similar values ranging between 48.8% and 54.4%. Strains with identical sequences were invariably found to have nearly identical GC contents. The strains of *Cladosporium trichoides/bantianum* have similar values, ranging from 51.4% to 51.7%. *Cladosporium devriesii* and *Cladophialophora arxii* were clearly different from this group but close to each other, having values of 52.0% and 52.4%, respectively. The strains labelled *Cladosporium carrionii* were found to be polyphyletic. The authentic strain, CBS 160-54, a second strain from chromoblastomycosis (CBS 362-70), and the two *Cladophialophora ajelloi* strains had similar

values ranging between 54.0% and 54.4%. A further chromoblastomycosis strain, CBS 164-54, had a GC% of 49.0%, which was nearly identical to that of *Exophiala jeanselmei* var. *lecanii-corni* (48.8%). CBS 454-82 had a GC% of 51.7%, compared to 51.5% for its nearest neighbour *Capronia pilosella*.

## Discussion

Some members of the well supported groups I and II (Fig. 2) are known to have teleomorphs which have been placed in two different families of bitunicate ascomycetes, viz. *Capronia* Sacc. (fam. Herpotrichiellaceae) and *Mycosphaerella* Johanson (fam. Mycosphaerellaceae). In the phylogenetic tree these are positioned far apart from each other. Additional species with known teleomorphs or supposed family relationships are *Hormoconis resiniae* (teleom.: *Amorphotheca resiniae*, fam. Amorphothecaceae) and *Aureobasidium pullulans*. The latter species is related to the ascomycete family Dothideaceae, judging from teleomorph connections of the closely similar genus *Hormonema* [19], 18 S rDNA sequences [20], karyology and thallus maturation [21] and coenzyme Q systems [22]. Therefore, the phylogenetic distances (Fig. 2) between *Capronia pilosella/Capronia mansonii*, *Aureobasidium pullulans*, *Hormoconis resiniae* and *Mycosphaerella tassiana* can be viewed as indicative of differences at the family level. The distance between *Capronia pilosella* and *Capronia mansonii* (39 differences, 6.8%) would thus indicate an intrafamilial relationship. On this basis, it is likely that all fungi clustered in group I are anamorphs of members of the Herpotrichiellaceae, as suggested by Untereiner's finding [23] of cleistothecium initials in



**Table 4** Mole% G + C of DNA of Herpotrichiellaceae and purported anamorphs placed in Group I with partial sequencing of LS rRNA

<i>Ca. mansonii</i>	CBS 101-67	53.2
<i>Ca. pilosella</i>	CBS 125-88	51.5
<i>Cl. ajelloi</i>	CBS 260-83	54.4
<i>Cl. ajelloi</i>	CBS 259-83	54.1
<i>Cl. arxii</i>	CBS 306-94	52.0
<i>C. carrionii</i>	CBS 160-54	54.0
<i>C. carrionii</i>	CBS 164-54	49.0
<i>C. carrionii</i>	CBS 362-70	54.4
<i>C. carrionii</i>	CBS 454-82	51.7
<i>C. devriesii</i>	CBS 147-84	52.4
<i>C. trichoides</i>	CBS 173-52	51.4
<i>C. trichoides</i>	CBS 328-65	51.5
<i>C. trichoides</i>	CBS 364-80	51.6
<i>C. trichoides</i>	IP 2246-94	51.7
<i>C. trichoides</i>	CBS 564-82	51.5
<i>E. dermatitidis</i>	CBS 207-35	52.6
<i>E. dermatitidis</i>	CBS 153-94	52.8
<i>E. lecanii-corni</i>	CBS 232-39	48.8
<i>E. jeanselmei</i>	CBS 528-76	50.2
<i>E. pisciphila</i>	CBS 537-73	50.0
<i>F. pedrosoi</i>	CBS 271-37	52.0
<i>F. pedrosoi</i>	IP 2247-94	52.0
<i>P. verrucosa</i>	CBS 286-47	54.2
<i>R. mackenziei</i>	CBS 650-93	50.3
<i>Rh. atrovirens</i>	CBS 317-33	50.5
<i>T. boppii</i>	CBS 126-86	54.6
<i>X. emmonsii</i>	CBS 678-79	51.5

*C* = *Cladosporium*, *Ca* = *Capronia*, *Cl* = *Cladophialophora*, *E* = *Exophiala*, *F* = *Fonsecaea*, *P* = *Phialophora*, *R* = *Ramichloridium*, *Rh* = *Rhinoctadiella*, *T* = *Taeniolella*.

several of these pathogenic species, and, *vice versa*, her recognition [in 20] of anamorphs of *Capronia* which fit the *Exophiala* complex. Similarly, the fungi of group II probably all belong to or are anamorphs of members of the Mycosphaerellaceae.

Most members of Mycosphaerellaceae are pathogens on plants with a restricted host range or are ubiquitous on dead plant material [2,3,24,25]. Despite the fact that the latter are abundantly present in the human environment, human mycoses caused by these species are extremely rare. The few cases described do not show any clear predilection for particular organs and the route of infection is variable [26]. These species can thus be regarded as typical opportunists, occasionally infecting and surviving inside the tissue of warm-blooded animals but having their normal ecological niche elsewhere.

