INTRODUCTION

This study was initiated with the aim to produce a world monograph of the genus *Pezicula* and its anamorphs, implying a complete revision of *Cryptosporiopsis*. Because of the suspected close relationship with *Pezicula*, the genus *Ocellaria*, and several taxa that had been excluded from *Dermea* by Groves (1946), were also taken into account. Several taxa of *Pezicula* had been studied previously in varying depth by taxonomists and phytopathologists. Taxonomists from Europe and America adopted different species and genus concepts, and no comprehensive taxonomic treatment was available.

The unusual morphological variation characterizing most taxa of *Pezicula* and *Cryptosporiopsis* has been a source of confusion, and inevitably a rather artificial, host-based taxonomy emerged. The introduction of new taxa based on single finds or anamorph taxa based exclusively on characteristics *in vitro* further complicated the situation.

In this study I tried to characterize the teleomorphs and anamorphs on the natural substratum as well as in culture, using newly collected material whenever possible. Subsequently, type-specimens were studied critically in order to settle nomenclature. Basically the same methods were already applied by J. W. Groves in his study of some American taxa during his working life in Ottawa. He built up an invaluable collection of American specimens, which I was previliged to receive on loan from DAOM practically in full. A short visit to North-America gave me an opportunity to collect and study some common and critical species from the same areas. The material available in culture was further characterized by RFLP of the nuclear ribosomal DNA. This extra information proved valuable in species delimitation and offered ways of checking the identity of old (ex-type) strains.

In the four years of studying this group, new insights have emerged about host specificity and applicability of teleomorph and anamorph names. The comparative morphological and molecular study of a fairly large number of isolates of the *P. cinnamomea* complex permitted a reliable characterization and delimitation of its most common and widespread species. However, some taxa still need to be further characterized morphologically and genetically, in particular the species of *Neofabraea*, and several species of *Cryptosporiopsis*. Some species described in *Pezicula* are unrelated and could be referred to other genera. Several specific names could not be evaluated because they are only known from incomplete descriptions, or the original material was found to be in such a bad condition that it could not be identified.

GENERAL PART

History

The Tulasne brothers erected the genus *Pezicula* in 1865 for species with clusters of brightly coloured apothecia bursting out of the bark, associated with an anamorph that forms ellipsoid conidia. They formally combined three species in *Pezicula*, viz. *P. coryli*, *P. amoena*, and *P. dissepta*, all originally described in *Dermatea* Fr. by Tulasne (1853). Tulasne & Tulasne (1865) regarded *Peziza carpinea* Pers. as the 'prototype' of the genus, but did not formally combine it in *Pezicula*, and considered *Peziza rhabarbarina* (= *P. rubi*) closely related. The generic name *Dermatea* was used for species characterized by very dark-coloured, hard apothecia, and anamorphs with 'linear-lanceolate' conidia, viz. *D. cerasi* and *D. seriata* (≡ *Godronia seriata* (Fr.) Seaver). *Peziza frangulae* Pers. was considered a transitional form to *Pezicula*, and placed in *Dermatea* as the sole representative of the '*Dermatellae*', while the typical forms were called '*Eudermateae*'. *Ocellaria* was published in the same work, but as a subgenus of *Stictis* Pers. It was raised to generic level by Karsten (1871) for *O. aurea* Tul. & C. Tul. (= *P. ocellata* (Pers. : Fr.) Seaver).

Before the introduction of *Pezicula* by the Tulasnes, its taxa had been classified in *Peziza* by Persoon (1801, 1822), De Candolle (in De Candolle & de Lamarck, 1815), and Desmazières (1850), in *Stictis*, *Dermatea*, and *Lachnella* by Fries (1822, 1849), and in *Patellaria* by Libert (1834).

Fuckel (1870) accepted *Pezicula* as proposed by Tulasne & Tulasne (1865), in '*Patellariacei*'. He formally combined *Peziza carpinea* (Pers.) Tul. & C. Tul. ex Fuckel into *Pezicula*, and also included *P. frangulae* (Pers. : Fr.) Fuckel. Fuckel assembled species developing globular or beaked conidiomata and dry, horny apothecia in *Cenangium*, including several taxa currently classified in *Cenangium*, *Tympanis* and *Dermea*. Some species with leathery apothecia but unknown anamorph were placed in *Dermatea*, including species of *Encoelia* (Fr.) P. Karst. (*Encoelia furfuracea* (Roth) P. Karst. and *E. fascicularis* (Alb. & Schw.) P. Karst.). *Peziza ocellata* Pers. was referred to *Habrostictis* in '*Stictei*' (Fuckel, 1871), a genus erected for *H. rubra* Fuckel by

Fuckel in 1870. Recently, Baral (1994) proposed to merge *Habrostictis* with *Orbilia* (*Orbiliaceae*).

Karsten (1871) erected the genus *Dermatella* for a single species, *Tympanis frangulae* Fr. : Fr. (= *Peziza frangulae* Pers. : Fr.), which now is regarded to be a member of *Pezicula*. The genus *Pezicula* as conceived by Karsten included *Helotiaceae* currently placed in *Allophylaria* (see excluded species, sub *P. subliciformis* (P. Karst.) P. Karst.), and two further species of *Pezicula*, originally described by Karsten in *Peziza*. Peck (1872) described a taxon in *Nodularia* Peck, which is here accepted as *Pezicula acericola* (Peck) Peck ex Sacc. & Berl.

Phillips (1887) and Rehm (1896) noted the diverging anamorphs, yet maintained a broad concept of *Dermatea*. Besides the type species, *Dermea cerasi* (Pers. : Fr.) Fr., Phillips (1887) included in the genus a few species of *Pezicula*, and also *D. ulicis* Cooke, which Nannfeldt (1939) considered a member of *Encoelia*. He regarded *P. ocellata* as a synonym of *Propolis lecanora*, and as congeneric with *Propolis versicolor* (Fr. : Fr.) Fr. (?*Rhytismataceae*). Rehm (1896) pointed out that the knowledge of the life-cycle was still incomplete for many species, and emphasized the unifying characters of the apothecia. He described several new species, forms and varieties in *Dermatea*, of which *P. aurantiaca* and *P. melastomatis* are accepted species of *Pezicula* in this monograph. Rehm (1896) divided *Dermatea* into three informal groups, *Eudermatea*, *Pezicula*, and *Dermatella*, corresponding with the genera originally proposed by the Tulasne brothers and Karsten. The family '*Dermateae*' further only comprised *Tympanis*. Rehm accepted *Ocellaria* and placed it together with genera such as *Propolis* Fr., *Trochila* Fr. and *Habrostictis* Fuckel in the family '*Eusticteae*', based on convergent characters, viz. waxy, finally widely opening apothecia immersed in the upper layers of the substratum. *Pezicula aurantiaca* was originally described in *Habrostictis* by Rehm, then placed in *Ocellaria*, and finally combined by him into *Pezicula* in 1912. Rehm (1912) made several other combinations when he accepted *Pezicula* at a generic level. Massee (1895) placed several species of *Pezicula* according to ascospore septation in *Cenangium* Fr. (no septum) and *Scleroderris* (Fr.) Rehm (three or more septa), together with species currently classified in *Cenangium* and *Dermea*.

In the classification of the discomycetes presented by Saccardo (1889), *Pezicula* was reserved for species with 0-septate ascospores, and *Dermatella* for species with septate or 'submuriform' ascospores (*Dermatina*). Saccardo did not see a distinct boundary between the two genera, and found a gradual transition also towards *Dermatea* and *Scleroderris*. Later, he referred *Patellaria livida* (= *P. cinnamomea*) to *Durella* (Saccardo, 1889).

Three further species were described by Durand (1904), Petrak in Sydow & Petrak (1922) and Farlow in Thaxter (1922), while Jackson (1913) proposed the generic name *Neofabraea* for the newly discovered teleomorph of the apple anthracnose fungus, by 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 trees. Nannfeldt (1932) recombined both into *Pezicula*, considering *Neofabraea*, *Dermatina* (Sacc.) Höhn. and *Phaeangium* (Sacc.) Sacc. synonyms of *Pezicula*. *Cenangium* subgen. *Phaeangium* was introduced by Saccardo (1889), and raised to generic rank by Saccardo & P. Sydow (1902), but this name is a later homonym of *Phaeangium* Patouillard 1894 (*Otideaceae*), and the identity of its type species is unclear.

Gregor-Wilson (1931) studied material from conifers and recognized three 'growth-forms', which she regarded as belonging to a single species, *Dermatea livida*. Wollenweber (1939) presented detailed descriptions and nomenclature for ten species and their anamorphs, including *P. ocellata* which was accepted under *Ocellaria*, and the newly proposed *Pezicula plantarium* Wollenw. (= *P. cinnamomea* (DC. : Fr.) Sacc.).

In a series of papers published from 1938 to 1947, Groves described several new species of *Pezicula*, including *P. corylina*, *P. hamamelidis* (with Seaver), and *P. subcarnea*. By studying most of these fungi on the natural substratum and in culture, he developed a sound holomorphic species concept. No formal names for the anamorphs were proposed. *Pezicula alnicola* Groves is found to be less related to *Pezicula*, and new names are proposed here for the teleomorph and anamorph, *Scleropezicula* and *Cryptosympodula*, respectively. Groves (1946) also compared the American fungi with European type material and some isolates, and studied several relevant types for his monographic study of the American species of *Dermea*.

Six new names were introduced by Kirschstein (1938, 1941, 1944), one by Wehmeyer (1940), and three by Johansen (1949), who studied eight Danish species. Dennis (1974) gave brief descriptions and valuable drawings, and provided a tentative key to the British species of *Pezicula*. He referred to the problems of the morphological variability and incompletely known host-ranges.

Seaver (1937, 1951; in Seaver & Velásquez, 1933) proposed three new species of *Pezicula* (one with his colleague Groves), and surveyed the American species in his floristic work on the North American Cup-fungi. Seaver (1951) accepted *Pezicula* with 23 species, and also *Dermatella* which, besides the type species *D. frangulae* (Fr. : Fr.) P. Karst., included five probably re-

mote Dermateaceous species with septate, yellow, green or brown ascospores. By combining *Peziza ocellata* into *Pezicula*, he effectively placed *Ocellaria* in synonymy with *Pezicula*. Although most European mycologists had recognized its close relationship with *Pezicula* by then, they preferred to uphold *Ocellaria* (Dennis, 1978). Groves (1940a) already stated: 'when a series of species including *O. ocellata*, *P. aurantiaca*, and *P. corni* are considered, it is found impossible to draw a clear-cut generic distinction between *Ocellaria* and *Pezicula*'. While monographing these fungi I have come to share this view.

Korf (1978), Zhuang & Korf (1988) and Iturriaga & Korf (1997) described new species of *Pezicula* from Macaronesia and Tasmania.

Donk (1957) regarded *Pezicula* Tul. & C. Tul. a nomen nudum, that was only validated by Saccardo (1884: 217), to whom the combination *Pezicula carpinea* was ascribed according to Cannon & Hawksworth (1983; as '*Pezicula carpinea* (Pers.) Tul. ex Sacc.'). However, this combination was in fact already made by Fuckel in 1870. I agree with Farr, Leussink & Stafleu (1979: 1301) and Cannon & Hawksworth (1983) that the generic name should be accepted as dating back from 1865. As such it has priority over *Ocellaria*.

