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Phylogeny of *Pezicula, Dermea* and *Neofabraea* inferred from partial sequences of the nuclear ribosomal RNA gene cluster

Edwin C. A. Abeln[1]
Marian A. de Pagter
Gerard J. M. Verkley

Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, The Netherlands

Abstract: The phylogenetic relationship between *Pezicula, Dermea* and *Neofabraea* species (Dermateaceae, Leotiales) was investigated using sequence analysis of part of the 18S ribosomal DNA, the internal transcribed spacer 1, the 5.8S ribosomal DNA and the internal transcribed spacer 2. DNA was isolated from 44 CBS-strains (7 *Dermea,* 3 *Neofabraea* and 34 *Pezicula* species). Parsimony analysis confirmed the morphology-based hypothesis that *Ocellaria ocellata* should be considered as a true *Pezicula* species. Furthermore, the position of two taxa (*Pezicula malicorticis* and *Pe. alnicola*) was found to be outside the *Pezicula* main cluster.

Key Words: anthracnose, *Cryptosporiopsis,* Dermateaceae, ITS, *Ocellaria, Phlyctema,* rDNA, *Scleropezicula,* taxonomy

INTRODUCTION

Pezicula Tul. & C. Tul. nom. cons. is a genus of inoperculate discomycetes classified in the family Dermateaceae (order Leotiales), with *Pezicula carpinea* (Pers.) Tul. & Tul. as the type species (Cannon and Hawksworth 1983). Most species occur in the northern temperate zone growing endophytically on shrubs and trees, whereas some are parasitic. Anamorph connections are known with *Cryptosporiopsis* Bubák & Kabát and *Phlyctema* Desm. (Wollenweber 1939, Johansen 1949, Seaver 1951, Dennis 1974). *Pezicula alba* Guthrie (Guthrie 1959) is connected to *Phlyctema vagabunda* Desm., an anamorph with banana-shaped conidia, phialidic conidiogenesis and eustromatic conidiomata (Zazzerini and Van der Aa 1979, Sutton 1980). Some taxa are known producers of secondary metabolites with antibiotic activity (Noble et al 1991, Schulz et al 1995). The morphological variation exhibited by many taxa in vivo and in vitro resulted in a rather artificial host-based classification. (Wollenweber 1939).

Traditionally, material on conifers is identified as *Pe. livida* and morphologically similar material on deciduous trees as *Pe. cinnamomea.* Kowalski and Kehr (1992) reported the presence of *Pe. cinnamomea* as an endophyte in both deciduous and coniferous hosts (e.g., *Picea abies*), suggesting a wider host range for this species. This was confirmed recently after the investigation of fresh fructifications and isolations from various hosts. (1999).

Several taxonomic questions within or related to *Pezicula* remain to be answered. *Ocellaria* was discriminated from *Pezicula* by most authors on the basis of an immersed, sessile apothecium with a white margin while the apothecium of *Pezicula* is typically more pronounced, lacking the white margin (Wollenweber 1939). However, Groves (1940) found it impossible to draw a clear-cut line between *Ocellaria* and *Pezicula* as he considered the apothecia of *Pe. aurantiaca* and *Pe. corni* to be intermediate forms. In his comprehensive monograph of *Pezicula,* Verkley (1999), also concluded that there were no grounds for maintaining *Ocellaria* as a separate genus, stressing the similarity between the *Cryptosporiopsis* anamorphs.

In his monograph of the American species of *Dermea,* Groves (1946) concluded that it was also impossible to draw a boundary between *Dermea* and *Pezicula,* regarding species such as *Pe. frangulae* and *Pe. alnicola* referable to either genus with equal justification. Although these genera have been classified in different subfamilies by Nannfeldt (1932a) and Korf (1973), the difference between certain *Dermea* and *Pezicula* species is indeed not always obvious nor is their evolutionary relationship within the Dermateaceae.

Jackson (1913) proposed the generic name *Neofabraea* for the newly discovered teleomorph of the apple anthracnose fungus, until then known as *Gloeosporium malicorticis* Cordley [= *Cryptosporiopsis curvispora* (Peck) Gremmen]. Jørgensen (1930) proposed the name *Neofabraea corticola* for another species causing a bark disease on apple and pear. Nannfeldt (1932b) recombined both into *Pezicula,* considering *Neofabraea* a synonym of *Pezicula.* Most taxonomists have followed this author, but many phytopathologists continued to use the name *Neofabraea.*

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[1] Email: abeln@cbs.knaw.nl

To clarify the generic boundaries of *Pezicula, Ocellaria* and *Dermea,* and to get a better insight in the evolutionary relations of these fungi, we compared a part of the gene coding for the 18S small ribosomal subunit RNA, both internal transcribed spacers, and the 5.8S gene.

MATERIAL AND METHODS

The strains used in this study are listed in TABLE I. Strains were transferred from agar cultures to 2 mL liquid medium (2% malt extract) and incubated on a rotary shaker (300 rpm) for 2–3 wk at RT.

DNA techniques.—Liquid cultures were transferred to 2-mL tubes, centrifuged and washed twice with sterile water. Subsequently, 0.05 g of silica (Merck, Darmstadt, Germany) and 300 μL of CTAB extraction buffer was added [200 mm tris(hydroxymethyl)aminomethane (Tris)-HCl, pH 7.5, 1.5 M NaCl, 20 mm EDTA, 2% hexadecyltrimethylammonium bromide (CTAB)]. Tissue was ground with a micropestle for 1 min, another 200 μL of CTAB extraction buffer was added and the lysate was incubated for 10 min at 65 C. A chloroform/isoamylalcohol (24:1) extraction was performed and 2 vol of ethanol were added, followed by an incubation at −20 C for 30 min. The precipitate was centrifuged 5 min and the pellet was washed with 70% ethanol. The pellet was allowed to dry and dissolved in 100 μL TE. Finally, 2.5 μL RNase solution (10mg/mL) was added and the solution was incubated for 5 min at 37 C.

