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Cylindrocarpon pauciseptatum sp. nov., with notes on *Cylindrocarpon* species with wide, predominantly 3-septate macroconidia

Hans-Josef SCHROERS[a,*], *Metka ŽERJAV*[a], *Alenka MUNDA*[a], *Francois HALLEEN*[b], *Pedro W. CROUS*[c]

[a]*Agricultural Institute of Slovenia, Hacquetova 17, p.p. 2553, 1001 Ljubljana, Slovenia*
[b]*ARC Infruitec-Nietvoorbij (The Fruit, Vine and Wine Institute of the Agricultural Research Council), P. Bag X5026, Stellenbosch 7599, South Africa*
[c]*Centraalbureau voor Schimmelcultures, P.O. Box 85167, NL-3508 AD Utrecht, The Netherlands*

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ABSTRACT

A *Cylindrocarpon* species with up to 10 μm wide, straight and predominantly 3-septate macroconidia, subglobose to ovoidal microconidia and chlamydospores, is described as *Cyl. pauciseptatum*. It is most similar to *Cyl. austrodestructans* but no chlamydospores and microconidia are formed in the latter. Similar macroconidia also occur in *Cyl. theobromicola*, which forms oval to ellipsoidal microconidia at least sparsely and has slightly curved macroconidia, and *Cyl. destructans* var. *crassum*, which forms abundant 1-celled microconidia. DNA sequence data of the internal transcribed spacer regions 1 and 2 plus the 5.8S rDNA and the partial beta-tubulin gene were used for phylogenetic inferences. *Cylindrocarpon pauciseptatum* and *Cyl. macrodidymum* are monophyletic and are closely related to other species of *Cylindrocarpon sensu stricto* including members of the *Cyl. destructans* (teleomorph, *Neonectria radicicola*) species complex, which accommodates *Cyl. liriodendri* (teleomorph, *Neon. liriodendri*), *Cyl. destructans* var. *crassum* and *Cyl. austrodestructans* (teleomorph, *Neonectria austroradicicola* comb. nov.). *Cylindrocarpon theobromicola* is distantly related to species of *Cylindrocarpon sensu stricto* or *Neonectria sensu stricto*. It clustered among cylindrocarpon-like species with curved macroconidia, of which some belong to the *Neon. mammoidea* group. Relatively voluminous cells in sporodochial conidiophores of *Cyl. theobromicola* resembled those described for *Campylocarpon*, which is closely related to members of the *Neon. mammoidea* group including *Cyl. theobromicola*. *Cylindrocarpon pauciseptatum* has been isolated from roots of *Vitis* spp. in South-eastern Europe (Slovenia) as well as New Zealand, where it also occurs on roots of *Erica melanthera*.

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Introduction

Cylindrocarpon and *Campylocarpon* are hypocrealean genera characterised by 1- to multiseptate macroconidia that may be associated with non-septate microconidia and brownish chlamydospores (Booth 1966; Halleen *et al.* 2004). Based on the occurrence of microconidia and chlamydospores, species of *Cylindrocarpon* have been segregated into four groups, within

* Corresponding author.
E-mail address: hans.schroers@kis.si

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which more than 30 species were identified mainly according to morphological details relating to conidium size, shape and number of septa (Booth 1966). Most teleomorphs of *Cylindrocarpon* are currently classified in *Neonectria* (Brayford *et al.* 2004; Rossman *et al.* 1999; Seifert *et al.* 2003). Based on anatomical characters of the perithecial wall and partly on characters of ascospores, however, at least five groups of teleomorphs within *Neonectria* have been delineated (Booth 1959; Brayford *et al.* 2004; Rossman *et al.* 1999; Samuels & Brayford 1993). Preliminary phylogenetic analyses revealed that the type species of *Cylindrocarpon* (*Cyl. cylindroides*) and *Neonectria* (*Neon. ramulariae*) are phylogenetically distant from the *Neon. mammoidea* species group and *Campylocarpon* having relatively voluminous cells in fasciculate sporodochia (Brayford *et al.* 2004; Halleen *et al.* 2004). Species groups or complexes in *Cylindrocarpon/Neonectria s. str.* centre mainly on *Cyl. cylindroides*, *Neon. coccinea*, *Neon. galligena*, *Neon. ditissima* (anamorph, *Cyl. heteronema*) and *Neon. radicicola* (purported anamorph, *Cyl. destructans*). The *Cyl. destructans* species complex is genetically diverse comprising various phylogenetic units (Seifert *et al.* 2003). Some of these have been raised to species level, namely *Cyl. liriodendri* (teleomorph, *Neon. liriodendri*) (Halleen *et al.* 2006), *Neon. macroconidialis* and *Neon. coprosmae* (Seifert *et al.* 2003).

Cylindrocarpon species are commonly known to be saprobes in soil, or occur on dead plant substrata, or act as weak pathogens of plants infecting wounds of roots and stems of various hosts (Brayford 1993). Specifically, *Cyl. liriodendri*, *Cyl. macrodidymum*, *Campylocarpon fasciculare* and *Camp. pseudofasciculare* were associated with black foot rots of grapevines (Halleen *et al.* 2004, 2006) and several phylogenetic species of the *Cyl. destructans* species complex, but not *Cyl. liriodendri*, with root disturbances of ginseng (Seifert *et al.* 2003).

Recently a species with wide macroconidia was isolated from roots of *Vitis* spp. that could not be identified using the current identification schemes, although a similar strain from New Zealand had been identified previously as *Cyl. theobromicola*. Results from comparisons of the wide-spored species with *Cyl. theobromicola*, *Cyl. austrodestructans*, *Cyl. destructans* var. *crassum*, and some other species for which wide-spored macroconidia have been described as well, are reported here.

Materials and methods

Isolation and handling of fungal strains

Grapevines showing symptoms of dieback as reported by Halleen *et al.* (2004), including symptoms of black foot rot, were removed from the field and transferred to the laboratory. Soil and stones were carefully washed off from 2–4 mm diam roots; 2 cm long pieces with dark lesions were excised and rinsed under running tap water for 5 min. Pieces were then treated with 70 % ethanol for 5 s and dried on sterile filter paper. Blocks of *ca* 1–2 mm were removed from the margins of lesions with a sterile scalpel and placed on the surface of a potato-dextrose agar (PDA) (Biolife, Ref. 4019352, Milan, Italy) dish containing streptomycin and penicillin (Gams *et al.* 1998). The PDA dishes were incubated at room conditions. Outgrowing colonies were subcultured within 7 d and kept in tubes with slanted synthetic nutrient-poor agar (SNA) medium (Gams *et al.* 1998) enriched with 50 g of small pieces of carrot (SNA-C). Newly isolated strains were kept in the collection at the Agricultural Institute of Slovenia (KIS) and deposited at the Centraalbureau voor Schimmelcultures (CBS, Utrecht, the Netherlands). Actively growing strains were obtained from the CBS and maintained in tubes with SNA-C. No living material of *Cyl. austrodestructans* was available at the CABI Genetic Resources Collection (IMI, Egham), American Type Culture Collection (ATCC, Maryland) and the Systematic Mycology and Microbiology Laboratory (BPI, USDA, Beltsville) [Vasia Dekou (ATTC), Matthew J. Ryan (CABI) and Gary J. Samuels (USDA), personnel communication. The following herbarium specimens of *Cyl. austrodestructans* from the New Zealand Fungal Herbarium (PDD) were re-examined: PDD 46395 (G.J.S.; USDA, ARS, BPI; 83-190), PDD 46334 (G.J.S. 83-154), PDD 46394 (G.J.S. 83-189) and PDD 47770 (G.J.S. 85-22).