Cannon & Hawksworth (1983) proposed to conserve *Pezicula* Tul. & C. Tul., typified by *Peziza carpinea* Pers., over *Pezicula* Paulet 1791. This proposal was ratified by the International Botanical Congress (Greuter *et al*, 1994: 157). *Pezicula* Paulet was lectotypified by *P. cornucopioides* (L.) Paulet, thus fixing this generic name as an earlier synonym of *Craterellus* Pers., *nom. cons*. As a result, the wide usage of *Pezicula* as a name of an important discomycete genus has been preserved.

The generic name *Cryptosporiopsis* was introduced by Bubák & Kabát (1912) for *C. nigra* on *Salix*. Petrak (1921) noted that this species had already been described as *Sphaerellopsis scutellata* by Otth (1868), and made the necessary combination. Like Höhnel (1906), Petrak suspected that *Ocellaria ocellata* was the alternate state, but the genetic connection was confirmed only after cultural studies by Wollenweber (1939).

Before the establishment of *Cryptosporiopsis*, the anamorphs of *Pezicula* were classified by various authors in a number of genera, as a reflection of the diversity in conidiomatal structure and different interpretations, viz. *Gloeosporium* Desm. & Mont., *Macrophoma* (Sacc.) Berl. & Voglino, *Myxosporium* Link, *Naemaspora* Pers., *Sphaeronaema* Fr., and *Sphaeropsis* Sacc. The conidiomata vary from acervular, i.e. stromatic forms which are plane or pustulate and sporodochioid, to eustromatic forms, i.e. 'pycnostromatal' or pycnidioid. Later, segregate genera *Pachydiscula* Höhn., *Lagynodella* Petr., and *Discosporiella* Petr. were proposed, but these generic names have been considered synonyms of *Cryptosporiopsis* since Petrak (1923), Wollenweber (1939), and von Arx (1957), respectively.

Desmazières (1847) proposed the name *Phlyctema vagabunda* for the anamorph of a species described as *Pezicula alba* by Guthrie (1959). The latter is here combined into *Neofabraea*. The name '*Phlyctaena*' is an orthographic change made by Montagne (1849) and subsequently incorrectly accepted by later authors (Sutton, 1977: 150).

Material and methods

LIVING MATERIAL.— Living material was collected in Europe (Netherlands, France, Germany, Denmark) and North America (New York, Washington, Oregon, and Ontario). Most collections were found on recently dead branches and twigs still attached to the host plant in the field. In addition, the stems of diseased or recently fallen trees were particularly rich substrata. Immersed conidiomata are difficult to find in the field, but often also occur in collections of the more conspicuous teleomorph. Conidia can be produced by stromata that support apothecia, or by separate conidiomata, in which case isolates of ascospores and conidia had to be compared, as more than one species may fruit on the same bark.

STRAINS.— A survey of the strains studied is given in Table 1. More details are given for each species under 'Additional specimens examined', following citation of the dried vaucher specimens that served as the source. A section 'Additional strains examined' is used for cases where no vauchers were available for study.

ISOLATION.— Single-spore isolates were obtained by dissecting fresh apothecia with mature asci from the stroma, and placing them on a small block of agar adhering to the cover of a Petri-dish with malt extract agar (MEA, 2–4%). Spores shot onto the agar surface were checked for germination with the light microscope. Under a dissecting microscope at high magnification (40 ×) small pieces of agar containing a single ascospore were excised with a scalpel and transferred to fresh media. When shooting could not be induced, mass-sexual isolates (MS) were obtained by making squash preparations of apothecial fragments in water, and streaking some material out on plates with MEA or oatmeal agar (OA) with penicillin-streptomycin or aureomycin (20–50 ppm). In some cases germination or growth appeared to be inhibited by the antibiotics, and cherry decoction agar (CHA) was used for isolation.

Conidial isolates were usually obtained by incubating the collected material overnight in a damp chamber, allowing the conidiomata to swell and release masses of conidia that could serve as inoculum.

HERBARIUM MATERIAL.— Dried specimens of personal collections are preserved in the CBS herbarium. Further material was examined from the following herbaria, including many types which were received on loan (herbaria visited personally are marked by an asterisk (*)): B, BPI, BR, C, Concarneau, CUP*, DAOM*, FH, G, GZU, H, IMI, K, L*, LPS, M, NY, NYS, O, OSC, PAD, PC, S, TRTC, UPS, W (abbreviations according to Holmgren *et al.*,1990).

MICROSCOPIC EXAMINATION.— Fresh material was mounted in tap-water. Herbarium material was first allowed to rehydrate in tap-water, and occasionally gently heated. Living and rehydrated material was studied using bright-field and differential-interference contrast (DIC) optics. Line drawings were made with a drawing tube. To observe reactions with iodine, a drop of Lugol's iodine solution (IKI; 0.3 g I_2, 0.6 g KI in 100 ml H_2O) or Melzer's reagent (Mlz; 3.75 g KI, 1.25 g I_2, in 50 ml H_2O and 50 ml chloralhydrate) was added to one side of the cover glass of a water mount, while at the other side water was sucked away with blotting paper.

Measurements of propagules were made on mature, 0-septate ascospores and liberated conidia. Ascospores of species of *Pezicula* are 0-septate when discharged under natural conditions. Later, they become septate, while their size can change under the influence of environmental conditions. Likewise, macroconidia are liberated from the conidiogenous cells in the aseptate stage. In living material on the natural substratum and in culture, only liberated, one-celled ascospores were measured. Whenever possible, measurements of living spores *in vivo* and *in vitro* (turgescent, T) and of rehydrated dead spores (non-turgescent, NT) were compared, and 30 spores were measured per specimen. If a reasonable number of spores could be measured and more than one specimen was examined in this way, the lowest and highest specimen average values are given ('range of averages') with extreme values over all specimens between parentheses, complemented with the total number of specimens (S) and spores (N) measured [(overall min) lowest specimen average – highest specimen average (overall max)]. When only a single specimen was investigated, the average ± Standard Deviation (SD) is given with extreme values between parentheses [(min) average – SD — average + SD (max)]. Whenever possible, asci containing the normal number of one-celled spores, were measured in the turgescent state (prior to dehiscence, T) and non-turgescent, rehydrated state (NT). For each collection, the L/W was calculated from the specimen average length and width of the ascospores. For species with more than one specimen examined, the highest and lowest value of the specimen average L/W is given.

Abbreviations used in the text:

bp	Base pair
CHA	Cherry decoction agar
CMA	Cornmeal agar
d	days
IKI	Lugol's iodine solution
L/W	Length/width ratio (of ascospores)
MA	Mass asexual isolate
MEA	Malt extract agar
Mlz	Melzer's reagent
MS	Mass sexual isolate
N	Sample-size (total number of spores measured)
NT	Non-turgescent, state of living (fresh), rehydrated and/or dead asci or propagules with reduced internal pressure
OA	Oatmeal agar
PCR	Polymerase chain reaction
PDA	Potato-dextrose agar
RFLP	Restriction fragment length polymorphism
S	Number of isolates or specimens examined for size of propagules
SS	Single sexual isolate (ascospore)
SA	Single asexual isolate (conidium)
T	Turgescent, state of living ascus or propagule with maximum internal pressure

Table 1. Isolates studied in this monograph ordered according to CBS accession number, with previous identification (if applicable), revised name, substratum, country of origin, accession number in other culture collections, and nomenclatural status if applicable (LT, lectotype; ST, syntype).

CBS nr.	Previous identification	Revised name	Substratum	Country	Other collections/status
101.96		*Peziculа* aff. *sporulosa*	*Abies alba* (necrosis)	NL	
102.96		*P.* aff. *sporulosa*	*Abies alba* (necrosis)	NL	
109.85	*Cryptosporiopsis* sp.	*Fusicoccum* sp.	*Picea abies* (needle)	?	
139.41	*P. malicorticis*	*Neofabraea perennans*?	*Malus sylvestris*	NL	
140.22	*P. corticola*	?	*Malus sylvestris*	UK	
141.22	*P. malicorticis*	*Neofabraea malicorticis*	*Malus sylvestris* (fruit)	?	ATCC 13903
179.75	*Cr. platanicola*	*Discula umbrinella*	*Platanus* sp.	NZ	LEV 8903
184.50	*Cr. balsamea*	*P. cinnamomea*	*Abies balsamea*	NOR	LT (dried)
185.50	*Cr. diversispora*	*Cr. diversispora*	*Picea abies*	NOR	ST (LT, dried)
191.39	*Cr. longispora*	*P. sporulosa*	*Pseudotsuga menziesii*	UK	LT (dried)
199.46	*P. alni*	*P. heterochroma*	*Alnus crispa*	CAN	ex T
200.46	*P. alnicola*	*Sclerepeziculа alnicola*	*Alnus incana*	CAN	
201.46	*P. aurantiaca*	*P. aurantiaca*	*Alnus crispa*	CAN	
202.46	*P. carnea*	?	? (*Acer* sp.)	CAN	
203.46	*P. subcarnea*	*P. subcarnea*	*Acer pennsylvanicum*	CAN	
203.82	*P. livida*	*P. cinnamomea*	*Chamaecyparis*	NL	
207.57	*P. malicorticis*	*P. perennans*?	*Malus sylvestris* (fruit)	NL	ATCC 13904
211.90	*P. livida*	*P. sporulosa*	*Pseudotsuga menziesii*	F	
215.97		*P. sporulosa*	*Fagus grandifolia*	NY	
219.78	*P. corticola*	*P.* aff. *cinnamomea*	*Malus sylvestris*	CH	
224.78	*P. plantarium*	*P. cinnamomea*	*Picea* sp.	D	
224.96		*P. sporulosa*	*Larix decidua*	NL	ex T
225.96		*P. sporulosa*	*Larix decidua*	NL	ex T
226.96		*P. cinnamomea*	*Fagus sylvatica*	F	
227.96		*P. cinnamomea*	*Fagus sylvatica*	F	
228.96		*P. cinnamomea*	*Betula pendula*	F	
230.79	*P. cinnamomea*	*P. eucrita*	*Acer platanoides*	D	
236.97		*P. cinnamomea*	*Acer saccharum*	NY	
238.96		*P. cinnamomea*	*Betula pendula*	F	
238.97		*P. cinnamomea*	*Carpinus caroliniana*	NY	
239.38	*P. acericola*	*P. acericola*	*Acer spicatum*	CAN	
239.96		*P. cinnamomea*	*Fagus sylvatica*	F	
240.96		*P. cinnamomea*	*Fagus sylvatica*	F	
239.97	*P. acericola*	*P. acericola*	*Acer spicatum*	CAN	
241.96		*P. cinnamomea*	*Picea abies*	F	
242.38	*P. corylina*	*P. corylina*	*Corylus rostrata*	CAN	
242.96		*P. cinnamomea*	*Picea abies*	F	
242.60	*P. cinnamomea*	?	*Quercus* sp.	NL	
243.38	*P. corylina*	*P. corylina*	*Corylus rostrata*	CAN	
243.97		*P. cinnamomea*	*Lindera benzoin*	NY	
245.97	*P. acericola*	*P. acericola*	*Acer spicatum*	CAN	
247.97	*P. acericola*	*P. acericola*	*Acer spicatum*	CAN	
249.97	*P. corylina*	*P. corylina*	*Corylus cornuta*	CAN	
251.97	*P. rubi*	*P. rubi*	*Rubus* sp.	NY	
253.97	*P. rubi*	*P. rubi*	*Rubus* sp.	NY	
256.32	*P. cinnamomea*	*P. cinnamomea*	*Quercus* sp.	DK?	
256.97	*P. livida*	*P. eucrita*	*Pinus sylvestris*	NY	
257.97	*P. livida*	*P. eucrita*	*Pinus sylvestris*	NY	
257.32	*P. livida*	*P.* aff. *cinnamomea*	*Tsuga canadensis*	?	
258.97	*P. livida*	*P. eucrita*	*Pinus sylvestris*	NY	
259.97	*P. livida*	*P. eucrita*	*Pinus sylvestris*	NY	
259.31	-	*P. corticola*	?	DK	ex T
260.31	*P. corticola*	?	*Pyrus malus*	DK	
260.97	*P. livida*	*P. eucrita*	*Pinus sylvestris*	NY	
261.97	*P. livida*	*P. eucrita*	*Pinus sylvestris*	NY	
261.31	*P. livida*	*P. sporulosa*	*Larix decidua*	UK	
262.31	*P. livida*	*P. sporulosa*	*Cupressus lawsoniana*	UK	
262.97	*P. livida*	*P. eucrita*	*Pinus sylvestris*	NY	
263.97	*P. livida*	*P. eucrita*	*Pinus sylvestris*	NY	
267.39	*Ocellaria ocellata*	*P. ocellata*	*Salix sp.*	D	
268.39	*O. ocellata*	*P. ocellata*	*Salix sp.*	D	
268.78	*P. pruinosa*	?	*Prunus domestica*	B	
275.29	*P. malicorticis*	*Neofabraea perennans*?	*Malus sylvestris* (fruit)	?	ATCC 13905
281.47	*P. cinnamomea*	?	*Alnus glutinosa*	?	
282.39	*P. carpinea*	*P. carpinea*	*Carpinus caroliniana*	CAN	
282.47	*P.* sp.	?	*Alnus glutinosa*	?	
283.39	*P. carpinea*	*P. carpinea*	*Carpinus caroliniana*	CAN	
284.39	*P. corni*	*P. corni*	*Cornus circinata*	CAN	
285.39	*P. corni*	*P. corni*	*Cornus circinata*	CAN	
286.39	*P. frangulae*	*P. frangulae* subsp. *frangulae*	*Rhamnus* sp.	D	