Sequence analysis. A part of the ribosomal RNA gene cluster was amplified by PCR using primers NS7 (5′-gag gca ata aca ggt ctg tga tgc) (White et al 1990) and LS266 (5′-tcc ctt tca aca att tca cg) (Masclaux et al 1995). The amplicon contains about 200 bp of the 3′ end of the gene coding for the 18S rRNA, the ITS1, the gene coding for the 5.8S rRNA, the ITS2 and about 100 bp of the gene coding for the 28S rRNA. PCR was performed in 50 μL reaction volumes and each reaction contained 10–100 ng of genomic DNA, 5 μM of each primer, 200 μM dNTP, 0.5 unit Supertaq DNA polymerase and 5 μL 10× PCR buffer (SphaeroQ, Leiden, the Netherlands). The amplification was performed in a thermocycler (Biomed, type 60) with the following program: 1 min 95 C, 30× [1 min 95 C, 1min 58 C, 1 min 72 C] followed by a final extension of 10 min at 72 C. PCR product was cleaned with sephadex S-300 columns (Amersham Pharmacia Biotech, Roosendaal, The Netherlands) and analyzed on a 2% agarose gel to estimate the concentration. PCR product was sequenced using internal primers Oli14 (5′-ata aca ggt ctg tga tgc cc, corresponding to base 1419–1437 of the 18S rRNA gene of *Saccharomyces cerevisiae)* and ITS4 (5′-tcc tcc gct tat tga tat gc) (White et al 1990). Sequencing was performed with the BigDye terminator chemistry (Part number 403049, PE Applied Biosystems, Foster City, California) following to the manufacturer's instructions. The sequencing products were separated and analyzed on an automated sequencer (ABI377; PE Applied Biosystems). Forward and reversed sequences were matched using SeqMan from the Lasergene package (DNAstar Inc., Madison, Wisconsin). Sequences from the different strains were aligned using MegAlign from the same package. Alignments are available at TreeBASE (S464).

Phylogenetic analysis was performed with a test version of PAUP (Swofford 1998). The data were divided into three character sets. The first character set, the SSU-set (character 79-776) consists of the 3′-end of the small subunit, the second character set (INDEL) comprises a large insert (character 374-757), and the third set (ITS-region) consists of the ITS1, the 5.8S, and the ITS2 (character 777-1265). Support for the branching topologies was evaluated using bootstrap analysis (Felsenstein 1985). Parsimony analysis was performed with exclusion of the INDEL, using a heuristic search with the following parameters: characters were unordered and had equal weight, gaps were interpreted as a fifth base. The maximum number of trees was set at 20 000. The swapping algorithm was tree bisection-reconnection (TBR), the steepest descent option was not in effect and to eliminate unsupported branches, branches collapsed if the maximum branch length was zero. Bootstrap analysis was performed using 1000 replicates and with maxtrees set at 100. In the analysis of the SSU and the ITS-region, *Sclerotinia sclerotiorum* (GenBank X69850 and Z73800) was selected as outgroup. A set of extra outgroup specimens was selected to get a better idea about the place of *Pe. alnicola.* The ITS sequences of the following species were retrieved from GenBank: *Calycellina punctata* (U57494), *Microscypha ellisii* (U57493), *Pycnopeziza sympodialis* (Z81445), *Myriosclerotinia scirpicola* (Z81440), *Pocolum henningsianum* (Z81442), *Scleromitrula calthicola* (Z80886), *Trichopezizella nidulus* (U57813), *Neodasyscypha cerina* (U57812), *Proliferodiscus alboviridis* (U57990), *Solenopezia solenia* (U57991), and *Lachnum nudipes* (U59003). Gaps in this alignment were handled as missing data.

RESULTS

The partial 18S region and the ITS region of 44 strains of *Pezicula* and its anamorphs were sequenced for a phylogenetic analysis. Conflicts between computerized basecalling occurred on average at two positions per contig; these conflicts could always be solved on basis of visual comparison of both electrophorograms. The automatic alignment resulted in a good match between all sequences, but occasionally manual corrections were performed. An insertion of 383 bp was found in four strains [*Pe. cinnamomea* (CBS 236.97, 625.96), *Pe. plantarium* (CBS 290.39) and *Pezicula* sp. (CBS 778.95)] at the end of the 18S rRNA gene (at position 1786 relative to the *S. cerevisiae* 18S rRNA gene). A FASTA search showed similarities of about 65% with other fungal sequences which all appeared to be group-I introns. The highest Z-score was obtained with a group-I intron from *Dactyella copepodii* (U51964), with a 64% identity over a region of 396 nucleotides, interestingly located in the same position in the gene. Other sequences with