DNA isolation, sequencing and phylogenetic analyses

Mycelium for DNA extractions was grown in tubes with 2 mL of malt extract liquid medium (Biolife, Milan, Italy) and lysed using a Qiagen Tissue Lyser (Hilden, Germany). DNA was extracted from the lysed material using either the BioSprint 15 or DNeasy Plant Mini Kits provided by Qiagen (Hilden, Germany). Dried mycelium and conidia were removed from *ca* 2 cm^2 of a dried PDA colony of specimen PDD 46334; DNA was extracted from this material using the DNeasy Plant Mini Kit. The partial beta-tubulin gene was amplified as described by O'Donnell & Cigelnik (1997). The ITS rDNA, spanning the internal transcribed spacer regions 1 and 2 plus the 5.8S rDNA, and the partial large subunit (LSU) rDNA was amplified with primers ITS5 (White *et al.* 1990) and NL4 (O'Donnell 1993) or VG9 (de Hoog & Gerrits van den Ende 1998), and LR5 (Vilgalys & Hester 1990). The PCR program for reactions using the latter primer pair was as described in Summerbell & Schroers (2002); when using the primers ITS5 and NL4 a program initially described by O'Donnell & Cigelnik (1997) was adopted but 30 cycles with an annealing temperature of 52 °C followed 10 cycles with an annealing temperature at 58 °C. A PCR product from the herbarium material was obtained only when using primers ITS3 and ITS4 (White *et al.* 1990) spanning the internal transcribed spacer region 2 (ITS 2). The PCR volume of 50 μL contained 1 μL genomic DNA extract, 25 pmol of each of the primers, 200 μM of each of the dNTPs (Promega), 1 U of recombinant Taq DNA polymerase or AmpliTaq Gold (Fermentas, Vilnius, Lithuania or Applied Biosystems, Foster City, CA), and 1 × standard PCR buffer supplied together with the polymerases. A rDNA fragment in strain CBS 218.67 was amplified with VG9 and LR5 only after 4 μL dimethyl sulfoxide (Serva 20385) had been added to the PCR mixture. PCR fragments were visualized after electrophoresis in a gel containing 1 % agarose and submitted to Macrogen Inc. (Seoul, South Korea) for clean-up procedures and sequencing. Primers used in sequence reactions were T1 and T2 (O'Donnell & Cigelnik 1997) for the partial beta-tubulin gene and NL1 (O'Donnell 1993), ITS5, ITS3, ITS4 as well as NL4 (O'Donnell 1993) and LR5 for the ITS and the partial LSU rDNA. Newly generated sequences and their

Table 1 – Taxa with *Cylindrocarpon*-like species, for which data were newly generated

Taxon	Strain no.	Location	Host or substrate	GenBank accession no.	
				ITS rDNA	Beta-tubulin
Cyl. austrodestructans	PDD 46334, G.J.S. 83-154	New Zealand (NZ), Arthur's Pass Natl. Park	bark of *Pseudopanax crassifolius*	EF607077	–
Cyl. destructans var. *crassum*	CBS 537.92	Belgium, Liège	wood of *Aesculus hippocastanum*	EF607079	EF607064
Cyl. destructans var. *crassum*	CBS 605.92	Germany, Hamburg	root of *Tilia petiolaris*	EF607078	EF607065
Cyl. macrodidymum	CBS 120170	Slovenia (Slo.), Nova Gorica, vineyard	inner wood of base of main stem of *Vitis* sp. showing foot rot symptoms and shrinking leaves	EF607091	–
Cyl. macrodidymum	CBS 120169	Slo., Krško, vineyard	partly decayed roots of 4-year-old, still living but badly shooting *Vitis* sp. in vineyard	–	EF607063
Cyl. pauciseptatum	CBS 100819	NZ, Tauranga	roots of *Erica melanthera*	EF607090	EF607067
Cyl. pauciseptatum	CBS 113550	NZ, Keesbury Estate	blackening areas in wood and base of trunk of *Vitis* sp.	EF607080	EF607069
Cyl. pauciseptatum	CBS 120171	Slo., Krško, vineyard	partly decayed roots of 4-year-old, still living but badly shooting *Vitis* sp.	EF607089	EF607066
Cyl. pauciseptatum	CBS 120173	Slo., Krško, vineyard	partly decayed roots of 4-year-old, still living but badly shooting *Vitis* sp.	EF607088	EF607068
Cyl. pauciseptatum	KIS 10763	Slo., Mrzlak, vineyard	brownish spots of healthy looking root of ca. 12-year-old, possibly dead *Vitis* sp.	EF607085	EF607071
Cyl. pauciseptatum	KIS 10775	Slo., Ivanovci, vineyard	strongly decayed, entirely black rootlets of 6-year-old, declining and dying *Vitis* sp.	EF607087	EF607072
Cyl. pauciseptatum	KIS 10778	Slo., Ivanovci, vineyard	decayed, partly blackish-brown and entirely rotten roots of 6-year-old, declining and dying *Vitis* sp.	EF607083	EF607073
Cyl. pauciseptatum	KIS 10780	Slo., Ljutomer, vineyard	decayed secondary roots with black areas of 3-year-old, dead *Vitis* sp.	EF607084	EF607074
Cyl. pauciseptatum	KIS 10798	Slo., Bogojina, vineyard	black areas on fine roots of partly decayed root system of 5-year-old, still living, wilting and dying *Vitis* sp.	EF607081	EF607075
Cyl. pauciseptatum	KIS 10799	Slo., Bogojina, vineyard	blackish lesions on roots of 5-year-old, still living, declining *Vitis* sp.	EF607082	EF607076
Cyl. pauciseptatum	CBS 120172	Slo., Žužemberk, vineyard	strongly decayed, blackish brown root of ca. 9-year-old, possibly dead *Vitis* sp. in vineyard	EF607086	EF607070
Cyl. theobromicola	CBS 218.67, ATCC 16546, IMI 112161a	Papua New Guinea, New Britain, Keravat	*Theobroma cacao*	EF607092	EF607062