CBS nr.	Previous identification	Revised name	Substratum	Country	Other collections/status
287.39	*P. hamamelidis*	?	*Hamamelis virginiae*	CAN	
289.39	*P. livida*	*P.* aff. *sporulosa*	*Picea sitchensis* (root)	UK?	
290.39	*P. plantarium*	*P. cinnamomea*	*Prunus avium*	D	ex T
291.39	*P. pruinosa*	*P. pruinosa*	*Amelanchier* sp.	CAN	
292.39	*P. pruinosa*	*P. pruinosa*	*Amelanchier* sp.	CAN	
293.39	*P. rubi*	*P. rubi*	*Rubus* sp.	CAN	
298.58	*P. cinnamomea*	*P. sporulosa*	*Quercus robur*	NL	
299.58	*P. livida*	?	*Larix leptolepis*	?	
306.49	*P. livida*	*P.* aff. *sporulosa*	*Picea abies* (needle)	NL	
315.96		*P. cinnamomea*	*Abies procera* (necrosis)	G	
316.96		*P. cinnamomea*	*Pseudotsuga menziesii*	F	
317.96		*P. cinnamomea*	*Pseudotsuga menziesii*	F	
318.96		*P. cinnamomea*	*Pseudotsuga menziesii*	F	
319.96		*P. cinnamomea*	*Betula pendula*	D	
320.96		*P. cinnamomea*	*Betula pendula*	D	
321.96		*P. cinnamomea*	*Fagus sylvatica*	D	
322.96		*P. cinnamomea*	*Fagus sylvatica*	D	
322.97		*P. eucrita*	*Picea abies*	D	
323.96		*P. cinnamomea*	*Picea abies*	D	
323.97		*P. eucrita*	*Picea abies*	D	
324.96		*P. cinnamomea*	*Picea abies*	D	
324.97	*P. carpinea*	*P. carpinea*	*Carpinus betulus*	NL	
325.96		*P. eucrita*	*Larix decidua*	D	
325.97	*P. carpinea*	*P. carpinea*	*Carpinus betulus*	NL	
326.96		*P. eucrita*	*Larix decidua*	D	
326.75	*Cr. inaequalis*	*Cr. inaequalis*	*Chamaecyparis* sp.	F	ex T
327.96		*P. cinnamomea*	*Tilia cordata*	D	
328.96		*P. cinnamomea*	*Tilia cordata*	D	
329.96		*P. cinnamomea*	*Tilia cordata*	D	
330.96		*P. cinnamomea*	*Tilia cordata*	D	
350.52	*P. livida*	*P.* aff. *cinnamomea*	*Pinus nigra*	I	
355.51	*P. betulae*	*P. cinnamomea*	*Malus sylvestris* (fruit)	PORT	
355.72	*P. malicorticis*	*Neofabraea malicorticis*	*Malus sylvestris* (fruit)	POR	
362.81	*P. livida*	*P. cinnamomea*	*Juniperus communis*	CH	
450.68	*P. corylina*	?	*Corylus avellana*	B	
452.64	*Phlyctaena vagabunda*	*Neofabraea alba*	*Malus sylvestris*	UK	
453.64	*P. malicorticis*	*Neofabraea perennans* ?	*Malus sylvestris* (fruit)	UK	
474.97	*P. alnicola*	*Scleropezicula alnicola*	*Alnus incana*	CAN	
481.97	*P. cinnamomea*	*P. cinnamomea*	*Betula pendula*	CAN	
482.97	*P. cinnamomea*	*P. cinnamomea*	*Betula pendula*	CAN	
593.96	*P. rubi*	*P. rubi*	*Rubus* sp.	NL	
625.96	*P. cinnamomea*	*P. cinnamomea*	*Quercus robur*	D	
626.96	*P. cinnamomea*	*P. cinnamomea*	*Quercus robur*	D	
627.96		*P. eucrita*	*Pinus sylvestris*	D	
628.96		*P. cinnamomea*	*Alnus glutinosa*	D	
629.96		*P. cinnamomea*	*Alnus glutinosa*	D	
630.96		*P. cinnamomea*	*Acer pseudoplatanus*	D	
631.96		*P. cinnamomea*	*Acer pseudoplatanus*	D	
632.96		*P. cinnamomea*	*Picea abies*	NL	
633.96		*P. cinnamomea*	*Picea abies*	NL	
634.96		*P.* aff. *sporulosa*	*Larix decidua*	NL	
635.96		*P.* aff. *sporulosa*	*Larix decidua*	NL	
636.96		*P.* aff. *sporulosa*	*Larix decidua*	NL	
640.94	*Cr. radicicola*	*Cr. radicicola*	*Quercus robur* (root)	POL	ex T
656.96		*P. eucrita*	*Picea abies*	D	
657.96		*P. eucrita*	*Picea abies*	D	
658.96		*P. eucrita*	*Pinus sylvestris*	NL	
659.96		*P. eucrita*	*Pinus sylvestris*	NL	
660.96		*P. eucrita*	*Pinus sylvestris*	NL	
661.96		*P. eucrita*	*Pinus sylvestris*	NL	
660.95		*P.* aff. *sporulosa*	*Pseudotsuga menziesii*	NL	
661.95		*P.* aff. *sporulosa*	*Pseudotsuga menziesii*	NL	
662.96		*P. eucrita*	*Pseudotsuga menziesii*	NL	
663.96		*P. eucrita*	*Pseudotsuga menziesii*	NL	
664.96		*P. eucrita*	*Pinus sylvestris*	NL	
665.96		*P. eucrita*	*Pinus sylvestris*	NL	
666.96		*P. eucrita*	*Pinus sylvestris*	NL	
667.96		*P. eucrita*	*Pinus sylvestris*	NL	
668.96		*P. eucrita*	*Pinus sylvestris*	NL	
669.96		*P. eucrita*	*Pinus sylvestris*	NL	
670.96		*P. eucrita*	*Pinus nigra*	NL	
671.96		*P. eucrita*	*Pinus nigra*	NL	

Table 1. Continued.

CBS nr.	Previous identification	Revised name	Substratum	Country	Other collections/status
681.83	*P. cinnamomea*	*Cr. radicicola*	*Quercus robur* (root)	A	
693.95		*Cr.* aff. *diplodiodes*	*Sorbus aria*, endophyte	D	
723.95		*P.* aff. *sporulosa*	*Pseudotsuga menziesii*	NL	
778.95		*P. cinnamomea*	*Larix decidua* (necrosis)	G	
778.96	*P. frangulae*	*P. frangulae* subsp. *frangulae*	*Rhamnus frangula*	NL	
779.95		*P.* aff. *sporulosa*	*Cryptomeria japonica*	NL	
898.97	*Cr. melanogena*	*Cr. melanogena*	*Quercus petraea* (root)	A	ex T
921.96	*P. carpinea*	*P. carpinea*	*Carpinus betulus*	D	
922.96	*P. carpinea*	*P. carpinea*	*Carpinus betulus*	D	
925.96	*P. carpinea*	*P. carpinea*	*Carpinus betulus*	D	
939.70	*P. livida*	*P. cinnamomea*	*Abies alba*	D	
949.97	*Ocellaria ocellata*	*P. ocellata*	*Salix* sp. ?	LUX	
950.97	*Ocellaria ocellata*	*P. ocellata*	*Salix* sp. ?	LUX	
100240	*P. cinnamomea*	*P. cinnamomea*	*Larix decidua*	DK	
100244	*P. frangulae*	*P. frangulae* subsp. *frangulae*	*Rhamnus frangula*	DK	
100245	*P. frangulae*	*P. frangulae* subsp. *frangulae*	*Rhamnus frangula*	DK	
100248		*P.* aff. *cinnamomea*	*Abies alba*	DK	
100249	*P. eucrita*	*P. eucrita*	*Larix decidua*	DK	
100275	*P. frangulae*	*P. frangulae* subsp. *frangulae*	*Rhamnus frangula*	DK	
100276	*P. frangulae*	*P. frangulae* subsp. *frangulae*	*Rhamnus frangula*	DK	
100416		*P.* aff. *sporulosa*	*Amelanchier lamarckii*	NL	
136.46	*Dermea cerasi*	*Dermea cerasi*	*Prunus* sp.	USA	

CULTURE DESCRIPTIONS.— Media were prepared according to CBS List of Cultures (Fungi and Yeasts, 34th ed., 1996). After isolation, 5 mm square blocks were taken from the margin of actively growing subcultures and placed upside down in the centre of fresh agar plates. Cultures on malt extract agar (MEA, Oxoid, 3 %), oatmeal agar (OA) and cherry decoction agar (CHA) were placed in an incubator under n-UV (12h light : 12h dark) at 18° C. Colony diameters were measured after 7, 14 and 21 days. Colours were described according to Rayner (1970). Cultures were checked for colour changes after the addition of a drop of 1N NaOH. Sporulating activity varied considerably between species and between isolates of the same species. Most isolates sporulated after (2–)4–8(–16) weeks, usually only with conidia. In some species, however, apothecia also developed, most often on slants with OA and lupin stem under diffuse daylight. Some strains only produced a teleomorph. Generally, spore morphology on OA most closely resembled that seen *in vivo*.