TABLE I. Strains studied

Number[a]	Species	Status	Accepted name[b]	Source	Origin	GenBank
CBS 185.50	*Cryptosporiopsis diversispora*	ex-type		*Picea abies*	Norway	AF141165
CBS 191.39	*C. longispora*	ex-type	*P. sporulosa*	*Pseudotsuga menziesii*	UK	AF141166
CBS 898.97	*C. melanigena*	ex-type		*Quercus petraea* (root)	Austria	AF141196
CBS 640.94	*C. radicicola*	ex-type		*Quercus robur* (root)	Poland	AF141193
CBS 161.38	*Dermea acerina*			*Acer rubrum*	Canada	AF141164
CBS 134.46	*D. ariae*			*Sorbus* sp.	Canada	AF141158
CBS 136.46	*D. cerasi*			*Prunus* sp.	USA	AF141159
CBS 137.46	*D. hamamelidis*			*Hamamelis virginiana*	Canada	AF141157
CBS 140.46	*D. padi*			*Prunus* sp.	USA	AF141160
CBS 143.46	*D. prunastri*			*Prunus* sp.	Canada	AF141162
CBS 145.46	*D. viburni*			*Viburnum* sp.	Canada	AF141163
CBS 267.39	*Ocellaria ocellata*		*P. ocellata*	*Salix* sp.	Germany	AF141199
CBS 949.97	*O. ocellata*		*P. ocellata*	?	Luxembourg	AF141181
CBS 239.97	*Pezicula acericola*			*Acer spicatum*	Canada	AF141198
CBS 245.97	*P. acericola*			*Acer spicatum*	Canada	AF141175
CBS 199.46	*P. alni*	ex-type	*P. heterochroma*	*Alnus crispa*	Canada	AF141167
CBS 200.46	*P. alnicola*		*Scleropezicula alnicola*	*Alnus incana*	Canada	AF141168
CBS 474.97	*P. alnicola*		*Scleropezicula alnicola*	*Alnus incana*	Canada	AF141169
CBS 201.46	*P. aurantiaca*			*Alnus crispa*	Canada	AF141170
CBS 921.96	*P. carpinea*			*Carpinus betulus*	Germany	AF141197
CBS 923.96	*P. carpinea*			*Carpinus caroliniana*	Germany	AF141191
CBS 625.96	*P. cinnamomea*			*Quercus robur*	Germany	AF141186
CBS 236.97	*P. cinnamomea*			*Acer saccharum*	USA	AF141185
CBS 285.39	*P. corni*			*Cornus circinata*	Canada	AF141182
CBS 259.31	*P. corticola*	ex-type		*Malus*	Denmark	AF141179
CBS 243.38	*P. corylina*			*Corylus rostrata*	Canada	AF141174
CBS 249.97	*P. corylina*			*Corylus cornuta*	Canada	AF141176
CBS 662.96	*P. eucrita*			*Pseudotsuga menziesii*	The Netherlands	AF141194
CBS 286.39	*P. frangulae*			*Rhamnus* sp.	Germany	AF141183
CBS 778.96	*P. frangulae*			*Rhamnus frangula*	The Netherlands	AF141195
CBS 262.31	*P. livida*		*P. sporulosa*	*Cupressus lawsoniana*	UK	AF141180
CBS 141.22	*P. malicorticis*		*Neofabraea malicorticis*	*Malus sylvestris* (fruit)	Unknown	AF141161
CBS 355.72	*P. malicorticis*		*Neofabraea malicorticis*	*Malus sylvestris* (fruit)	Portugal	AF141189
CBS 290.39	*P. plantarium*		*P. cinnamomea*	*Prunus avium*	Germany	AF141184
CBS 292.39	*P. pruinosa*			*Amelanchier* sp.	Canada	AF141188
CBS 593.96	*P. rubi*			*Rubus* sp.	The Netherlands	AF141192
CBS 251.97	*P. rubi*			*Rubus* sp.	USA	AF141177
CBS 253.97	*P. rubi*			*Rubus* sp.	USA	AF141178
CBS 778.95	*Pezicula* sp.		*P. cinnamomea*	*Larix decidua*	Germany	AF141187
CBS 101.96	*Pezicula* sp.		*Pezicula* sp. 1	*Abies alba*	The Netherlands	AF141173
CBS 224.96	*Pezicula* sp.	ex-type	*P. sporulosa*	*Larix decidua*	The Netherlands	AF141172
CBS 100416	*Pezicula* sp.		*Pezicula* sp. 1	*Amelanchier lamarckii*	The Netherlands	AF141200
CBS 203.46	*P. subcarnea*			*Acer pennsylvanicum*	Canada	AF141171
CBS 452.64	*Phlyctema vagabunda*			*Malus sylvestris*	UK	AF141190

[a] CBS = Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands. http://www.cbs.knaw.nl

[b] Verkley 1999.