GenBank accession numbers are listed in Table 1. They were compared with sequences downloaded from GenBank (www.ncbi.nlm.nih.gov) and by using preliminary published alignments (Halleen *et al.* 2004), to which newly generated sequences were added. Phylogenetic relationships were estimated from the aligned sequences by the maximum parsimony (MP) criterion as implemented in PAUP 4.0b10 (Swofford 2003). Heuristic searches for most parsimonious trees were done using starting trees obtained *via* stepwise and random, 100 times repeated sequence addition, the tree-bisection-reconnection (TBR) branch-swapping algorithm, a MAXTREES setting to 1000, and MULTREES option in effect. Only parsimony informative and unweighted characters were used in tree searches. Branch support was tested on 1000 bootstrapped datasets using the same parsimony settings but a 10 times repeated sequence addition per repeat. The dataset consisting of ITS rDNA sequences was also analyzed according to the Bayesian phylogeny (BP) inference. The substitution model implemented in the BP analysis was selected according to the akaike information criterion (Posada & Buckley 2004) as implemented in modeltest 3.7 (Posada & Crandall 1998). Selected was a general time reversible substitution model with unequal base frequencies, 6 rate categories, a proportion of invariable sites and a gamma distribution of the remaining sites (GTR + I + G). The program MrBayes 3.1.2 (Huelsenbeck *et al.* 2001) was run for 5 M generations with four markov chains sampled every 100 generations starting from a randomly selected tree. A 50 % majority rule consensus tree and posterior probabilities for each split were calculated after excluding the first 10 000 generations from the set of sampled trees. Another BP analysis was done only on data of the internal transcribed spacer region 2 for inferring the phylogenetic position of *Neon. austroradicicola*. A total of 146 characters of the ITS rDNA from indels present only in some of the included taxa or regions difficult to align were excluded from all analyses.

Morphological examinations

Strains were grown on media including SNA with and without the addition of a 1×3 cm piece of filter-paper to the colony surface (Nirenberg 1976), SNA-C, PDA, oatmeal agar (OA; Gams *et al.* 1998) and potato carrot agar (PCA; Gams *et al.* 1998). In order to induce formation of perithecia, strains and crosses of strains were also grown on water agar (WA; Gams *et al.* 1998) with the addition of a piece of sterile carnation leaf and autoclaved *Pinus* needles — but no perithecia were observed in any cross of strains of *Cyl. pauciseptatum*. For general morphological characterisations, cultures were incubated in darkness at 20 °C and 25 °C. Microscopical features were described from SNA, SNA-C or PCA after 14–21 d or up to five wks; macroscopical features, e.g. pigments of colonies and surfaces of colonies were described from PDA after 14 d of incubation in darkness using colour codes from Kornerup & Wanscher (1978); all but particularly badly sporulating strains were grown on PCA for several wks; colony diameters were measured on PDA after 7 or 14 d at 5 (+/− 2), 10, 15, 20, 25, 30, 33, and 35 °C. The effect of incubation of PDA cultures under continuous near-UV light (400–315 nm, Sylvania Blacklight-Blue) at 20 °C was measured. Images were taken from slides mounted in water or lactic acid. Petri dishes were sealed with Parafilm or kept unsealed in sets of eight in perforated plastic bags. Herbarium material was mounted in lactic acid and examined microscopically.

Results

The maximum parsimony analysis of sequences spanning both ITS regions yielded 468 equally most parsimonious trees that had a length (TL) of 328 steps, consistency index (CI) of 0.546, retention index (RI) of 0.867 and homoplasy index (HI) of 0.454. 103 characters were parsimony informative. Most of the equally parsimonious trees (74 %) had an overall topology as the 50 % majority rule consensus tree from BP inferences shown in Fig 1. The group of ''*Cyl. cylindroides* and other species'', whose monophyly was only weakly supported in bootstrap analyses, formed a paraphyletic assemblage in 26 % of equally parsimonious trees. The equally parsimonious trees showed also variable branching patterns within the *Neon. radicicola* and the *Neon. mammoidea* groups (details not described). Heuristic parsimony analyses of aligned partial beta-tubulin intron and exon sequences yielded six equally most parsimonious trees, of which one is shown in Fig 2. The analysis was based on 179 parsimony-informative characters. The trees were 428 steps long and had a CI of 0.675, a RI of 0.938 and a HI of 0.325.

Generic names of taxa with purportedly unsettled generic classification are hereafter placed in quotation marks. In sequence analyses, the ex-type isolate of ''Cyl.'' *theobromicola* (CBS 218.67, isolated from *Theobroma cacao*) clustered together with ''Neon.'' *lucida*, ''Neon.'' *trachosa* and ''Cyl.'' *ianthothele* (anamorph of ''Neon.'' *discophora*), all belonging to the ''Neon.'' *mammoidea* group (clade III of Brayford *et al.* 2004), and ''Cyl.'' *olidum*. Members of this clade are closely related to the genus *Campylocarpon*, established for species not forming microconidia and slightly but consistently curved macroconidia (Halleen *et al.* 2004) (Figs 1 and 2). The ''Neon.'' *mammoidea/Campylocarpon* clade is distantly related to *Neonectria/Cylindrocarpon s. str.* (Figs 1 and 2). *Cylindrocarpon s. str.* includes *Cyl. cylindroides* and closely related species, *Cyl. macrodidymum* and *Cyl. pauciseptatum* and members of the *Cyl. destructans* complex including *Cyl. destructans* var. *crassum*, *Cyl. liriodendri* (teleomorph, *Neon. liriodendri*), *Cyl. macroconidialis* (teleomorph, *Neon. macroconidialis*) and *Cyl. coprosmae* (teleomorph, *Neon. coprosmae*) (Figs 1 and 2). A BP analysis only based on sequences of the ITS 2 placed *Cyl. austrodestructans* (teleomorph, *Nectria austroradicicola*) into the *Neon. radicicola* species complex (Fig 1). The BP and MP analyses of the ITS rDNA (Fig 1) and the MP analysis of the beta-tubulin gene (Fig 2) strongly supported sister grouping of *Cyl. pauciseptatum* and *Neon. macrodidyma* and moderately supported that these two species are closely related to the *Neon. radicicola* species complex. The strains from New Zealand and Slovenia are similar in morphology and have identical ITS rDNA sequences but differ from each other by 7 nucleotides in the partial beta-tubulin gene analysed in Fig 2.