All descriptions are based on fresh isolates. It is important to note that they are valid for species identification provided the above standardized conditions are strictly followed. In addition, cultures were incubated on the laboratory bench under diffuse daylight at room temperature; these are described if different.

Materials and methods used in molecular work

STRAINS AND INCUBATION CONDITIONS.— The strains used for this study are listed in Table 2. Cultures were obtained from the slant stock kept at 4° C or the liquid-nitrogen stock of CBS. After growth on oatmeal slants at room temperature for 1–3 weeks, identity and purity were checked. The strains were transferred to tubes with 100 ml liquid 4% malt extract medium, and incubated at 20° C in darkness for 7 d, rocked in a nearly horizontal position at 50 rev. min^{-1}.

DNA EXTRACTION.— The mycelium was centrifuged in an Eppendorf centrifuge at 14000 rpm, washed twice with sterile water, and frozen at –20 °C for 10 min. DNA was extracted using a miniprep protocol (Möller *et al.*, 1992), starting with the addition of CTAB (Sigma) in high salt concentration. After homogenization with a pistil, the mycelium was incubated at 65°C for 10 min, followed by a chloroform extraction. After centrifugation at 14000 rpm for 5 min, DNA was precipitated with 96% ethanol. The resulting DNA pellet was dissolved in TE-buffer (10 mM Tris-HCl, 10 mM EDTA, pH 8.0).

DNA AMPLIFICATION.— The PCR reaction mixture (50 µl volume) contained *ca* 50 ng DNA, 0.2 mM of each deoxynucleotide triphosphate, 40 pmol of each primer (NS1 and NS24; ITS1 and ITS4; White *et al.*, 1990), and 0.5 U of Taq DNA polymerase (Goldstar, Eurogentec), in 10 mM TRIS, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.01 % gelatin (pH 8.3). Forty amplification cycles were performed, each consisting of 1 min 94°C, 2 min 48°C, and 3 min 74°C, with an initial delay of 2 min at 94°C, in a thermocycler (Biomed type 60). Amplification products were resolved by electrophoresis (4 V cm^{-1}) in 2 % agarose gels in 0.5 % TBE buffer (Sambrook, Fritsch & Maniatis, 1989), supplemented with ethidium bromide. Gels were photographed under UV-

light. Amplicon lengths were estimated using the ImageMaster system (Pharmacia).

RESTRICTION ANALYSIS.— The amplicons were digested for at least 2 h, using 1 U of the following restriction enzymes in separate reactions: *Dde* I, *Hae* III, *Hha* I, *Msp* I, *Rsa* I, and *Taq* I (Pharmacia). Digests were subjected to electrophoresis and photographed as described above. Length of fragments was determined from these photographs, using the ImageMaster system.

CLUSTER ANALYSIS.— Cluster analyses were performed using UPGMA and Neighbor Joining algorithms of PAUP (4.0 d64; Swofford, 1998). The strains that were selected for these analyses are marked with an asterisk in Table 2. For each recognized species or group with identical results on NS1/NS24 and ITS1/ITS4, a reference strain was selected for the data matrix. Strains regarded as belonging to a single species but with slightly different RFLP patterns were also included. A small number of strains with uncertain identity was also included. Each profile differing by one or more bands was scored as a separate character state. For both UPGMA and Neighbor Joining analyses, the strain CBS 200.46 of *Scleropeziculа alnicola* was selected as outgroup, and the question marks in Table 2 were treated as unknown data.

Notes on the morphology of *Pezicula*

Teleomorph

STROMA.— The stroma can develop between a single and (in some species) over 40 apothecia, depending on its size. Stromatal size in *P. cinnamomea* appears to be correlated with the size of the supporting branch. On thin twigs stromata are small, bearing a few apothecia, while on branches and stems they are larger, bearing many more. In contrast, several other species only develop small stromata with mostly a single apothecium, even on large branches.

APOTHECIA.— The apothecia can be entirely sessile, subsessile (i.e., seated on a broad base), or short-stalked. Apothecia of *P. eucrita* that develop underneath flakes of pine bark can form unusually long stalks to fully expose the disc. *Pezicula puberula* also forms long-stalked apothecia. In many species, apothecia develop from old conidiomata. Conidial production is usually arrested when apothecia appear, but in *P. carpinea* and *P. aesculea* it often continues even when apothecia are fully mature. In *P. puberula*, conidiomata and apothecia may develop simultaneously from the same stroma.

DISC.— The disc is circular, but can be deformed by crowding. Initially it is concave to flat, surrounded by a slightly elevated margin. In a later phase, the disc strongly expands and becomes convex in most species. The margin may be damaged in the process, and no longer protrude beyond the periphery of the hymenium. A more strongly developed and persistent margin is diagnostic of some species. The surface of the disc is characteristically pruinose due to protruding tips of paraphyses; in fresh apothecia also the tips of turgescent asci protrude.

RECEPTACLE.— The receptacles are usually of the same colour as the disc, but somewhat darker towards the base. The surface is often minutely pruinose to downy due to free club-shaped cells. Small masses of globular cells in the ectal excipulum can render a pulverulent surface. It should be noted that these features may not fully develop in densely crowded apothecia.

BASAL STROMA, MEDULLARY EXCIPULUM, ECTAL EXCIPULUM.— The colour of these tissues is described from approximately 50–100 µm thick free-hand sections of apothecia observed in water under a dissecting microscope or light microscope at low magnification. Pigments can be distributed homogeneously over the cell walls, or as intercellular granular particles. The degree of pigmentation of these tissues is variable within a species. The ectal excipulum is usually darker than the medullary excipulum, with a gradual transition.

Compared to other genera of the family *Dermateaceae*, the tissue anatomy is unusually variable within a species. At least in species like *P. cinnamomea* the excipulum is rather poorly differentiated into ectal and medullary regions. In *P. ocellata*, *P. puberula*, *P. heterochroma*, and *P. sepium*, however, the regions are more clearly differentiated. The transition in the anatomy from the basal stroma to the medulla also is gradual.

The basal stroma is voluminous in *P. aesculea* and *P. carpinea*, but only weakly developed in *P. melastomatis* and *P. linda*. In species that develop apothecia from old conidiomata, the basal stroma can consist of the former conidiomatal wall to a greater or lesser degree. The structure of the conidiomatal wall is always described separately.

ASCI.— The asci arise from croziers and are inoperculate. They are typically clavate or cylindrical–clavate, and relatively wider than in most species of *Dermea* and *Neofabraea*; but the shape can be quite variable in some species, such as *P. cinnamomea* and *P. acericola*, both *in vivo* and *in vitro*. Other species, like *P. subcarnea* and *P. hamamelidis*, have characteristic broadly clavate asci with very short stalks, and the ascal width distinguishes these species from others found in the

same area. The asci of *P. ocellata* are widest in the lower part, and usually much less abundant. *Pezicula carpinea* can be noted for its long asci, ranging between 150–190 µm.

The apex is rounded, often somewhat conical or truncate. The ascus wall typically is rather stout. The apical apparatus is always well-developed; it becomes fully compressed in mature asci prior to dehiscence at maximum internal pressure (T, turgescent). It is everted upon dehiscence. The apical apparatus consists of a well-developed apical thickening, which is largely occupied by an annulus or ring. The reaction of the annulus with iodine can be positive or negative; it can vary within collections, even within a single apothecium. As already noted by Baral (1992), it also depends on the age of the herbarium specimens. In IKI, the annulus in fresh or recently dried collections mostly shows several layers of material which give a red reaction ('hemiamyloid' *sensu* Baral, 1992). In older herbarium specimens this material often turns distinctly blue with IKI. In Melzer's reagent, a blue reaction is observed in fresh or recently dried material only after pretreatment with KOH. Occasionally old herbarium specimens give a blue reaction without KOH pretreatment. Of several herbarium collections of *Pezicula* spp. examined by Baral (1992), only the oldest (dating from 1877) gave a pale blue reaction in Mlz without pretreatment.

Under normal conditions, most species form eight ascospores per ascus. *Pezicula eucrita* and *P. frangulae* are characterized by 4-spored asci. Like in other members of the genus, eight nuclei are formed and delimited by double membranes, but four degenerate subsequently, and may remain visible as highly refractive bodies adjacent to the four normally developing ascospores. In some collections of *P. eucrita* the four did not degenerate, but remained viable though smaller than the normal ones. In *P. sporulosa*, under optimal conditions most asci contain 8 spores, and some 6 or 4. Instability of sporogenesis was occasionally observed in other species. It is unclear whether this is due to adverse environmental conditions or some kind of genetic degeneration.

The position of the spores within the ascus depends on the state of internal pressure. The terms 'irregularly biseriate' or 'mono-distichous' are applicable to the arrangement of ascospores in non-turgescent or dead asci, 'biseriate' or 'distichous' to that in living asci shortly before dehiscence.

ASCOSPORES.— The ascospores can be heteropolar or homopolar, and are almost always bilateral due to a slight curvature ('inequilateral'). They vary in shape from broadly to elongated ellipsoid, allantoid or fusoid. Spore length and width vary over a wide range within most species of *Pezicula*. As a result, the size-ranges of several taxa overlap. Dennis (1974) already noted that the shape of the ascospores was of taxonomic importance, but difficult to convey in a description. I have chosen the mean length/width ratio (L/W) determined from one-celled spores to describe their shape. This proved to be one of the more valuable characters for species identification. In the unicellular stage, the ascospores have rather thin walls, except in *P. crataegicola* and *P. sepium*, where the walls of 0-septate, hyaline ascospores are up to 0.5 µm thick. In *P. ocellata* they can even be up to 1.0 µm thick. In most species the (released) ascospores hereafter develop several transverse septa, and the thin walls become constricted at the septa, at least in turgescent spores. Subsequently, they can form oblique and longitudinal septa, while the outer wall gradually thickens and becomes pigmented, occasionally minutely roughened.

For purposes of identification, it normally suffices to measure the length and width of 20–30 randomly sampled, one-celled ascospores; the standard deviations usually do not exceed 10 % of the mean length and width. In rehydrated material, ascospores outside and within asci can be measured. In living (and fully hydrated) material it is better to measure free, mature ascospores, which are abundantly present if the material is mature. The high internal pressure of the asci otherwise influences the shape of the contained ascospores.

The sporoplasm of living ascospores contains many guttules or oil droplets. After cell death, the droplets eventually fuse to one or a few irregular oil bodies.

Apothecia collected in the field or grown in culture only ejected one-celled spores, of which over 90 % germinated on MEA. Septate ascospores can germinate from any compartment. When conditions are unfavorable for mycelial growth, the ascospores of some species produce small conidia, which are morphologically similar to typical microconidia of the anamorph. Germination hyphae can differentiate into discrete or integrated conidiogenous cells or the conidia are produced directly from minute openings in the ascospore wall. Such ascospores are common on the hymenium of older apothecia. In *P. eucrita*, spores in disfunctional asci germinate with hyphal outgrows that penetrate the ascus apex and then differentiate into branched conidiophores protruding above the hymenium.