In addition to the above-described differences in ecology and pathogenicity, the mycosphaerellaceous *Cladosporia* are characterized by having differentiated conidiophores and protruding, dark conidial scars [4]. They are mostly unable to grow at temperatures above 30 °C [27] and can liquefy gelatin [28].

Within the Mycosphaerellaceae, *Cladosporium herbarum* was proven to represent the anamorph of *Mycosphaerella tassiana*, as supposed earlier by several authors, e.g. [4]. *Cladosporium macrocarpum* Preuss and *C. cladosporioides* (Fres.) de Vries had the same partial 26 S rRNA sequences, even including identical hypervariable domains D1 and D2. *C. macrocarpum* differs morphologically in having larger, more rough-walled conidia [1,2], while *C. cladosporioides* has conidiophores without nodes and smaller, less ornamented conidia. In contrast, *C. sphaerospermum* Penz. was found to have 14 base differences (12 transitions, 2 transversions).

If we consider the taxa clustered in the monophyletic Group I to be anamorphic members of the family Herpotrichiellaceae, a remarkably high percentage of isolates in this small family are seen to originate from vertebrates. Several species are aetiologic agents of characteristic mycoses, with almost consistent clinical symptoms. Among these are *Fonsecaea pedrosoi*, *F. compacta* (Carrion) Carrion, *Phialophora verrucosa*, *Cladosporium carrionii* and *Cladophialophora ajelloi* (all chromoblastomycosis), and *Cladosporium trichoides* and *Ramichloridium mackenziei* Campbell *et al.* (both neurotropic in humans). Several others are nearly exclusively known from mammals: *Cladophialophora arxii*, *Cladosporium devriesii*, *Exophiala (Wangiella) dermatitidis* (Kano) de Hoog and *Exophiala jeanselmei* var. *lecanii-corni*. The species *Exophiala jeanselmei* resides in the environment, but is also frequently involved in skin disorders in humans.

Some peculiarities are noted in the major systemical agents, *Cladosporium trichoides*, *Ramichloridium mackenziei* and *Exophiala dermatitidis*. The latter species is known to provoke fatal neurotropic mycoses in otherwise healthy, Asian patients but not in Caucasians [25]. *Cladosporium trichoides* causes neurotropic infections which lead to fatal brain lesions. *Ramichloridium mackenziei* seems restricted to the Middle-East [29]. It may thus be supposed that the clinical course in these mycoses is determined by rather subtle racial differences in the human immune system and/or by environmental differences. This suggests that the fungi concerned might have a highly evolved ecological strategy which includes vertebrate hosts.

Both groups I and II contain anamorphs currently classified in the genus *Cladosporium*. Our data indicate that they belong to different ascomycete families, and it is, therefore, confusing to maintain them all in a single genus. The genus *Cladosporium* should be divided along phylogenetic lines, as advocated by Morgan-Jones & Jacobsen [30] and actually proposed by de Hoog *et al.* [31]. The genus *Cladophialophora* Borelli is redefined to accommodate von Arx's [4] fourth group of *Cladosporia*,

viz. human-pathogenic species characterized by undifferentiated conidiophores and conidia with unpigmented, unthickened scars. An earlier proposal by McGinnis *et al.* [32] to classify *Cladosporium bantianum* and *C. trichoides* in *Xylohypha* should not be followed, for several reasons. Firstly, species of *Xylohypha s. str.* proved phylogenetically unrelated to the herpotrichiellaceous Cladosporia. This coincides with differences in conidial scars and germination patterns [27]. The studied *Xylohypha* species seem unrelated to each other (Fig. 2). Furthermore, *Cladosporium carrionii*, that had not been reclassified in *Xylohypha* by McGinnis *et al.* [32], clusters in group I with *C. trichoides*, and should thus be classified in the same genus.

*Cladosporium elatum* (Harz) Nannf. proved to be unrelated to either mycosphaerellaceous or herpotrichiellaceous anamorphs. It has undifferentiated conidiophores and conidia with unpigmented scars. It is known from wood [3,33] and only a single human case of subcutaneous phaeohyphomycosis has been described [34]. As its affinities are unknown, its provisional classification remains in *Cladosporium*.

Within the Herpotrichiellaceae and their purported anamorphs, the following taxonomic remarks can be made. *Cladosporium trichoides* var. *chlamydosporum* has the same partial sequence as *C. trichoides* and proved identical in DNA/DNA reassociation experiments (Table 2). The variety was originally described on the basis of the presence of thick-walled chlamydospores [35]. Similar chlamydospores are known in other strains of *C. trichoides* [32,36], *Cladophialophora arxii* [38] and in *Cladosporium carrionii* [31]. The occurrence of chlamydospores appears to be of physiological rather than of taxonomic interest. We therefore consider this taxon to be redundant [31].