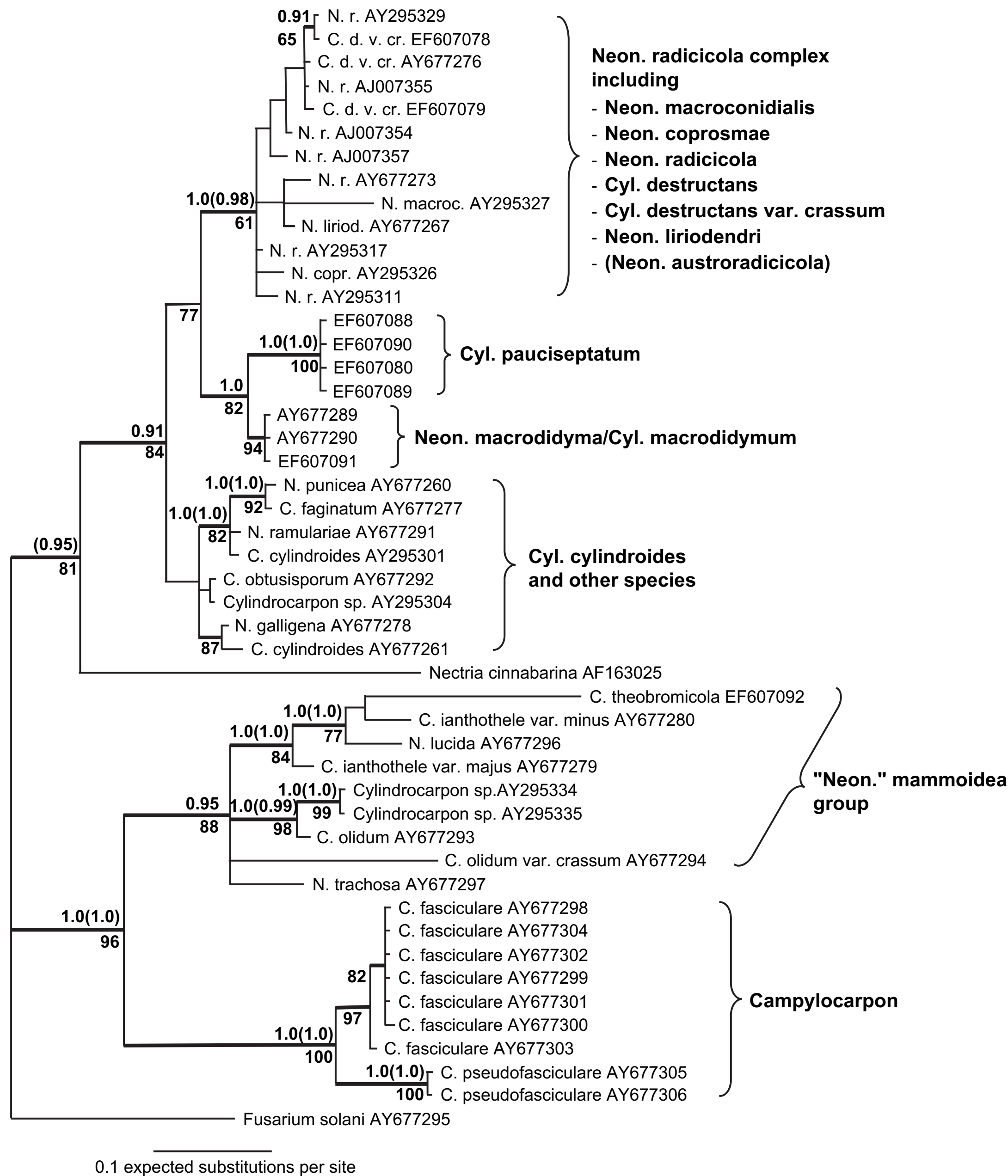

Fig 1 – 50 % majority rule consensus tree of Bayesian Markov chain Monte Carlo sampling inferred from sequences of the ITS 1, 5.8S rDNA and ITS 2 for *Cylindrocarpon* and *Campylocarpon* taxa using a sequence of *Fusarium solani* as outgroup. The numbers above branches indicate Bayesian posterior probabilities higher than 0.90. Branches present in all equally parsimonious trees of a parallel full heuristic maximum parsimony analysis are thickened. Numbers below the branches are bootstrap support values higher than 60 of a parallel parsimony analysis. Numbers in brackets above branches refer to a parallel Bayesian phylogeny analysis inclusive of *Neon. austroradicicola* based on data of the internal transcribed spacer region 2.

Taxonomy

Cylindrocarpon pauciseptatum Schroers & Crous, **sp. nov.** (Fig 3)

MycoBank no.: MB 504972

Cylindrocarpo destructanti simile sed macroconidiis 3-septatis, (37–)42–45–47(–54) × (7.0–)8.5–9.0–9.5(–10.0) μm, long.: lat. (4.0–)4.7–5–5.4 (–6.2) et microconidiis subglobosis ali ovoideis (3.5–)4.4–5–5.4 (–7.8) × (2.4–)2.9–3.3–3.7(–4.6) μm, long.: lat. (1.1–)1.3–1.5–1.7(–2.3) distinguendum. *Cylindrocarpo theobromicolae* etiam simile quod macroconidiis quasi rectis et conidiophoris fasciculatis differt. Conidiophora simplicia vel, si sporodochialia, compluries irregulariter ramosa. Cellulae basales stipitis cylindricae.

Typus: **Slovenia**: Krško. From partly decayed roots of 4-year-old, still living but badly shooting *Vitis* sp. in vineyard, May 2005, *M.*