PARAPHYSES.— In some species, the distal parts of the paraphyses entangle in an extracellular matrix containing granular pigment, giving the upper part of hymenium a yellow to brownish colour ('epithecium'). Living apical cells can contain large oil droplets. The level of branching of the paraphyses and the width of intermediate and apical cells are of some diagnostic value.

Anamorph

CONIDIOMATA— The anamorphs of several species of *Pezicula* are difficult to distinguish morphologically. Two types of conidiomata can be distinguished according to ontogenetic criteria, stromatic acervular conidiomata and eustromatic conidiomata. Conidiomata of the first type are open from the beginning, composed of a simple stroma which can remain plane or grow out to a pulvinate mass from which conidiophores arise all over the upper surface. Normally, this type is first still covered by the tissue of the host. Eustromatic conidiomata (term introduced by Sutton, 1980) are initially closed, with a single or several separate or merged cavities, but later they open by small cracks or pores in the upper conidiomatal wall, or they open more widely, sometimes becoming cupulate. In old material these wide open forms can be difficult to distinguish from the pulvinate conidiomata of the first type, as the centre of the stroma may grow out to form a convex surface. *In vivo*, the structure of the eustromatic conidioma and the tissues that constitute its walls vary considerably within several species. But *C. pruinosa* (*P. pruinosa*) and *C. coryli* (*P. corylina*) both form cylindrical to conical conidiomata with a characteristic wall anatomy. Unfortunately, these features are not fully expressed in agar cultures. Species that form acervular conidiomata *in vivo*, often produce very similar acervuloid conidiomata *in vitro*. On sterilized pieces of host substratum the conidiomata resemble those *in vivo* more closely (Groves, 1938b, 1939). Isolates of *Neofabraea*, especially the *N. malicorticis/perennans* complex, may (only) form conidiogenous cells directly on mycelial hyphae, and synnema-like structures may also be formed. Isolates of *Pezicula* will normally only produce conidia from stromata.

CONIDIOGENESIS.— The conidiogenous cells are either discrete, i.e. arising directly from the inner cells of the conidiomatal wall, or integrated in conidiophores. In culture the conidiophores tend to be longer and more branched, while discrete conidiogenous cells rarely occur. In most species, cells producing macroconidia and those producing microconidia are formed in different conidiophores. The conidiogenous cells of either type are described as determinate and phialidic if no proliferation (elongation) is observed during the formation of consecutive conidia. Periclinal thickenings are often seen and in general more pronounced *in vivo*. Conidiogenous cells may also form consecutive conidia at progressive levels, leaving a series of close or more distant scars on the cell apex. These cells are described as indeterminate and proliferating percurrently. These two types of conidiogenesis are distinguished here for practical purposes only. I have repeatedly observed that a single macroconidiogenous cell can show either type of conidiogenesis. Therefore I conclude that both types are probably based on the same cytological process of wall formation, which probably occurs in many coelomycetes. For example, it was observed in a study of the conidiogenesis of two species of *Septoria* using transmission electron microscopy (TEM) by Verkley (1998).In contrast to *Cryptosporiopsis*, conidiogenous cells can be polyblastic in *Septoria*. Sutton & Sandhu (1969) studied the conidiogenesis in *Cryptosporiopsis* sp. using TEM. The conidia of this fungus seceded progressively, almost at the same level, or retrogressively. These authors interpreted the conidiogenous cells as 'annellophores', annelides in the current sense, not annellophores *sensu* Wang (1990).

Microconidiogenous cells are determinate and phialidic, except in *P. rubi*, which was found to form indeterminate and percurrently proliferating cells *in vitro*. Conidiogenesis of these small cells is difficult to assess with the light microscope. To date, microconidia are unknown in a number of species.

CONIDIA.— The shape of the macroconidia is fairly constant within the genus, typically ellipsoid with a rounded apex and base, the latter with a distinct scar. A few species can be recognized by particular conidial properties: the unnamed anamorph of *P. puberula* by cylindrical and mostly strongly curved macroconidia; *C. phaeosora* (teleomorph *P. rubi*) by pointed apices; *C. corticola* by relatively narrow width, and *C. fasciculata* and *C. coryli* by typically broadly ellipsoid (ovoid–pyriform) conidia, those of the latter with a relatively wide and somewhat flattened apex. The conidial measurements are of limited diagnostic value, as the size ranges overlap for many species. The macroconidia of *P. cinnamomea*, *P. sporulosa*, *P. eucrita* and *P. acericola* are practically indistinguishable. Culture media influence shape and size of the macroconidia. Even when incubated under identical conditions, conidial sizes may vary considerably between isolates of the same species, or even subcultures of the same isolate. Nevertheless, under standard conditions recent isolates of *P. cinnamomea*, *P. acericola*, and *P. eucrita* can be identified by characteristics of the colony within 2 weeks, which is usually well before sporulation occurs. Remarkably, isolates from American and European populations belonging to the same species agree closely in vegetative traits of the colony.

Macroconidia show the same process of septation and subsequent formation as described above for the ascospores.

The length of microconidia is a useful character for distinguishing a few species that often occur on conifers, e.g. *P. sporulosa* and *P. eucrita*. The latter was

the only species found to form two structurally distinct conidiomatal types, one producing only microconidia, the other predominantly macroconidia. In other species the same conidiomata produce both conidial types, starting with microconidia, later forming predominantly macroconidia. I did not observe germination of conidia from ascospores or microconidia in culture. In fresh material on the natural substratum these small conidia are difficult to discriminate from morphologically similar conidia of other fungi. It is unknown which structures could serve as the nuclear receptors for these propagules. Gregor-Wilson (1931) once observed a germ tube arising from one end of a conidium originating from an ascospore, while Bayliss Elliott & Chance (1920) stated that the majority of such conidia germinated in hanging drops.

Relation with other genera and systematic position

The relation with *Dermea* and similar genera

Pezicula (including *Ocellaria*) and *Neofabraea* share several macroscopic and microscopic features with *Dermea*, indicating a close relationship. These genera are characterized by the development of a largely hyaline stroma, from which one or more conidiomata and/or apothecia emerge, breaking through the outer layers of the bark of a recently dead host. Microscopically, 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. Similar growth-forms are found in other inoperculate discomycetes that occupy a similar ecological niche, e.g. *Durandiella* Seaver, *Pragmopora* A. Massal., and *Godronia* Moug. & Lév.; but these genera differ in the structure of the asci and ascospores and apothecial anatomy; therefore, *Pragmopora* and *Godronia* have been referred to the family *Helotiaceae* nom. cons. (*Leotiaceae* sensu lato). Another similarity between *Pezicula*, *Neofabraea* and *Dermea* is the conidial dimorphism, viz. the occurrence of macro- and microconidia. The frequently observed consecutive development of the anamorph and teleomorph on the same stroma indicates that both states play an important role in the life-cycle of many species of *Pezicula* and *Dermea*. In contrast, the teleomorphs of *Neofabraea* are rarely collected. Species of *Pezicula* and *Dermea* occasionally have developed parasitic relationships with their hosts, but they usually are harmless endophytes or only weak pathogens. In contrast, species of *Neofabraea* are more explicitly pathogenic.

When Groves (1946) monographed the North American species of *Dermea*, he found it impossible to draw a sharp line between *Dermea* and *Pezicula*, and that there were a few species whose generic position remained more or less a matter of opinion. He referred to *Pezicula frangulae* and *P. alnicola* in this respect (Groves, 1940b, 1946); the former is, however, apart from the dark-coloured excipulum, a typical member of *Pezicula* as already envisaged by Wollenweber (1939); the latter can only be remotely related to either genus on the ground of some unique features, as will be explained below.

Apart from the similarities outlined above, there are a number of differences in teleomorphs and anamorphs of these three genera that must be appreciated. In *Dermea*, apothecia are typically black or very dark brown and hard or leathery, while in *Neofabraea* and *Pezicula* they are generally brighter in colour, and softer and fleshy or waxy. *Neofabraea* is unique in having apothecia with less differentiated excipular tissues, 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 more strongly and it is much darker than in the other two genera. Rather than the anatomy, the contrast in pigmentation of the excipular tissues is diagnostic at generic level. Only in *Dermea* the inner tissues are conspicuously brighter than the outer tissues.

The genus *Ocellaria* was introduced for *Pezicula ocellata*, which has orange, sessile apothecia with a prominent white margin and asci which are broadest in their lower part. However, *Ocellaria* cannot be upheld, because (i) the anamorph is very typical of *Pezicula* spp. both *in vivo* and *in vitro*, and (ii) other species with intermediate (almost) sessile apothecia are known that otherwise agree with typical species of *Pezicula*.

Most anamorphs of *Pezicula* and *Dermea* spp. have 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*. The shape of the macroconidia is fairly constant within *Pezicula* and *Neofabraea*, in the former typically straight or slightly curved and ellipsoid with a scar at the base, in the latter more strongly curved and cylindrical. There are two species that form aberrant conidia: *P. puberula* forms cylindrical and strongly curved macroconidia, and *P. rubi* has ellipsoid–fusoid macroconidia with pointed tips,

yet in both the apothecia are typical of *Pezicula*. In *Dermea* conidial shape varies over a wider range. Groves (1946) arranged the accepted species into four informal groups according to size and shape of the conidia: (i) (sub)filiform with sharply pointed ends and over 35 µm long, e.g. in *D. cerasi*, the type species of the genus; (ii) similar in shape but rarely exceeding 35 µm in length, e.g. in *D. padi* (Alb. & Schw. : Fr.) Fr.; (iii) elongate-fusiform, e.g. in *D. prunastri* (Pers. : Fr.) Fr.; and (iv) ellipsoid, in *D. acerina* (Peck) Rehm. 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 percurrently proliferating in *Pezicula*. So far, only phialidic conidiogenesis has been reported in *Dermea* and *Neofabraea*.

The various conidiomatal forms were described under a number of anamorph genera. Groves (1946) concluded that in principle all anamorphs connected to *Dermea* were to be regarded as *Micropera* states, with the exception of the anamorph of *D. acerina*. He did not propose names for anamorphs that appeared to be undescribed nor did he make new combinations in *Micropera* for those already described under other generic names. A similar approach has been taken here to *Pezicula*. The generic name *Cryptosporiopsis* is used in a broad sense, for anamorphs of *Pezicula* and *Neofabraea*, or similar anamorphs without a known teleomorph, covering a wide range of conidial morphology and conidiomatal structure. An exception is made for the anamorph of *Scleropezicula alnicola*, which is unique among the coelomycetes by its eustromatic conidiomata, sympodial conidiogenesis and *Micropera*-like conidia provided with a basal appendage, and for which the name *Cryptosympodula* is proposed.

The relation of *Pezicula* to other genera in the *Dermateaceae*

The genus *Pezicula* has been classified in the family *Dermateaceae* Fr. by most authors (Nannfeldt, 1932; Korf, 1973; Dennis, 1978). *Pezicula* and *Dermea* were placed in separate subfamilies by Nannfeldt (1932) and Korf (1973). Nannfeldt (1932) placed *Pezicula*, *Ocellaria* (= *Pezicula*), *Habrostictis* (= *Orbilia*), *Ploettnera* Henn., and *Cryptodiscus* Corda in the subfamily *Peziculoideae*, and *Dermatea* (= *Dermea*) and *Bulgariastrum* H. Sydow in the *Dermateoideae*. Korf (1973) accepted the *Peziculoideae fide* Nannfeldt (1932), and emended the *Dermateoideae* to accommodate besides *Dermea* also *Atropellis* Zeller & Goodd., *Dermateopsis* Nannf., *Waltonia* Saho, *Encoeliopsis* Nannf., *Ascocalyx* Naumov, and *Crumenulopsis* J. W. Groves. In more recent classifications the last three genera have been referred to the *Leotiaceae* (now *Helotiaceae* nom. cons.), while *Cryptodiscus* is now generally considered a member of the *Stictidaceae* (*Ostropales*).