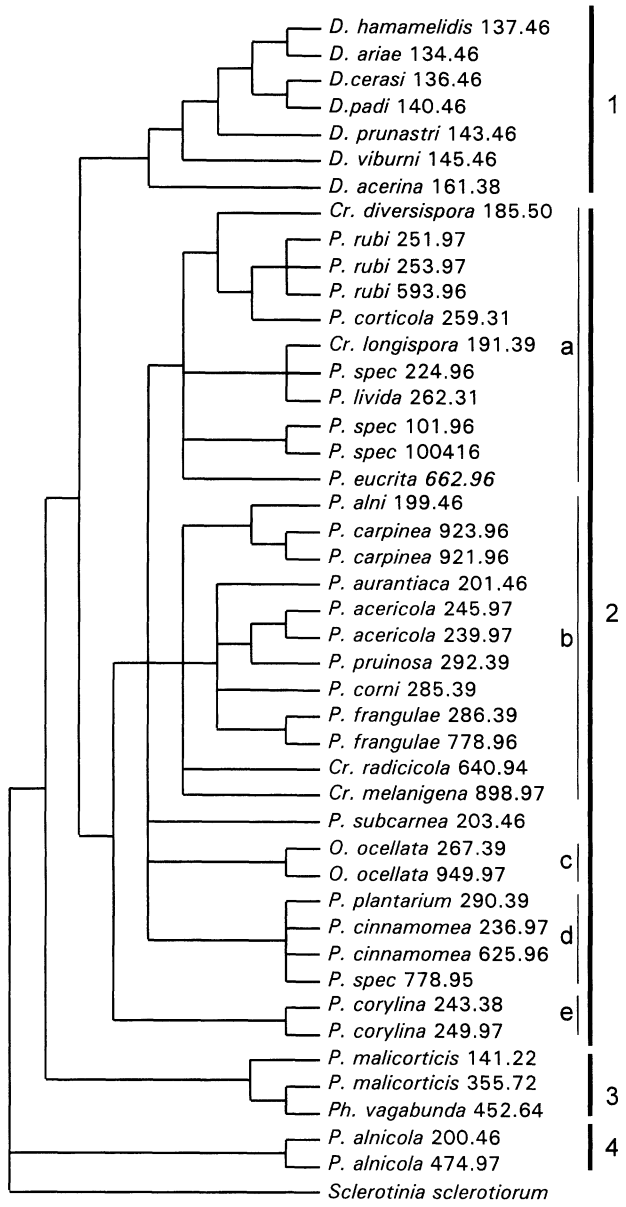

FIG. 1. Strict consensus of 22 most parsimonious trees of 580 steps based on the alignment of the 18S rDNA and the ITS regions, showing the relations between different *Pezicula* species (clade 2–4) and *Dermea* (clade 1).

high identity were group-I introns from predominately ascomycetes and some basidiomycetes.

Included in the phylogenetic analysis were 315 bp of the 3′ part the 18S rRNA gene, the ITS1, the 5.8S gene and the ITS2, giving a total of 803 bp of which 553 (69%) were constant. Of the remaining bases, 172 (21%) were parsimony informative. A parsimony analysis resulted in 22 most parsimonious trees of 580 steps with a consistency index (CI) of 0.57, a retention index (RI) of 0.74 and a rescaled consistency index (RC) of 0.42. The strict consensus of the 22 most parsimonious trees is shown in FIG. 1. All included *Dermea* species end in a monophyletic group (clade 1) while the largest group (clade 2) comprises the vast majority of *Pezicula* strains as well as the *Cryptosporiopsis* and both *Ocellaria ocellata* strains. Within this *Pezicula* core group, several subgroups (clades 2a–2e) can be recognized, providing some extra information about the relationships within *Pezicula*. Clade 2a suggests a closer relationship between *Cryptosporiopsis diversispora* (CBS 185.50), *Pe. rubi, Pe. corticola, Cr. longispora, Pe. livida, Pezicula* sp. (CBS 101.96, 224.96, 100416) and *Pe. eucrita*. Clade 2b comprises *Pe. alni, Pe. carpinea, Pe. aurantiaca, Pe. acericola, Pe. pruinosa, Pe. corni, Pe. frangulae, Cr. radicicola,* and *Cr. melanigena*. Smaller subgroups within the *Pezicula* core group are clades 2c, 2d and 2e. Both *Ocellaria ocellata* strains form one clade (clade 2c) while the strains from *Pe. plantarium* and *Pe. cinnamomea* and strain CBS 778.95 form clade 2d. Clade 2e, comprising the strains from *Pe. corylina,* is placed further outside the *Pezicula* core group, suggesting a more distant relationship. There are two small *Pezicula* clades outside the *Pezicula* core group, which are even outside the *Dermea* clade. The first one (clade 3) comprises the *Pe. malicorticis* strains and one strain of *Ph. vagabunda,* (CBS 452.64; teleomorph *Pe. alba,*). Secondly, clade 4 is even more remotely connected to the *Pezicula* core group and this group comprises both *Pe. alnicola* strains.

The support for the branches was evaluated by bootstrap analysis and is shown in FIG. 2. The *Pezicula* core group is strongly supported as a monophyletic group with a bootstrap of 99%. Within the *Pezicula* core group obvious species clades can be observed, like *O. ocellata, Pe. frangulae* and *Pe. corylina* all with high bootstrap values (97–100%). Other subclades join different species like the clade with *Pe. plantarium* and *Pe. cinnamomea* with strong support (97%). The larger subclades within the *Pezicula* core group, as found in the strict consensus tree, are not supported by high bootstrap values.

Five *Pezicula* strains do not group with the main *Pezicula* clade but form two separate clades both with a bootstrap of 100. Two strains of *Pe. malicorticis* (CBS 141.22, 355.72) form a clade with the *Ph. vagabunda* strain (CBS 452.64; teleomorph *Pe. alba,*) relatively far apart from the *Pezicula* core group. Both *Pe. alnicola* strains (CBS 200.46, 474.97), form a separate clade, similar to the strict consensus tree (FIG. 1). The occurrence of these two separate *Pezicula* clades does not support a monophyletic origin of the genus *Pezicula* as treated by most authors. The seven investigated *Dermea* strains form a separate, paraphyletic assemblage intermediate between the *Pezicula* main group and the *Pe. malicorticis* clade. A strong relationship between *D. hamamelidis* and *D. ariae* is suggested.