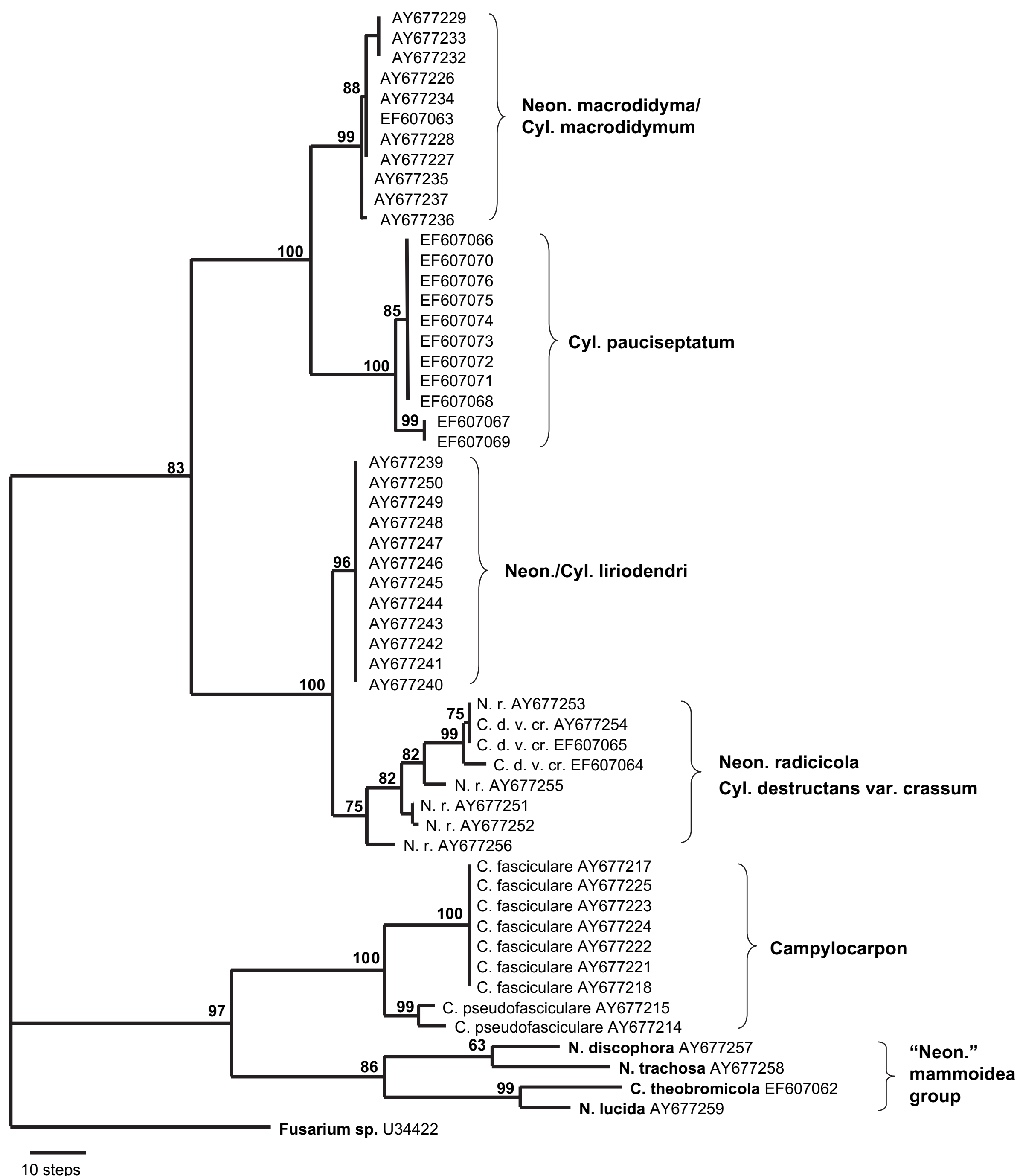

Fig 2 – One of 6 equally parsimonious phylograms for *Cylindrocarpon* and *Campylocarpon* isolates inferred from sequences of partial β-tubulin gene exons and introns using a sequence of *Fusarium solani* as outgroup. Subclades within *Cyl. pauciseptatum* distinguish strains from New Zealand and Slovenia. Bootstrap values higher than 60 are indicated near the nodes.

Žerjav (CBS herb. H-19890–holotypus; dried PDA culture ex CBS 120171).

Etym.: From Latin "*pauci*" meaning few and Latin "*saeptum*" meaning fence, septum, referring to relatively few septa in the macroconidia.

Cardinal temperatures for growth: Colonies on PDA not or hardly growing at 5 °C; optimum temperature 25 °C, when colonies reach 35–50 mm diam after 7 d in the dark; maximum temperature around 30 °C, when colonies reach 10–15 mm; no growth detectable at 33 and 35 °C after 14 d. *Colonies* at 20 °C reaching 13–20 mm diam after 7 d.

Aerial mycelium on SNA and PCA typically absent or sparsely formed, sometimes accumulating near the inoculum or on filter paper, typically white or with age yellowish or with some brownish aspect due to the formation of

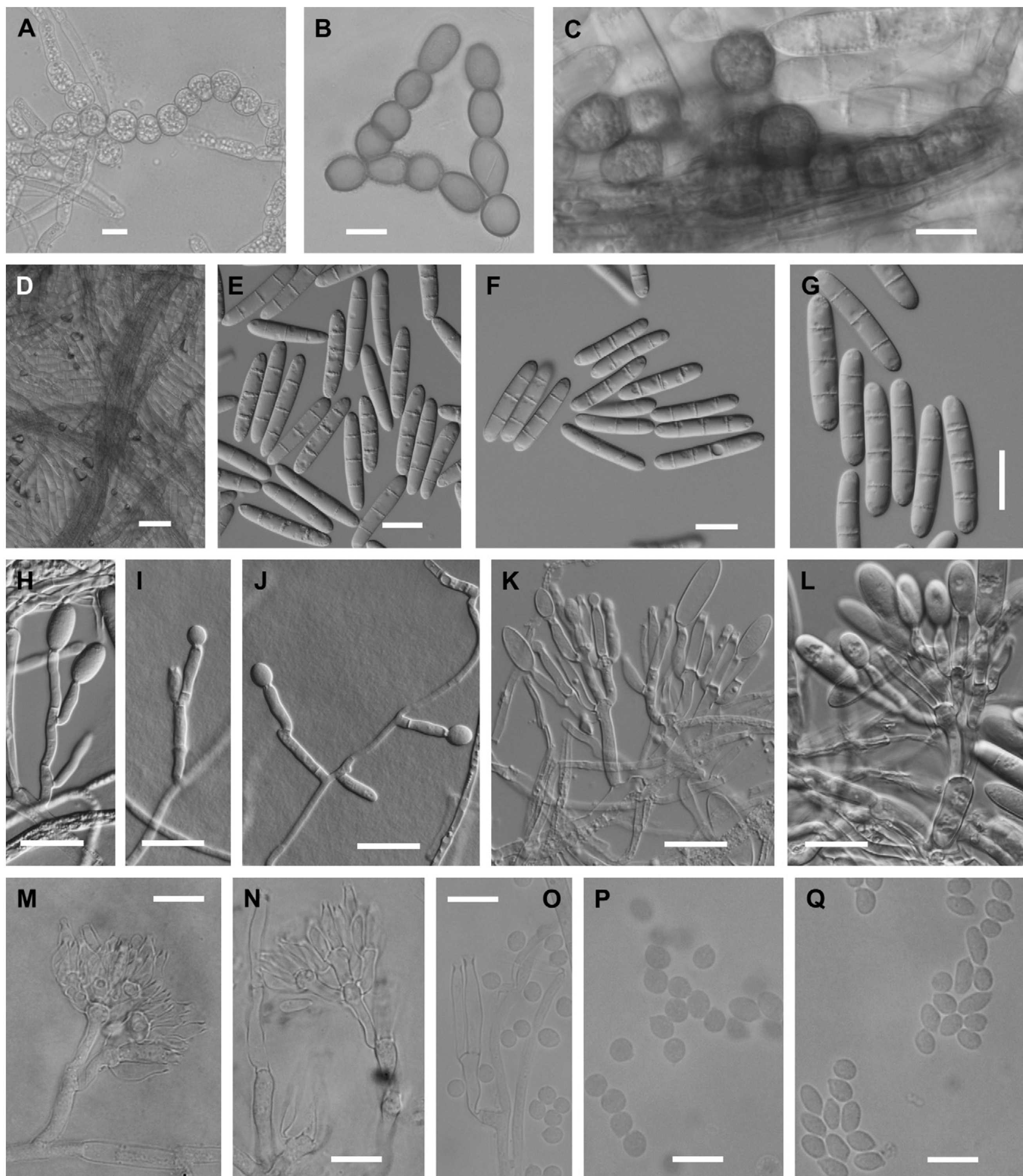

Fig 3 – *Cylindrocarpon pauciseptatum*. (A–C) Chlamydospores. (D) Hyphal strands of aerial mycelium. (E–G) Macroconidia. (H–J) Simple, sparsely branched conidiophores of the aerial mycelium forming macroconidia. (K–L) More frequently branched, ter- to quinqueverticillate conidiophores of sporodochia forming macroconidia. (M–Q) Penicillate or sparsely branched conidiophores (M–O) forming subglobose to ovoidal microconidia (P–Q). (A–D) from OA, (E–L) from two to three-week-old SNA cultures, (M–Q) from five week-old-SNA cultures. (A, C–E, K–L, N–O) from CBS 120171, (B, F–G, Q) from CBS 120173, (H–J, M, P) from CBS 100819. Bars: (A–C, M–Q) = 10 μm, (D) = 30 μm, (E–L) = 20 μm.

chlamydospores; on PDA abundantly formed, but lacking sometimes in sectors, in the centre of older colony arranged in up to 100 μm thick strands, white to greyish brown or golden-brown due to the formation of chlamydospores, particularly when arranged in strands. *Colony surface* on SNA smooth, with time irregularly dotted due to small sporodochia, generally unpigmented or, beneath or on filter-paper pale yellow and later becoming slightly golden-brownish; on PDA white to greyish brown and felty due to aerial mycelium, or in dark orange to brownish hues in sectors without aerial mycelium; on PCA dotted due to small sporodochia, typically without other structures, not pigmented. *Colony reverse* brownish yellow (5C7) to cinnamon-brown (5E6–6E6), typically with a pale yellow to amber-yellow (3A5–4B6) margin, particularly when grown at 20 or 25 °C.