In view of the arguments mentioned above, *Pezicula*, *Neofabraea* and *Dermea* should be classified more closely together. These genera are very different from the mollisioid fungi, and could form the core of a family *Dermateaceae sensu stricto*, primarily characterized by taxa with an ectal excipulum composed of *textura angularis* or the same mixed with hyphal elements, and, furthermore, by the frequent occurrence of a stroma and coelomycetous anamorphs with phialidic and/or percurrent conidiogenesis. In fact, various workers have recognized that the present *Dermateacae* is still a heterogeneous family (Hawksworth, 1994). *Mollisia* (Fr.) P. Karst. and related genera, which are characterized by a dark-walled ectal excipulum composed of *textura globulosa–angularis*, and associated with *Phialophora* or similar anamorphs, essentially the *Mollisioideae sensu* Korf (1973), could serve as the backbone of a new family. Both groups may have affinities to certain *Hyaloscyphaceae* Nannf. For example, *Polydesmia* Boud. comes close to *Pezicula* in morphology of the asci and ascospores.

The position of the genera *Scleropezicula* and *Mycosphaerangium* is still uncertain, and both are considered *Helotiales incertae sedis* at this time. In the *Helotiales*, many taxa still need to be critically revised. Molecular studies of only a limited number of selected species could be used as a short-cut to proposals for new suprageneric taxa. However, only a combined phenotypical and genotypical characterization based on larger sets of carefully selected taxa will lead to a stable classification in the future.

A close relationship between *Pezicula* and *Pseudopezicula* Korf was suspected by Korf *et al.* (1986). *Pseudopezicula* was erected to accommodate two species causing an angular leaf scorch disease of grapes ('Rotbrenner'), *Pseudopezicula tracheiphila* (Müller-Thurgau) Korf & W.-Y. Zhuang (basionym *Pseudopeziza tracheiphila* Müller-Thurgau), and a new species from North-America, *Pseudopezicula tetraspora* Korf, R. C. Pearson & W.-Y. Zhuang. The affinity was suspected on the basis of certain common microscopic features, viz. large thin-walled, slightly bent ascospores 'somewhat flattened on one side', the wide, 4- or 8-spored asci provided with an apical apparatus intensely blueing in IKI or Melzer's reagent if pretreated with 2–10% KOH, and the frequently deformed and apically branched 'propoloid' paraphyses, causing the powdery to pruinose appearance of the hymenium. I have not seen material of this genus. Korf *et al.* (1986) also

Table 2. Survey of amplicon lengths and restriction patterns of strains tested with RFLP of the ribosomal RNA gene cluster using primer pairs NS1 and NS24, and ITS1 and ITS4, and six restriction enzymes.

CBS nr.	Previous identification	Revised name	Substratum	Country	NS1 - NS24 Amplicon bp	*Dde* I	*Rsa* I	*Msp* I	*Hae* III	*Hha* I	*Taq* I	ITS1 - ITS4 Amplicon bp	*Dde* I	*Rsa* I	*Msp* I	*Hae* III	*Hha* I	*Taq* I
939.70	*P. livida*	*P. cinnamomea*	*Abies alba*	D	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
362.81	*P. livida*	*P. cinnamomea*	*Juniperus communis*	CH	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
203.82	*P. livida*	*P. cinnamomea*	*Chamaecyparis* sp.	NL	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
241.96	*P.* sp.	*P. cinnamomea*	*Picea abies*	F	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
*625.96	*P. cinnamomea*	*P. cinnamomea*	*Quercus robur*	D	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
224.78	*P. plantarium*	*P. cinnamomea*	*Picea* sp.	D	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
290.39	*P. plantarium*	*P. cinnamomea*	*Prunus avium*	D	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
256.32	*P. cinnamomea*	*P. cinnamomea*	*Quercus* sp.	DK?	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
628.96	*P.* sp.	*P. cinnamomea*	*Alnus glutinosa*	D	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
355.51	*P. betulae*	*P. cinnamomea*	*Betula verrucosa*	PORT	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
319.96	*P.* sp.	*P. cinnamomea*	*Betula pendula*	D	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
481.97	*P. cinnamomea*	*P. cinnamomea*	*Betula pendula*	CAN	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
327.96	*P.* sp.	*P. cinnamomea*	*Tilia cordata*	D	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
238.97	*P.* cf. *cinnamomea*	*P. cinnamomea*	*Carpinus caroliniana*	NY	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
239.96	*P.* sp.	*P. cinnamomea*	*Fagus sylvatica*	F	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
243.97	*P.* sp.	*P. cinnamomea*	*Lindera benzoin*	NY	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
236.97	*P. cinnamomea*	*P. cinnamomea*	*Acer saccharum*	NY	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
631.96	*P.* sp.	*P. cinnamomea*	*Acer pseudoplatanus*	D	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
778.95	*P.* sp.	*P. cinnamomea*	*Larix decidua (necrosis)*	G	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
315.96	*P.* sp.	*P. cinnamomea*	*Abies procera (necrosis)*	G	1800	?	?	?	?	A	A	550	A	A	A	A	B	A
184.50	*Cr. balsamea*	*P. cinnamomea*	*Abies balsamea*	NOR	1800	A	A	A	A	A	A	550	A	A	A	A	B	A
350.52	*P. livida*	*P.* aff. *cinnamomea*	*Pinus nigra*	I	1800	A	A	A	A	A	A	550	A	A	A	A	C	A
257.32	*P. livida*	*P.* aff. *cinnamomea*	*Tsuga canadensis*	?	1800	A	A	A	A	A	A	550	A	A	A	A	C	A
*100248		*P.* aff. *cinnamomea*	*Abies alba*	DK	1800	A	A	A	A	A	A	550	A	A	A	A	C	A
*664.96	*P. livida*	*P. eucrita*	*Pinus sylvestris*	NL	1800	A	A	A	A	A	A	550	B	A	A	A	A	A
256.97	*P. livida*	*P. eucrita*	*Pinus sylvestris*	NY	1800	A	A	A	A	A	A	550	B	A	A	A	A	A
258.97	*P. livida*	*P. eucrita*	*Pinus sylvestris*	NY	1800	A	A	A	A	A	A	550	B	A	A	A	A	A
262.97	*P. livida*	*P. eucrita*	*Pinus sylvestris*	NY	1800	A	A	A	A	A	A	550	B	A	A	A	A	A
260.97	*P. livida*	*P. eucrita*	*Pinus sylvestris*	NY	1800	A	A	A	A	A	A	550	B	A	A	A	A	A
662.96	*P.* sp.	*P. eucrita*	*Pseudotsuga menziesii*	NL	1800	A	A	A	A	A	A	550	B	A	A	A	A	A
656.96	*P.* sp.	*P. eucrita*	*Picea abies*	D	1800	A	A	A	A	A	A	550	B	A	A	A	A	A
230.79	*P. cinnamomea*	*P. eucrita*	*Acer platanoides*	D	1800	A	A	A	A	A	A	550	B	A	A	A	A	A
262.31	*P. livida*	*P. sporulosa*	*Cupressus lawsoniana*	UK	1800	A	A	A	A	A	A	550	A	B	A	B	A	A
211.90	*P. livida*	*P. sporulosa*	*Pseudotsuga menziesii*	F	1800	A	A	A	A	A	A	550	A	B	A	B	A	A
*224.96	*P.* sp.	*P. sporulosa*	*Larix decidua*	NL	1800	A	A	A	A	A	A	550	A	B	A	B	A	A

298.58	*P. cinnamomea*	*P. sporulosa*	*Quercus robur*	NL	1800	A	A	A	A	A	A	550	A	B	A	B	A	A
215.97	*P.* sp.	*P. sporulosa*	*Fagus grandifolia*	NY	1800	A	A	A	A	A	A	550	A	B	A	B	A	A
261.31	*P. livida*	*P. sporulosa*	*Larix decidua*	UK	1800	A	A	A	A	A	A	550	A	B	A	B	A	?
191.39	*Cr. longispora*	*P. sporulosa*	*Pseudotsuga menziesii*	UK	1800	A	A	A	A	A	A	550	A	B	A	B	A	A
*201.46	*P. aurantiaca*	*P. aurantiaca*	*Alnus crispa*	CAN	1800	A	A	A	A	A	A	550	A	A	A	A	A	A
*203.46	*P. subcarnea*	*P. subcarnea*	*Acer pennsylvanicum*	CAN	1800	A	A	A	A	A	A	550	A	A	A	A	A	A
*101.96	*P.* sp.	*P.* aff. *sporulosa*	*Abies alba* (necrosis)	NL	1800	A	A	A	A	A	A	550	A	A	A	A	A	A
306.49	*P. livida*	*P.* aff. *sporulosa*	*Picea abies* (needle)	NL	1800	A	A	A	A	A	A	550	A	A	A	A	A	A
289.39	*P. livida*	*P.* aff. *sporulosa*	*Picea sitchensis* (root)	UK?	1800	A	A	A	A	A	A	550	A	A	A	A	A	A
100416	*P.* sp.	*P.* aff. *sporulosa*	*Amelanchier lamarckii*	NL	1800	A	A	A	A	A	A	550	A	A	A	A	A	A
259.31	*P. corticola* (ex type)	*P. corticola*	?	DK	2000	G	G	I	G	F	F	550	A	A	A	D	G	A
*219.78	*P. corticola*	*P.* aff. *cinnamomea*	*Malus sylvestris*	CH	1800	A	?	A	A	A	A	550	A	?	A	A	D	A
*293.39	*P. rubi*	*P. rubi*	*Rubus* sp.	CAN	1800	A	A	A	A	A	A	550	D	B	A	D	F	A
*253.97	*P. rubi*	*P. rubi*	*Rubus* sp.	NY	1800	A	A	A	A	A	A	550	A	A	A	D	G	A
251.97	*P. rubi*	*P. rubi*	*Rubus* sp.	NY	2300	F	H	J	H	H	G	550	A	A	A	D	G	A
593.96	*P. rubi*	*P. rubi*	*Rubus* sp.	NL	2300	F	H	J	H	H	?	550	A	A	C	D	G	A
*286.39	*P. frangulae*	*P. frangulae*	*Rhamnus* sp.	D	1800	A	A	A	A	A	A	550	A	A	A	A	A	A
*778.96	*P. frangulae*	*P. frangulae*	*Rhamnus frangula*	NL	1800	A	A	D	A	A	A	550	A	A	?	?	?	A
*242.60	*P. cinnamomea*	?	*Quercus* sp.	NL	1800	A	A	D	A	A	A	550	A	A	A	A	A	A
*202.46	*P. carnea*	?	? (*Acer* sp.)	CAN	1800	A	A	D	A	A	A	550	A	A	A	A	D	A
*199.46	*P. alni*	*P. heterochroma*	*Alnus crispa*	CAN	1800	A	D	B	D	A	A	550	A	A	A	A	A	A
*282.39	*P. carpinea*	*P. carpinea*	*Carpinus caroliniana*	CAN	1800	A	D	B	D	A	A	550	A	C	B	C	B	A
*921.96	*P. carpinea*	*P. carpinea*	*Carpinus betulus*	D	1800	A	D	B	D	A	A	550	A	A	A	A	B	A
923.96	*P. carpinea*	*P. carpinea*	*Carpinus betulus*	D	1800	A	D	B	D	A	A	550	A	A	A	A	B	A
*452.64	*Phlyctaena vagabunda*	*Neofabraea alba*	*Malus sylvestris*	UK	1800	A	D	B	D	?	A	550	A	F	A	D	J	A
*140.22	*P. corticola*	?	*Malus sylvestris*	UK	1800	A	D	B	D	?	A	550	A	C	A	A	A	A
*139.41	*P. malicorticis*	*Neofabraea perennans?*	*Malus sylvestris*	NL	1800	A	D	B	D	A	A	550	E	B	A	E	H	A
275.29	*P. malicorticis*	*Neofabraea perennans?*	*Malus sylvestris* (fruit)	?	1800	A	D	A	D	A	A	550	A	B	A	E	H	A
207.57	*P. malicorticis*	*Neofabraea perennans?*	*Malus sylvestris* (fruit)	NL	1800	A	D	A	D	A	A	550	A	B	A	E	H	A
453.64	*P. malicorticis*	*Neofabraea perennans?*	*Malus sylvestris* (fruit)	UK	1800	A	D	A	D	A	A	550	A	B	A	E	H	A
*355.72	*P. malicorticis*	*Neofabraea malicorticis*	*Malus sylvestris* (fruit)	POR	1800	A	D	A	D	A	A	550	A	B	A	E	H	A
141.22	*P. malicorticis*	*Neofabraea malicorticis*	*Malus sylvestris* (fruit)	?	2100	H	I	K	J	D	H	550	A	B	A	E	H	A
*243.38	*P. corylina*	*P. corylina*	*Corylus rostrata*	CAN	1800	A	D	A	D	A	A	550	A	C	A	A	A	A
249.97	*P. corylina*	*P. corylina*	*Corylus cornuta*	CAN	1800	A	D	A	D	A	A	550	A	C	A	A	A	A
450.68	*P. corylina*	?	*Corylus avellana*	B	2300	D	E	G	F	?	G	550	C	C	A	G	B	A