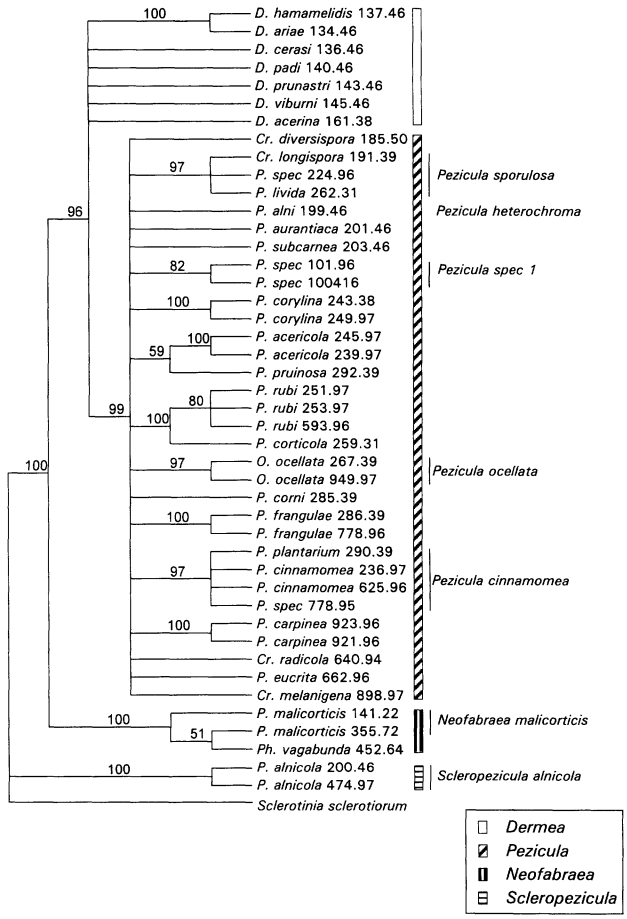

FIG. 2. Parsimony analysis from the 18S rDNA and ITS regions. Bootstrap percentages higher then 50% based on 1000 replications are shown on branches. Bars indicate the different genera. Proposed new names are indicated in the text.

To investigate whether *Pe. alnicola* and the *Pe. malicorticis* have closer relatives among the Leotiales, an alignment of the ITS-region of a selection of the investigated strains was performed with other taxa of this order. Of 544 characters 309 were constant, 71 were parsimony uninformative and 164 were parsimony informative. A parsimony analysis was performed and resulted in two equally parsimonious trees of 569 steps with CI of 0.58, a RI of 0.61 and a RC of 0.36. The support for the branches was evaluated by bootstrapping as shown in FIG. 3. Four main clades can be recognized in this unrooted tree. All investigated strains of the Dermateaceae except the *Pe. alnicola* strain form a clade supported by a bootstrap of 74%. *Pezicula malicorticis* is the remotest branch in this clade but is not closer to any of the other included species. Also all investigated members of the Sclerotiniaceae form a strongly supported monophyletic group (100%). The two other major clades both comprise species of the Hyaloscyphaceae. The *Pe. alnicola* strain falls in one of these latter two groups, closest to *Calycellina punctata,* suggesting a phylogenetic relation closer to this clade of the Hyaloscyphaceae than to the Dermateaceae or the Sclerotiniaceae.

DISCUSSION

The fungi that were included in this study show several common features. They all develop a well-differentiated stroma, from which one or more conidiomata or apothecia emerge, breaking through the outer layers of the bark of a recently dead host. The structure of the cylindrical–clavate to clavate asci with a well-developed apical apparatus and the ellipsoid to fusoid ascospores are similar in these fungi. Furthermore, conidial dimorphism, i.e., the occurrence of macro- and microconidia, has been found in many species. The frequently observed consecutive development of the anamorph and teleomorph on the same stroma indicates that both states play a significant role in the life cycle.

The name *Ocellaria* was introduced by the French mycologists Tulasne, as a subgenus of *Stictis* Pers. (Tulasne and Tulasne 1865) for *Stictis ocellata.* It was later raised to generic level by Karsten (Karsten 1871) for *O. aurea* Tul. & C. Tul. [= *Pe. ocellata* (Pers.: Fr.) Seaver]. In early classifications, the genus was placed far from *Pezicula* or *Dermea,* (Tulasne and Tulasne 1865, Fuckel 1870, 1871, Rehm 1896), mainly on the basis of the immersed habitat of the apothecia. Only after the connection of *Ocellaria* to the anamorph *C. scutellata* became firmly established and the similarity to the anamorphs of typical *Pezicula* spp. was noted (Wollenweber 1939) *Ocellaria* was referred to the Dermateaceae, to join *Pezicula* (Korf 1973, Dennis 1978). In fact, *Cryptosporiopsis* Bubák & Kabát, the generic name now used for these anamorphs, was introduced for the anamorph of *O. ocellata,* as *C. nigra* Bubák & Kabát [= *C. scutellata* (Otth) Petr.; (Petrak 1921)]. Most taxonomists regarded *Ocellaria* a discrete genus, primarily because of the sessile apothecia and the prominent, white apothecial margin, unknown in *Pezicula.* However, Groves (1940), having studied *Pe. aurantiaca,* a species with almost sessile apothecia (comparable with *O. ocellata*) but otherwise very typical of *Pezicula,* saw little reason to maintain *Ocellaria* as a separate genus. Seaver (1951) agreed with Groves, and proposed the combination *Pezicula ocellata,* effectively reducing *Ocellaria* into synonymy with *Pezicula.* Most authors of floristic works, however, continued to use the name under *Ocellaria.* The parsimony analysis in the present study justifies the conclusion that *Ocellaria ocellata* is congeneric with *Pezicula carpinea,* the type species of *Pezicula,* and that therefore the name *Ocellaria* should not be accepted.