Sporulation by macroconidia and microconidia. *Conidiophores* forming macroconidia simple or complex or sporodochial. Simple conidiophores sparsely formed, arising laterally or terminally from aerial mycelium, solitary, unbranched or sparsely branched, consisting only of phialides or up to three supporting cells bearing 1–3 phialides; phialides monophialidic, more or less cylindrical but slightly tapering in the upper part towards the tip, 11.5–18.5 μm long, 2.5–4.0 μm wide at the base, 3.0–4.5 μm at the widest point, and 2.5–3.5 μm near the aperture. Complex conidiophores aggregated in small sporodochia, repeatedly and irregularly branched; cells of the branched part 15–30 × 4.5–9.0 μm; phialides 12–25 × 3.5–4.5 μm; phialides more or less cylindrical but slightly tapering in the upper part towards the tip or narrowly flask-shaped, mostly with widest point near the middle, (12.5–)14.5–16.5–19.0(–25.5) μm long, (2.5–)3.0(–4.5) μm wide at the base, (3.0–)4.0(–4.5) μm at the widest point, and (2.0–)2.5(–3.0) μm near the aperture. *Conidiophores* forming microconidia encountered in 5 wks old SNA or WA cultures solitary, formed by mycelium near the agar surface, short stiped, simple with one terminal whorl of phialides or penicillately, mostly bi-verticillately branched; phialides monophialidic, densely adpressed, narroly flask-shaped, typically with widest point near the middle, 9–12 μm long, 2–3 μm wide at the base, 2.5–4 μm at the widest point, and 1–2 μm near the aperture.

Macroconidia on SNA and PCA formed in flat domes of slimy masses, predominantly 3, rarely 1-, 2- or 4-septate, straight or minutely curved, more or less cylindrical, with both ends more or less broadly rounded, mostly without a visible hilum; 3-septate macroconidia, (37–)42–45–47(–54) × (7.0–)8.5–9.0–9.5 (–10.0) μm, with a length: width ratio of (4.0–)4.7–5–5.4(–6.2). Masses of conidia slimy, off-white, beige or sometimes with a golden brownish aspect due to conidia whose cells transfer into golden brown chlamydospores. *Microconidia* on SNA formed in heads, 0-septate, subglobose to ovoidal, rarely ellipsoidal, mostly with a visible, centrally located or slightly laterally displaced hilum, (3.5–)4.4–5–5.4(–7.8) × (2.4–)2.9–3.3–3.7(–4.6) μm, with a length: width ratio of (1.1–)1.3–1.5–1.7 (–2.3). *Chlamydospores* golden-brown, formed in intercalary chains or in aggregates, globose to ellipsoidal, 8–15 × 7–14 μm.

Other material examined: **New Zealand**: Tauranga. From rotten roots of *Erica melanthera*, April 1998, *H.M. Dance* (CBS 100819). Keesbury Estate from blackening areas in wood and base of grapevine trunk, April 2003 as NZ C 72 (CBS 113550). — **Slovenia**: Krško. From partly decayed roots of 4-year-old, still living but badly shooting *Vitis* sp. in vineyard, May 2005, *M. Žerjav* (CBS 120173). Žužemberk. From strongly decayed, blackish brown roots of *ca* 9-year-old, possibly dead *Vitis* sp. in vineyard, June 2005, *M. Žerjav* (CBS 120172). Mrzlak. From isolated brownish spots of healthy looking root of *ca* 12-year-old, possibly dead *Vitis* sp. in vineyard, June 2006, *H.-J. Schroers* (KIS 10763). Ivanovci. From strongly decayed, entirely black rootlets of 6-year-old, declining and dying *Vitis* sp. in vineyard, June 2006, *M. Žerjav* (KIS 10775). Ivanovci. From decayed, partly blackish-brown and entirely rotten roots of 6-year-old, declining and dying *Vitis* sp. in vineyard, June 2006, *M. Žerjav* (KIS 10778). Ljutomer. From partly decayed secondary roots with black areas of 3-year-old, dead *Vitis* sp. in vineyard, June 2006, *M. Žerjav* (KIS 10780). Bogojina. From black areas on fine roots of partly decayed root system of 5-year-old, still living, wilting and dying *Vitis* sp. in vineyard, June 2006, *M. Žerjav* (KIS 10798). Bogojina. From blackish lesions on roots of 5-year-old still living, declining *Vitis* sp. in vineyard, June 2006, *M. Žerjav* (KIS 10799).

Cylindrocarpon theobromicola C. Booth, *Mycol. Pap.* **104**: 19 (1966). (Fig 4)

Cardinal temperatures for growth: Colonies on PDA not or hardly growing at 10 °C; optimum temperature 25–30 °C, when colonies reach 30 mm diam after 7 d in the dark; maximum temperature around 33 °C, when colonies hardly grow; colonies at 20 °C reaching around 10 mm diam after 7 d.

Aerial mycelium on SNA and PDA abundantly produced, white. *Colony surface* on PDA cottony because of aerial mycelium, smooth towards the margin, on SNA-C or OA with time irregularly granular due to the formation of small fascicular sporodochia. *Colony reverse* on PDA cinnamon-brown (5E6–6E6) or dark brown (7F4–7F8) with some reddish brown aspects; margin of colonies unpigmented.

Sporulation on pionnotal sporodochia or sporodochial fascicles formed laterally or terminally on supportive hyphae growing near agar surface; supportive hyphae consisting of voluminous angular cells measuring 13–30 × 9–16 μm; central cells of sporodochia similarly voluminous but subglobose; conidiophores arising from central sporodochial cells sparsely branched, mainly consisting of metulae bearing *ca* three phialides; phialides more or less cylindrical but slightly tapering in the upper part towards the tip or narrowly flask-shaped, 10–16 μm long, 2.0–3.5 μm wide at the base, 3.5–4.5 μm at the widest, and 2–3 μm near the aperture.

Microconidia rarely observed, formed by simple, sparsely branched conidiophores on the aerial mycelium, oval, 8.5–9.5 × 5.0–5.5 μm. *Macroconidia* formed on sporodochial fascicles predominantly 3-septate, very slightly and regularly curved, with broadly rounded ends, 37–42–45 × 7.2–8.0–8.7 μm. *Chlamydospores* not observed.