*Cladosporium trichoides* [= *Xylohypha bantiana* (Sacc.) McGinnis *et al.*] and *Xylohypha emmonsii* Padhye *et al.* [17] were also found to be identical using rRNA sequencing as well as nDNA/DNA reassociation techniques. *Xylohypha emmonsii* has thus far been distinguished by its ability to grow at 40 °C. However, de Hoog *et al.* [31] found strain CBS 678-79, used by Padhye *et al.* [17] as *X. emmonsii*, to grow at 40 °C. Morphological criteria for distinction, viz. conidial size and pigmentation and branching patterns of conidial chains, are all found to be highly dependent on cultural conditions. *Xylohypha emmonsii* is known from subcutaneous rather than systemic phaeohyphomycosis and it is found in tissue with irregular yeast-like cells rather than cylindrical hyphae [17,27,38]. The histopathological difference may be explained by a higher degree of innate cellular immunity in subcutaneous tissue. We therefore conclude that only a single species, *Cladophialophora bantiana* [31], should

be maintained. Infections by this species are normally acquired by inhalation, but, as with any fungus which occurs in nature, it may also be traumatically inoculated and then cause a primary (sub)cutaneous mycosis.

*Cladophialophora arxii* [37] and *Cladosporium devriesii* [39,40] were confirmed to be separate though closely related species. Interestingly, they are represented by the only two *Cladosporium* strains isolated from systemical infections rather than from the central nervous system. The two species are morphologically distinct but physiologically identical except for temperature tolerance [31].

The two *Fonsecaea pedrosoi* strains tested here, both agents of human chromoblastomycosis, cluster together and are found to be identical. The morphology of *Fonsecaea* resembles that of human-pathogenic species of *Cladosporium*, but differs in having conidia in short chains, in addition to non-catenate conidia.

The genus *Exophiala* Carmichael clusters amidst the human-pathogenic cladosporia, despite its mostly yeast-like, rather than dry, morphology. Conidiogenesis is prevalently annellidic. Dry chains similar to those of *C. bantiana* are occasionally observed in *Exophiala dermatitidis* [18], indicating that dry vs. yeast-like propagation may be found in closely related taxa. This further supports the classification of *Exophiala* in the Herpotrichiellaceae, close to the human-pathogenic species of *Cladosporium*, now [31] placed in *Cladophialophora*. The clustering of the sympodial species, *Rhinocladiella atrovirens*, amidst *Exophiala* species is not surprising because it has an *Exophiala* synanamorph and, conversely, sympodial conidiogenesis is known in *Exophiala jeanselmei* [41] and *E. dermatitidis* [42].

The type strain of *Cladophialophora ajelloi* had sequences identical to those of *C. carrionii* CBS 362-70 and proved identical in a DNA/DNA reassociation experiment (Table 3). This confirms the suggestion by Honbo *et al.* [43] that these two species should not be maintained in separate taxa. However, strains listed as *C. carrionii* appeared remarkably heterogeneous. This was also found in characters of nutritional physiology [31]. The nearest neighbour of strain CBS 454-82, identified as *C. carrionii*, though still with 25 base differences (4.33%), was *Capronia pilosella*. The latter teleomorph species was described as having a *Cladosporium*-like anamorph [7], which in our studies [31] proved morphologically indistinguishable from *C. carrionii*. *Capronia pilosella* is only known from wood in temperate climates, while CBS 454-82 was a culture contaminant (Table 1). These two groups may be a distinct, paraphyletic group of *C. carrionii* (Fig. 3). The *Exophiala* cluster contains two remarkably dimorphic, *Cladosporium*-like strains. One of these, CBS 164-54, had been labelled *C. carrionii*, isolated from human skin; it turned out to be identical to

*Exophiala jeanselmei* var. *lecanii-corni*. The second, CBS 153-94, is a strain identified as *Exophiala dermatitidis*, occasionally displaying a catenate synanamorph similar to *Cladophialophora bantiana* [18].

The type strain of *Taeniolella boppii* clustered in the Herpotrichiellaceae rather than close to the generic type species, *T. exilis* (P. Karst.) S. Hughes, and *T. alta* (Ehrenb.:Fr.) S. Hughes. Since the catenate species, *T. boppii*, is confirmed as quite different from the remaining species accepted in *Taeniolella*, this species may also be transferred to *Cladophialophora* [31]. *Taeniolella exilis* was recently described from a case of phaeohyphomycosis [43], but is normally found on wood [2].

## Conclusions

The melanized human-pathogens studied form a single, monophyletic group. The anamorph genera are morphologically diverse, being distinguished on the basis of conidium ontogeny. However, synanamorphs are known to occur in a single strain. The occurrence of *Phialophora* anamorphs in strains of *Fonsecaea*, *Cladosporium* and *Exophiala* is well-established. *Exophiala* is connected to *Rhinochrysiella* [40] and was recently found to be connected to *Cladosporium* [17]. The pleomorphism of these taxa underlines their close inter-relationship. *Capronia* strains were placed in the group with *Exophiala* and *Cladosporium* anamorphs. In addition, *Capronia*-like teleomorph initials have been found in strains of human-pathogenic *Exophiala* species [22].

The affiliation of the melanized human-pathogens to a single ascomycete family, Herpotrichiellaceae, is unambiguously supported by the results outlined in this article.

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