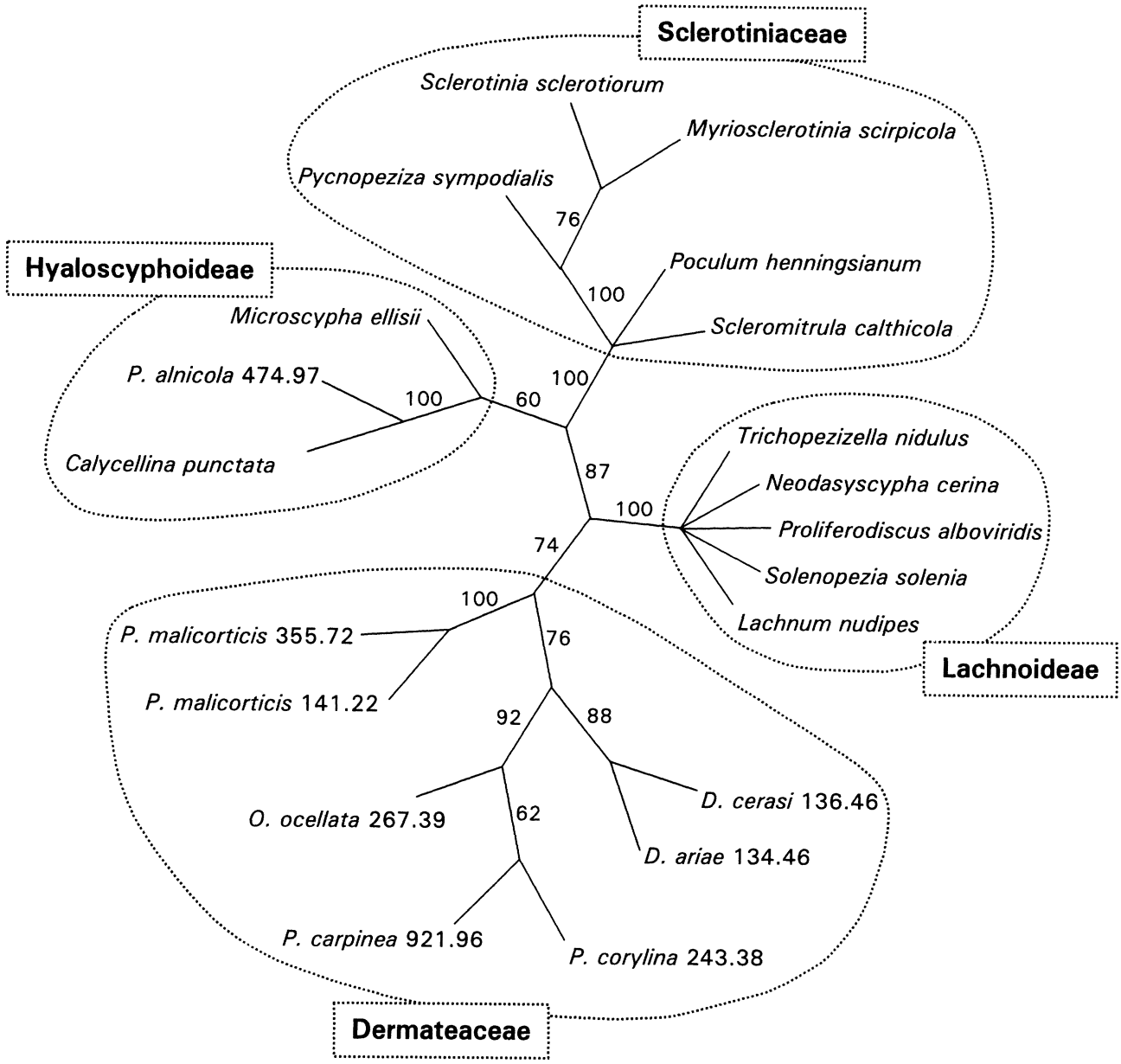

FIG. 3. Unrooted consensus tree of a parsimony bootstrap analysis, using 1000 replicates, based on an ITS-region alignment. Bootstrap values are printed on the branches. Representatives of three families of the Leotiales were included: Dermateaceae, Sclerotiniaceae and Hyaloscyphaceae. Two subfamilies of the latter were included: Hyaloscyphoidea and Lachnoideae.

The phylogenetic analysis further confirms that some of the taxa, formerly assigned to *Pezicula,* belong to different evolutionary lineages that should be recognized at generic level. The first lineage includes two taxa that are pathogens of apple and pear trees: *Pe. malicorticis,* the causal agent of an anthracnose canker and Bull's eye rot of fruit, and *Pe. alba* (anamorph *Ph. vagabunda*), which causes a similar fruit rot, but is also a common saprophyte (Zazzerini and Van der Aa 1979). The former was originally described as the first species in the genus *Neofabraea.* This generic name was accepted by Verkley (1999) for use for these teleomorphs that can be distinguished from *Pezicula* by the lack of well differentiated sterile tissues and apothecial discs merging into indefinite complexes. The anamorphs that occur in *Neofabraea* will be discussed below. No strains of the perennial canker fungus *Pe. perennans* were included in the present study. Whether this fungus is specifically distinct from *Pe. malicorticis* is still under debate (Dugan et al 1993). A recently initiated sequencing study including several strains aims to elucidate this problem. Our results indicate that the ex-type strain of *Neofabraea corticola* (CBS 259.31) (Jørgensen 1930), also causing cankers in apple trees,

is a natural member of *Pezicula.* This is also confirmed by recent analysis of its morphology. Nannfeldt (1932b) already combined this name into *Pezicula.*