Material examined: **Papua New Guinea**, New Britain, Keravat. From *Theobroma cacao*, 1965, *P.G. Hicks* (living strain CBS 218.67 = ATCC 16546 = IMI 112161a).

Notes: The description provided here focuses on characters not, or differently, described by Booth (1966). He observed numerous diffuse pionnotal sporodochia after 10 d, while we observed only few pionnotal sporodochia or sporodochial fascicles after several weeks on SNA-C and OA, and no sporulation at all on various other media. Most likely this poor sporulation is the result of degeneration of the culture over the past 40 y. Booth (1966) placed "Cyl." *theobromicola* in group 2, whose members are characterised by absence of

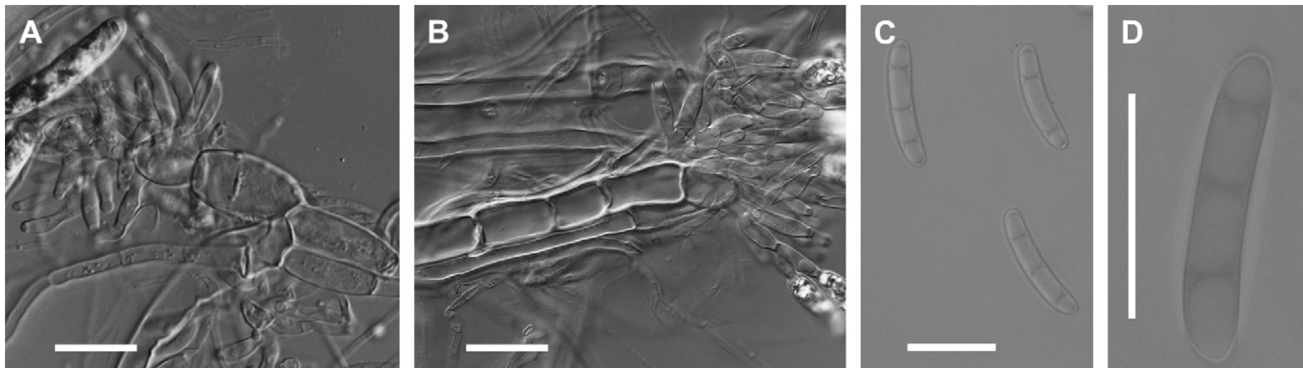

Fig 4 – *Cylindrocarpon theobromicola*. (A–B) Fascicles of once- or twice-branched conidiophores arising from relatively large sized angular to subglobose cells. (C–D) Macroconidia. All from *ca* five-week-old SNA-C cultures of CBS 218.67. Bars: (A–B) = 20 μm, (C–D) = 30 μm.

chlamydospores and microconidia, although he noted that this species forms microconidia at least rarely from sparsely branched and rarely observed conidiophores formed by the aerial mycelium. The few microconidia observed during this study have the same shape and similar range of size as reported by Booth (1966).

The macroconidia illustrated and described by Booth (1966) were 50–70 × 7–11 μm, but during this study, shorter and narrower macroconidia were encountered. Booth also did not mention hyphae with voluminous cells bearing clusters of sparsely branched conidiophores that also occurred in fascicles of *Campylocarpon* (Halleen *et al.* 2004; fig 10 h, m, n). Because they were formed even in presumably degenerated colonies of the ex-type strain, it is possible that such fascicular sporodochia with voluminous cells would also be formed in fresh cultures, but probably only after longer incubation times, because they were not mentioned by Booth (1966). Whether ‘‘*Cyl.*’’ *theobromicola* produces macroconidia of two different size ranges, namely, wide-spored and long macroconidia in relatively young cultures (Booth 1966), and shorter, narrower macroconidia on fascicular sporodochia (Fig 4a–d), can only be determined when new cultures become available.

Neonectria austroradicicola (Samuels & Brayford) Schroers, **comb. nov.**

MycoBank no.: MB 504973.

Basionym: *Nectria austroradicicola* Samuels & Brayford, *Mycol. Res.* **94**: 441, (1990).

Anamorph: *Cylindrocarpon austrodestructans* Brayford & Samuels, *Mycol. Res.* **94**: 441, (1990).

Discussion

Phylogenetic structure of cylindrocarpon-like species

Phylogenetic analyses revealed emerging evidence that hypocrealean taxa with a cylindrocarpon-like morphology are polyphyletic. Species groups most distantly related to the type species of *Neonectria* or *Cylindrocarpon* are found in *Campylocarpon* and representatives of the ‘‘*Neon.*’’ *mammoidea* group. In this study, both taxa form a monophyletic unit in ITS rDNA and beta-tubulin gene trees (Figs 1 and 2), but they appeared paraphyletic based on partial LSU rDNA gene phylogenies (Halleen *et al.* 2004). Two other groups of *Neonectria* or *Cylindrocarpon* species, closely related to each other, are found, respectively, in the (i) *Neon. galligena/coccinea* group, which also includes the type species of *Cylindrocarpon* and *Neonectria* and (ii) *Neon. radicicola* or *Cyl. destructans* group (Brayford *et al.* 2004; Halleen *et al.* 2004), of which the latter is considered to constitute a complex of phylogenetic species (Halleen *et al.* 2006; Seifert *et al.* 2003). Based on the analyses in the current study we conclude that ‘‘*Cyl.*’’ *theobromicola* is a member of the ‘‘*Neon.*’’ *mammoidea* group. Within the ‘‘*Neon.*’’ *mammoidea* group, ‘‘*Cyl.*’’ *theobromicola* is closely related with ‘‘*Cyl.*’’ *ianthothele* (anamorph of ‘‘*Neon.*’’ *discophora* var. *rubi*) and ‘‘*Neon.*’’ *lucida* (Figs 1 and 2).

The type species of *Campylocarpon*, *Camp. fasciculare*, is characterised by distinctive sporodochial fascicles whose densely arranged phialides, and phialide-supporting cells originate from particularly voluminous cells (Halleen *et al.* 2004; fig 10 h, m, n). Similar structures have also been described for ‘‘*Cyl.*’’ *ianthothele* var. *majus* (Booth 1966; fig 14, as ‘‘*Cyl.*’’ *ianthothele* var. *rugulosum*) and ‘‘*Cyl.*’’ *ianthothele* var. *ianthothele* (Wollenweber 1913; fig 14, as *Hypomyces rubi* Osterw. (= *Neon. discophora* var. *rubi*) and, in this paper, in the ex-type strain of ‘‘*Cyl.*’’ *theobromicola* (Fig 4a, b). The morphological characters of the sporodochial fascicles therefore support its phylogenetic relationships as inferred from sequence data of the ITS rDNA (Fig 1) and the beta-tubulin gene (Fig 2). Relatively rarely formed microconidia in ‘‘*Cyl.*’’ *theobromicola* also qualify this species as a member of group 2 of Booth (1966), which comprises several anamorphic species of the ‘‘*Neon.*’’ *mammoidea* group (see also Brayford *et al.* 2004) and *Campylocarpon* (Halleen *et al.* 2004).