Table 2. Continued.

CBS nr.	Previous identification	Revised name	Substratum	Country	NS1 - NS24 Amplicon bp	*Dde* I	*Rsa* I	*Msp* I	*Hae* III	*Hha* I	*Taq* I	ITS1 - ITS4 Amplicon bp	*Dde* I	*Rsa* I	*Msp* I	*Hae* III	*Hha* I	*Taq* I
*285.39	*P. corni*	*P. corni*	*Cornus circinata*	CAN	1800	C	C	C	C	B	C	550	B	A	A	A	A	A
*200.46	*P. alnicola*	*Scleropezicula alnicola*	*Alnus incana*	CAN	2300	I	J	L	K	E	I	550	H	D	E	F	A	B
474.97	*P. alnicola*	*Scleropezicula alnicola*	*Alnus incana*	CAN	2300	I	J	L	K	E	I	550	H	D	E	F	A	B
239.38	*P. acericola*	*P. acericola*	*Acer spicatum*	CAN	2300	E	F	H	I	G	E	550	F	C	D	A	E	A
239.97	*P. acericola*	*P. acericola*	*Acer spicatum*	CAN	2300	E	F	H	I	G	E	550	F	C	A	A	A	A
245.97	*P. acericola*	*P. acericola*	*Acer spicatum*	CAN	2300	E	F	H	I	G	E	550	F	C	D	A	A	A
247.97	*P. acericola*	*P. acericola*	*Acer spicatum*	CAN	2300	E	F	H	I	G	E	550	F	C	D	A	A	A
292.39	*P. pruinosa*	*P. pruinosa*	*Amelanchier* sp.	CAN	2300	?	?	?	?	I	?	550	A	A	F	A	A	A
*267.39	*Ocellaria ocellata*	*P. ocellata*	*Salix* sp.	D	1800	A	?	A	A	A	A	550	A	A	A	I	C	A
949.97	*Ocellaria ocellata*	*P. ocellata*	?	LUX								550d	A	A	A	A	C	A
*326.75	*Cr. inaequalis*	*Cr. inaequalis*	*Chamaecyparis* sp.	F	1800	A	?	B	A	A	A	550	E	B	A	D	G	A
*185.50	*Cr. diversispora*	*Cr. diversispora*	*Picea abies*	NOR	1800	A	A	D	A	A	A	550	A	A	A	I	A	A
*898.97	*Cr. melanigena*	*Cr. melanigena*	*Quercus petraea* (root)	A	1800	A	?	A	A	A	A	550	A	A	A	A	A	A
681.83	*P. cinnamomea*	*Cr. radicicola*	*Quercus robur* (root)	A	1800	A	D	A	A	A	A	550	G	A	B	A	E	A
*640.94	*Cr. radicicola*	*Cr. radicicola*	*Quercus robur* (root)	POL	1800	A	D	A	A	A	A	550	G	A	B	A	E	A
281.47	*P. cinnamomea*	?	*Alnus glutinosa*	?	1800	A	B	F	A	C	D	535	J	?	H	K	M	D
282.47	*P.* sp.	?	*Alnus glutinosa*	?	1800	A	A	D	A	A	A	550	A	A	A	?	?	A
109.85	*Cryptosporiopsis sp.*	*Fusicoccum* sp.	*Picea abies* (needle)	?	1800	A	D	E	E	A	A	550	H	B	A	H	K	A
299.58	*P. livida*	?	*Larix leptolepis*	?	1800	B	B	B	B	C	B	530	I	G	G	J	L	C
*136.46	*Dermea cerasi*	*Dermea cerasi*	*Prunus* sp.	USA	1800	A	D	E	B	A	A	550	H	E	A	F	I	A

* = *strain selected for cluster analysis.*

noted the differences in ecology and the anamorphs, which in *Pseudopezicula* is a *Phialophora*. Furthermore, the apothecia of *Pseudopezicula* do not arise from a basal stroma. Whether such differences weigh more heavily than the above-mentioned similarities remains to be verified. Molecular analyses can provide additional information on the relationships of these discomycetes.

Molecular characterization of the cultured species by RFLP of ribosomal DNA

Introduction

In order to characterize and delimit species within the genus *Pezicula*, especially the morphologically variable taxa of the *P. cinnamomea* complex, PCR-based restriction fragment length polymorphisms (RFLP) of the small subunit and the internal transcribed spacer regions of the nuclear ribosomal RNA gene cluster were compared for 90 strains to supplement the data of morphology *in vivo* and *in vitro*. Furthermore, some old strains that have degenerated morphologically and for which no vauchers are preserved, can thus be compared with more recent, morphologically well-defined strains using RFLP. The NS1/NS24 amplicon, comprising the 18 S rDNA, and the ITS1/ITS4 amplicon, containing 5.8 S rDNA and the internal transcribed spacers ITS1 and ITS2, were digested using six restriction enzymes.

In the course of the project the possibility arose to sequence part of the small subunit and the ITS regions of selected strains of the genera under consideration, viz. *Pezicula*, *Dermea*, *Scleropezicula* and *Neofabraea*, and construct a phylogeny for the species available in culture on the basis of these data. The results of that study will be published separately (Abeln, de Pagter & Verkley, in prep.).

Results and discussion

The amplicon lengths obtained are listed in Table 2. The NS1/NS24 amplicons were about 1800 base-pairs (bp) in most strains, but they were about 2300 bp in CBS 292.39 (*P. pruinosa*), the strains of *P. acericola* CBS 239.38, 239.97, 245.97, 247.97, and furthermore CBS 450.68 (sub *P. corylina*, but identity now uncertain). Amplicon length was also 2300 bp in CBS 200.46 and 474.97 of *Scleropezicula alnicola*, a fungus considered generically distinct and remotely related to *Pezicula*. In the ex-type strain of *P. corticola*, CBS 259.31, it was estimated to be 2000 bp long. Varying amplicon lengths were recorded within *P. rubi* and the *Neofabraea malicorticis/perennans* complex. In strains of *P. rubi*, amplicon lengths of 1800 bp were found in CBS 293.39 and 253.97, and about 2300 in 251.97 and 593.96, without any correlation to geographic origin or morphological data. The isolates of the *Neofabraea malicorticis/perennans* complex showed 1800 bp amplicons, except CBS 141.22 (ATCC 13903, *Neofabraea malicorticis*) with 2100 bp.

The ITS1/ITS4 amplicons were generally 550 bp long. No intraspecific variation was recorded in species of which more than a single strain was included.The amplicon was 530 bp in CBS 299.58 (sub *P. livida*), and 535 bp in CBS 281.47 (sub *P. cinnamomea*), but, due to

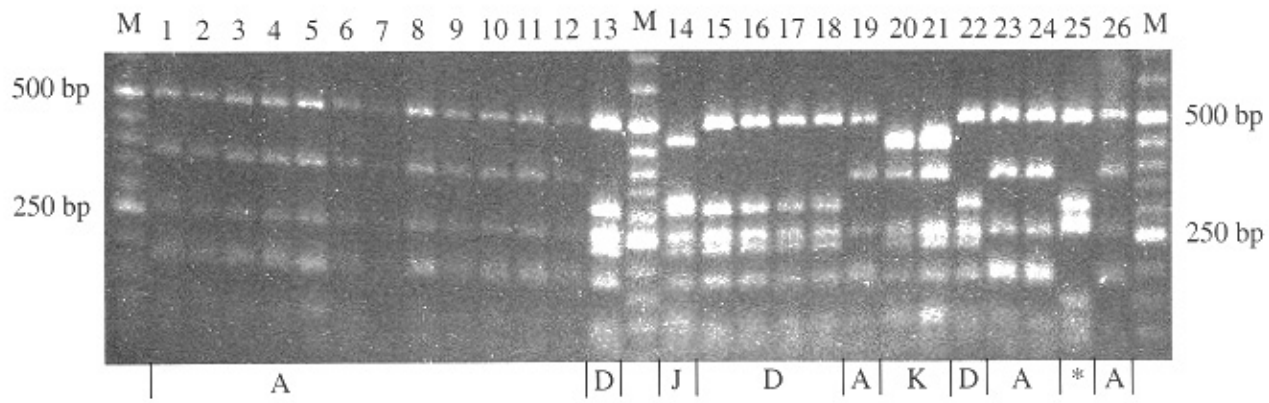

Fig. 1. Electrophoresis gel of the NS1/NS4 amplicon digested with restriction enzyme *Hae* III. Lane **1**, CBS 625.96 *Pezicula cinnamomea*; **2**, 238.97 *P. cinnamomea*; **3**, 241.96 *P. cinnamomea*; **4**, 656.96 *P. eucrita*; **5**, 662.96 *P. eucrita*; **6**, 101.96 *P.* aff. *sporulosa*; **7**, 224.96 *P. sporulosa*; **8**, 628.96 *P. cinnamomea*; **9**, 281.47 *P. cinnamomea*; **10**, 256.32 *P. cinnamomea*; **11**, 242.60, *P. cinnamomea*; **12**, 298.58 *P. sporulosa*; **13**, 921.96 *P. carpinea*; **14**, 141.22 *Neofabraea malicorticis*; **15**, 275.29 *N. perennans*?; **16**, 207.57 *N. perennans*?; **17**, 453.64 *N. perennans*?; **18**, 355.72 *N. malicorticis*; **19**, 224.78 *P. cinnamomea*; **20**, 200.46 *Scleropezicula alnicola*; **21**, 474.97 *S. alnicola*; **22**, 249.97 *P. corylina*; **23**, 778.96 *P. frangulae* subsp. *frangulae*; **24**, 286.39 *P. frangulae* subsp. *frangulae*; **25**, 287.39 *P. hamamelidis*, identity wrong (see text); **26**, 203.46 *P. subcarnea* (M = marker). Pattern identifyers below the gel correspond with those given in Table 2 and 3. The patterns J and K are based on amplicons of 2100 and 2300 bp respectively, all others 1800 bp.