A second clade, which seems less related to *Pezicula* comprises *Pe. alnicola.* Groves (1940) considered this species to be an intermediate between *Pezicula* and *Dermea,* but the sequencing data suggest a position far away from either genus. The isolated phylogenetic position of *Pe. alnicola* is further confirmed by the morphology of the anamorph which is unique amongst the eustromatic coelomycetes: conidiogenous cells that proliferate sympodially, and multiseptate conidia provided with a short, blunt cellular appendage, forming their first septa already before liberation. Compared to the cell walls in the stroma of *Pezicula* or *Dermea,* those in *Pe. alnicola* are extremely thickened. On the basis of morphological studies, Verkley (1999) proposed a new generic name for the teleomorph, *Scleropezicula alnicola,* appreciating several morphological peculiarities. The large sequence difference with the other strains and the fact that this clade even falls outside the *Dermea* cluster (FIG. 3) made us wonder if this species should be considered as a member of the heterogeneous family of the Dermateaceae. Searches in GenBank using the taxonomy browser and BLAST showed that the availability of comparable sequences is restricted to the ITS-region of predominantly other families of the Leotiales and not of the Dermateaceae itself. Two of these families have recently been investigated: the Sclerotiniaceae (Holst-Jensen et al 1997) and the Hyaloscyphaceae (Cantrell and Hanlin 1997). Although the use of ITS sequence data may be limited at this taxonomic level, the outcome of the analysis provided extra information about the possible place of *Scleropezicula.* The parsimony analysis showed that this species is more closely related to the Hyaloscyphoideae (a subfamily of the Hyaloscyphaceae) than to any of the included species of the Dermateaceae and the Sclerotiniaceae. In a recent phylogenetic study on basis of morphology and the ITS region of the Hyaloscyphaceae by Cantrell and Hanlin (1997), three different subfamilies are recognized: Hyaloscyphoideae, Arachnopezizoideae and Loachnoideae. There appears to be no obvious support in morphology for the homology in the ITS-region of *Scleropezicula alnicola* with the Hyaloscyphoideae. It has to be emphasized that only a limited number of genera of the Dermateaceae were used in this analysis. Sequences of other important genera of this family like *Mollisia, Pyrenopeziza, Tapezia, Niptera* and *Catinella* were not available, though their inclusion will provide a better understanding of the Dermateaceae.

The monophyly of *Dermea* is not supported by high bootstrap values. However, in the strict consensus tree the strains form a monophyletic clade. The large difference between the sequences of the *Dermea* strains can also explain the weak support for their monophyletic origin. A high amount of mutations makes the presence of homoplasy more likely. Furthermore, the short branch length between the strains of *D. hamamelidis* and *D. ariae* suggests a very close relationship; however, this is not supported by morphology (see below).

The separation of *Dermea, Pezicula,* and *Neofabraea* as inferred from the sequence data is supported by morphological differences. In *Dermea,* the apothecia are typically dark brown to black and hard or leathery (Groves 1946), while in *Neofabraea* and *Pezicula* they are generally brighter in color, soft fleshy or waxy (Verkley 1999). *Neofabraea* is unique in having apothecia that can merge into indefinite complexes and may even contain conidiophores. *Dermea* is characterized by relatively narrow and cylindrical asci, and in this respect resembles *Neofabraea* more closely than *Pezicula. Pezicula* has relatively wide, typically cylindrical–clavate asci. The tips of the paraphyses are entangled and/or glued together in these genera to form an epithecium. In *Dermea* this epithecium is glued together much more strongly and it is much darker than in the other two genera. Only in *Dermea,* the inner sterile tissues of the apothecium are conspicuously brighter than the outer tissues. In this particular feature, *Pe. heterochroma* is an exception to the rule, resembling more the species of *Dermea.*

Within *Pezicula, Neofabraea,* and *Dermea* the conidiomatal structure is quite diverse. Most taxa form eustromatic conidiomata, which are simple or complex, either immersed or erumpent and cylindrical or conical (pycnidioid). Plane or pulvinate acervular conidiomata also occur, particularly in *Pezicula* and *Neofabraea. Dermea hamamelidis* appears to be the only species of *Dermea* forming such acervuli in vivo (Groves 1946). Many of the diagnostic features of conidiomata are lost after isolation in culture. This does not hold for the macroconidia formed in cultures, particularly on oatmeal agar; their shape is fairly constant within *Pezicula* and *Neofabraea.* In the former the macroconidia are typically straight or slightly curved and ellipsoid with a scar at the base, in the latter they are more strongly curved and cylindrical. However, taxa with aberrant macroconidia do occur, e.g., *Pe. rubi,* which has ellipsoid-fusoid macroconidia with pointed tips (Verkley 1999). Groves (1946) demonstrated an even wider range of conidial shapes in *Dermea.* He arranged the accepted species into four informal groups according to size and shape of the conidia, viz. (i) the Cerasi group, with (sub)filiform conidia with sharply pointed ends and over 35 μm long, e.g., in *D. cerasi,* the type species of the genus