Morphological and molecular characters identifying Cylindrocarpon pauciseptatum

The genus *Cylindrocarpon* has been used for hypocrealean species with cylindrical, multiseptate macroconidia that can be longer than 100 μm, and up to 15 μm wide (Samuels & Brayford 1993). Generally, the number of macroconidial septa of many *Cylindrocarpon* species correlates with their length. For example, 3–4-septate macroconidia in several *Cylindrocarpon* species are frequently around 50 μm long, while 7–9-septate macroconidia

sometimes of the same species are longer than 100 μm. Macroconidia that are 10 μm or more wide are often encountered when conidia are relatively long, and have more than four septa (Booth 1966; Samuels & Brayford 1993). However, there are many *Cylindrocarpon* species known, for example, *Cyl. album* (anamorph of *Neon. punicea*) and *Cyl. faginatum* (anamorph of *Neon. faginata*), forming particularly long but relatively narrow macroconidia. A width of up to 10 μm in just 3-septate macroconidia as encountered in *Cyl. pauciseptatum* had been described earlier for only a few species such as *Cyl. destructans* var. *crassum*, ''Cyl.'' *theobromicola*, ''Cyl.'' *ianthothele* (Booth 1966), *Cyl. septosporum* (Samuels & Brayford 1993) and *Cyl. austrodestructans* (Samuels & Brayford 1990). Macroconidia of *Cylindrocarpon pauciseptatum* are typically straight and have rounded ends (Fig 3e–g), while those of *Cyl. septosporum* are clearly curved showing a basal cell with a short, protuberant basal abscission scar (Samuels & Brayford 1993; fig 26). *Cylindrocarpon pauciseptatum*, *Cyl. destructans* var. *crassum* and ''Cyl.'' *theobromicola* form brown pigments in cultures and can be distinguished from those of ''Cyl.'' *ianthothele*, which forms deep purple pigments (Booth 1966; Brayford *et al.* 2004). Very slightly but consistently curved macroconidia also distinguish ''Cyl.'' *theobromicola* from mostly straight macroconidia in *Cyl. pauciseptatum* and *Cyl. austrodestructans*. However, re-examinations of dried herbarium cultures of *Cyl. austrodestructans* revealed that *Cyl. pauciseptatum* and *Cyl. austrodestructans*, which both occur in New Zealand (Table 1), might be morphologically most similar and difficult to distinguish. Relatively wide macroconidia were also described for *Cyl. fraxini*, whose type could not be located in the herbaria of Georgia (Mirian Gvritishvili, pers. commun.). But the few conidia illustrated for this species have a length: width ratio of 5–10 (Mamukashvili 1977), while macroconidia of *Cyl. pauciseptatum* have a length: width ratio of 4–6.

Cylindrocarpon pauciseptatum is the closest phylogenetic sister-taxon of *Cyl. macrodidymum* and both these species are closely related to the *Cyl. destructans* species complex, which also includes *Cyl. macroconidialis*, *Cyl. coprosmae* (Seifert *et al.* 2003), *Cyl. liriodendri* (Halleen *et al.* 2006) and *Cyl. destructans* var. *crassum* (Figs 1 and 2). Straight and predominantly 3-septate macroconidia as well as chlamydospores (but not in *Cyl. coprosmae* and *Cyl. macroconidialis*) and brownish pigmented colonies characterise all these species, whose close phylogenetic relationship is statistically supported (Figs 1 and 2). *Cylindrocarpon austrodestructans* is characterised by the lack of microconidia, while ovoidal to ellipsoidal microconidia are formed at least sparsely or moderately in *Cyl. macroconidialis* (Samuels & Brayford 1990) and *Cyl. macrodidymum* (Halleen *et al.* 2004) and mostly in abundant amounts in *Cyl. liriodendri* (Halleen *et al.* 2006), *Cyl. destructans* var. *crassum* and in most other taxa of the *Cyl. destructans* species complex. The subglobose to ovoidal microconidia formed by *Cyl. pauciseptatum* differ from the ellipsoidal microconidia known in many other *Cylindrocarpon* species. Ovoidal microconidia have been described, however, also for *Cyl. liriodendri* (Halleen *et al.* 2006), but this species also forms ellipsoidal microconidia, which are absent in *Cyl. pauciseptatum*. Three-septate macroconidia formed by *Cyl. austrodestructans* are largely indistinguishable in size and shape from those of *Cyl. pauciseptatum*. *Cylindrocarpon austrodestructans* differs from *Cyl. pauciseptatum* only slightly by macroconidia with higher and more variable numbers of septa, lack of chlamydospores and slower growth at 20 °C (Samuels & Brayford 1990). Specifically, *Cyl. austrodestructans* (*Neon. austroradicicola*) fell into a subclade of the *Cyl. destructans* (*Neon. radicicola*) species complex, while *Cyl. pauciseptatum* was placed outside of this complex (Fig 1). Therefore, the earlier classification of *Cyl. austrodestructans* as a member of the *Cyl. destructans* (*Neon. radicicola*) species complex on the basis of morphological characters (Samuels & Brayford 1990) is confirmed. As a consequence of the phylogenetic placement but also supported by other characters, for example, the perithecia of *Cyl. austrodestructans* (Samuels & Brayford 1990), the new combination *Neon. austrodestructans* is proposed above (see Taxonomy).

This study showed that *Cyl. pauciseptatum* occurs on grapevines in Slovenia and New Zealand. In the latter country, it was also associated with roots of *Erica melanthera* (CBS 100819). The strains of *Cyl. pauciseptatum* so far studied have identical ITS rDNA sequences and are quite homogeneous in their micro- and macromorphology. Consistent nucleotide differences, however, encountered in the beta-tubulin gene introns, distinguish currently known strains from Slovenia and New Zealand (Fig 2). Future studies based on more strains and additional molecular characters may determine, whether the nucleotide differences within *Cyl. pauciseptatum*, as it is defined here, describes intra- or interspecific variation of a single species or several cryptic species, respectively. *Cylindrocarpon pauciseptatum* has been isolated from darkly pigmented, blackish, small lesions on roots and from blackening areas in wood and trunk bases of badly developing *Vitis* sp. rootstocks and sometimes from the roots of apparently dead looking grapevines. The symptoms encountered on the diseased grapevines partly resemble those of the black foot disease of *Vitis* caused by some other cylindrocarpon-like species (Halleen *et al.* 2004), but the specific role of *Cyl. pauciseptatum* as a root pathogen has yet to be determined.

The strains CBS 605.92, 537.92 and 773.83, which had been identified as *Cyl. destructans* var. *crassum* based on morphological characters, originated from roots of *Tilia petiolaris*, wood of *Aesculus hippocastanum* or water, respectively. They were paraphyletic in respect to some isolates of *Cyl. destructans* or *Neon. radicicola*, some of which cluster near strains that had been placed in a clade called ''III b'' based on sequences of the ITS rDNA or the partial beta-tubulin gene (Seifert *et al.* 2003). The strains of this clade included by Seifert *et al.* (2003) originated from various woody hosts such as *Cornus*, *Malus*, *Prunus* and *Panax*.

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