Dermea, and *D. viburni* J. W. Grov.; (ii) the Padi group, with conidia similar in shape but rarely exceeding 35 μm in length, e.g., in *D. padi* (Alb. & Schw.: Fr.) Fr., and *D. ariae* (Pers.: Fr.) Tul. ex P. Karst.; (iii) the Prunastri group, conidia elongate-fusiform, e.g., in *D. prunastri* (Pers.: Fr.) Fr., and *D. hamamelidis* (Peck) J. W. Grov.; (iv) conidia ellipsoid, only in *D. acerina* (Peck) Rehm. These informal groups are not supported by the current data set, however, and a definite conclusion can not be drawn since the sequence divergence is large and the number of included *Dermea* strains was limited. Groves noted that the conidia of *D. acerina* closely resemble those of most *Pezicula* spp., but nevertheless regarded its apothecia as fairly typical of *Dermea,* where it is also classified today. Conidiogenous cells are determinate and phialidic, or indeterminate and proliferating in *Pezicula.* So far, only phialidic conidiogenesis has been reported in *Dermea* and *Neofabraea.*

About the internal structure of the *Pezicula* clade, several remarks can be made. Wollenweber (1939) placed *Pe. acericola* and *Pe. aurantiaca* in synonymy of *Pe. cinnamomea,* but Groves (1938, 1940) considered all three as distinct species. *Pezicula cinnamomea* is a species with an extremely wide host-range, including many conifers and deciduous trees such as *Acer* spp. and *Alnus* spp., the typical hosts of *Pe. acericola* and *Pe. aurantiaca,* respectively (Kowalski and Kehr 1992, Verkley 1999). Our isolates of *Pe. acericola* originate from the type substratum *Acer spicatum,* and comply with Groves' morphological description of *Pe. acericola* in culture, which is very different from typical strains of *Pe. cinnamomea.* The *Pe. aurantiaca* strain CBS 201.46 isolated by Groves from *Alnus* sp. can be distinguished by its sessile apothecia with a persistent margin (Groves 1940, Verkley 1999). The results presented here confirm that *Pe. acericola* and *Pe. aurantiaca* are separate species, and that both are genetically distinct from *Pe. cinnamomea.* Likewise, the strain of *Pe. subcarnea,* isolated from *Acer pennsylvanicum,* is genetically distinct although its morphology, apart from the ascopore size and shape, approaches *Pe. cinnamomea* closer than the other taxa (Groves 1941). Groves collected a specimen from *Alnus,* which he considered conspecific with type material he saw of *Pe. alni* (Rehm) Rehm (Groves 1940). Verkley (1999) include that this material is specifically distinct, and proposed the name *Pe. heterochroma,* of which Groves' original herbarium material is the holotype and CBS 199.46 is the ex-type strain. The sequence analysis suggests a relationship close to *Pe. carpinea,* rather remote from *Pe. cinnamomea.* According to Wollenweber (1939), his *Pe. plantarium* from *Prunus avium* is closely related to *Pe. cinnamomea,* but distinguishable by the larger apothecia, asci and ascospores. The measurements however fall well within the range of *Pe. cinnamomea* (Verkley 1999), and the sequencing data confirm that *Pe. plantarium* is conspecific with *Pe. cinnamomea.* The included strains of *Pe. plantarium* as well as *Pe. cinnamomea* contain a totally identical group-I intron, located at exactly the same position in the 18S rRNA gene, but this is not present in any of the other investigated strains. This provides evidence for a recent common evolutionary history and supports the conspecificity of *Pe.cinnamomea* and *Pe. plantarium.* The sequence differences between both *Pe. cinnamomea* strains and between *Pe. plantarium* and *Pe. cinnamomea* are comparable (in both cases five).

A well supported clade consisting of three isolates from conifers, viz. CBS 191.39, the ex-type strain of *Cryptosporiopsis longispora* (van Beyma) von Arx, CBS 262.31, formerly identified as *Pe. livida,* and CBS 224.96 belong to a taxon that was recently recognized at species level as *Pe. sporulosa* (Verkley 1999).

Our results indicate that the *Quercus* root endophytes *Cryptosporiopsis radicicola* and *C. melanigena* are natural members of the *Pezicula* lineage. These species have only been characterized in vitro and may lack a teleomorph entirely. They agree in the presence of chlamydospores, seta-like vegetative hyphae, and the morphology of the macroconidia, although the latter are more distinctly curved in *C. melanigina.* Very little is known about the endophytic populations in roots of woody plants, and further screening of the rhizosphere is likely to reveal more specialist taxa belonging to this fungal group.

Various approaches for the interpretation of gaps introduced in the alignment have been proposed (Bruns 1992, Gu and Li 1995) but so far there is no consensus. When gaps are treated as a 5th base in the phylogenetic analysis, the amount of phylogenetic information may be overestimated since an evolutionary event, e.g., involving a deletion of insertion of 5 nucleotides, is falsely considered as multiple events. Conversely, phylogenetic information may be omitted from the analysis if a deletion or insertion of only one nucleotide, which can be considered a single evolutionary event, is considered as unknown. Since the number of gaps which comprised only one base in the alignment with the 18S rDNA and the ITS-region was relatively high, gaps were interpreted as a 5th base. In the ITS-region alignment with the large set of outgroup taxa gaps were treated as unknown since the frequency of multiple nucleotide gaps was relatively high in this alignment.

In conclusion, the phylogenetic analysis of a part of the ribosomal RNA locus (partial 18S, ITS1, 5.8S, and ITS2) enabled us to elucidate the evolutionary relations between several taxa of the Dermateaceae (FIG. 3). The

position of this family close to certain Hyaloscyphoideae as suggested by ITS data, needs to be corroborated by sequence analysis of more conserved loci. The combination of the morphology and the molecular analysis justifies the introduction of a new genus (*Scleropezicula*) and confirmed the hypotheses that *Ocellaria* belongs to the same genus as *Pezicula* and that *Neofabraea* is a separate evolutionary lineage.

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