Table 3. Survey of restriction patterns found (letters labeled with the same as in Table 2) for amplicons NS1/NS24 and ITS1/ITS4. Amplicon length and length of fragments obtained after digestion with restriction enzymes *Dde* I, *Rsa* I, *Msp* I, *Hae* III, *Hha* I and *Taq* I are given. Fragments shorter than 50 base-pairs are not listed. Some fragments were consistently found to be incompletely cut for unknown reasons (* pattern incomplete).

Profile	NS1-NS24 Amplicon (bp)	Fragments (bp)							NS1-NS24 Amplicon (bp)	Fragments (bp)						
		***Msp* I**								***Hae* III**						
A	1800	700	380	270	260	100	70		1800	510	380	260	180	170	90	
B	1800	730	350	270	260	100	70		1800	500	310	250	90			
C	1800	580	440	350	310	100			1800	570	300	260	200	180	160	90
D	1800	640	380	270	260	100	60		1800	510	310	260	240	170	90	60
E	1800	680	440	270	260	120			1800	510	320	280	200	100		
F	1800	710	320	280	230	130			2300	580	380	300	260	180	90	
G	2300	710	510	380	270	260	100	70	2000	510	390	290	240	170	160	
H	2300	1380	380	270	210	100	50		2000	510	390	260	170	80		
I	2000	790	640	270	260	50			2300	570	380	250	180	90		
J	2300	890	380	270	260	200	100	60	2100	470	340	260	230	190	100	80
K	2100	570	510	360	270	260	120		2300	460	400	260	230	180	100	80
L	2300	640	520	380	310	270	120									
		***Rsa* I**								***Dde* I**						
A	1800	1140	200	180	140	110			1800	940	340	230	130			
B	1800	960	400	160	140	110			1800	940	330	280	110			
C	1800	840	310	200	180	140	110		1800	870	330	190	140	110		
D	1800	1140	400	140	120				2300	1260	340	230	110			
E	2300	850	710	200	170	140	120		2300	890	340	280	230	180	120	
F	2300	1140	730	200	150				2300	890	340	260	230	190	120	
G	2000	1010	330	200	180	150	110		2000	940	340	260	230			
H	2300	840	760	200	180	150	110		2100	880	350	260	230	200	100	
I	2100	1050	490	410	140	100			2300	800	330	280	240	110		
J	2300	830	720	410	140	100										
		***Taq* I**								***Hha* I**						
A	1800	840	480	200	160	120			1800	1050	430	160	130			
B	1800	840	440	210	180	150			1800	1400	400					
C	1800	840	480	330	150				1800	1050	260	200	150			
D	1800	810	440	310	200	150			2100	1020	450	180	120			
E	2300	790	520	490	210	170	140		2300	870	690	440	200	140		
F	2000	520	490	470	210	170	140		2000	1100 *						
G	2300	1200	490	210	170	140	80		2300	1440	430	330	120			
H	2100	1200	490 *						2300	1480	430	170	120			
I	2300	970	370	330	200	130			2300	940	440	430	190	170	130	

incomplete knowledge of their morphologies, the identity of these strains remains obscure. Likewise, CBS 287.39 with an amplicon of 650 bp (formerly sub *P. hamamelidis*) probably is not the original isolate deposited by Groves. Attempts to amplify the ITS region of CBS 203.46 (*P. subcarnea*) failed for unknown reasons, and the data given in Table 2 are based on ITS sequences (Abeln *et al.*, in prep.).

The digestions of the two amplicons generated comparable diversities in patterns. A maximum of 14 different patterns was obtained by digestion with *Hha* I of the ITS1/ITS4 amplicon.

The digestion profiles are listed in Table 2. Lengths of the restriction fragments for these profiles are listed in Table 3. The size of restriction fragments with less than 50 bp could not be determined with

Table 3. Continued.

Profile	ITS1-ITS4 Amplicon (bp)	Fragments (bp)			
		***Msp* I**			
A	550	210	110	100	80
B	550	210	130	100	80
C	550	200	190	100	50
D	550	200	110	90	60
E	550	250	100	90	
F	550	180	160	100	70
G	530	200	160	100	60
H	535	390	140		
I					
J					
		***Hha* I**			
A	550	180	140	110	100
B	550	300	140	110	
C	550	170	130	70	50
D	550	170	130	90	
E	550	250	180	120	
F	550	440	130		
G	550	250	180	140	
H	550	250	170	120	
I	550	240	180	120	
J	550	240	170	120	
K	550	300	170	100	
L	550	300	250		
M	535	290	140	100	
		***Rsa* I**			
A	550	260	180	120	
B	550	390	170		
C	550	260	120	100	70
D	550	550			
E	550	250	240	70	
F	550	390	130		
G	530	530			

ITS1-ITS4 Amplicon (bp)	Fragments (bp)			
	***Dde* I**			
550	250	180	120	
550	230	180	130	
550	420	130		
550	210	200	120	
550	250	160	120	
550	190	180	120	60
550	250	120	110	60
550	380	180		
530	260	160	80	
535	515			
	***Hae* III**			
550	190	180	100	80
550	180	170	100	80
550	200	160	100	80
550	470	110		
550	450	100		
550	310	100	90	60
550	380	180		
550	430	120		
550	290	120	110	
530	500			
535	535			
	***Taq* I**			
550	230	220	60	
550	240	140	100	80
530	230	220		
535	250	210	80	

the same accuracy and they have therefore been omitted.

NS1/NS24.— An electrophoresis gel of the NS1/NS4 amplicon of a selection of strains digested with *Hae* III is shown in Fig. 1. At least 48 strains form an 'NS core group' with identical NS1/NS24 restriction patterns for all enzymes used (all profile 'A'). These include the four species of the *P. cinnamomea* complex as newly delimited in this study by morphology *in vivo* and *in vitro*, viz. *P. cinnamomea*, *P. eucrita*, *P. sporulosa*, and *P. aurantiaca*. In addition, two further groups can be recognized by the RFLP data, of which the reference strains CBS 100248 and 101.96 are morphologically close to *P. cinnamomea* and *P. sporulosa*, respectively, but they still need further study to assess their taxonomic status. Also included are 203.46 (*P. subcarnea*),

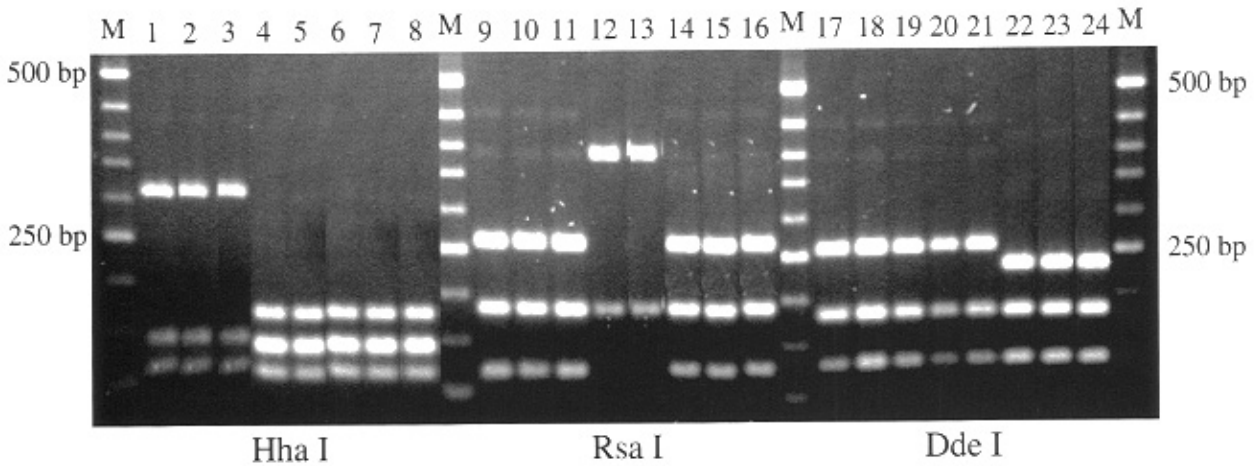

Fig. 2. Electrophoresis gel of the ITS1/ITS4 amplicon of *Pezicula*-strains digested with restriction enzymes Hha I (lane 1–8), Rsa I (lane 9–16), and Dde I (lane 17–24). *P. cinnamomea*, CBS 625.96 (lane 1, 9, 17), 290.39 (lane 2, 10, 18), 184.50 (lane 3, 11, 19); *P. sporulosa*, CBS 224.96 (lane 4, 12, 20), 191.39 (lane 5, 13, 21); *P. eucrita*, CBS 664.96 (6, 14, 22), 256.97 (lane 7, 15, 23), and 662.96 (8, 16, 24).

286.39 (*P. frangulae*) and the two strains of *P. rubi* with 1800 bp amplicons (293.39, 239.97). A recent European and an American isolate of *P. rubi* (CBS 593.96 and 251.97) cannot be compared due to longer amplicons.

The root isolates of *Cryptosporiopsis radicicola*, the ex-type strain CBS 640.94, and 681.83 (formerly sub *P. cinnamomea*) only differ from the 'NS core-group' by the *Rsa* I profile 'D'. Unfortunately, no *Rsa* I profile was obtained of the type strain CBS 898.97 of *C. melanogena*, a species close to *C. radicicola*, both morphologically and ecologically. CBS 267.39, the only strain of *P. ocellata* (syn. *Ocellaria ocellata*) that yielded sufficient PCR product, agrees in the profiles of five enzymes, but no data were obtained for *Rsa* I either. CBS 202.46 (*P. carnea*), 778.96 (*P. frangulae*), 242.60 (*P. cinnamomea*), and 185.50 (syntype of *C. diversispora*) differ from the core-group only by the *Msp* I profile 'D', but as these cultures now are degenerated and no key features are provided in the literature, their identity cannot be assessed.

An 'NS group II' is characterized by a unique combination of profiles 'D' for *Rsa* I, 'B' for *Msp* I, and 'D' for *Hae* III. Besides the isolates of *P. carpinea*, it comprises CBS 199.46, the ex-type strain of the newly proposed species *P. heterochroma* (formerly sub *P. alni*), and 139.41 (*N. malicorticis*). CBS 452.64 (*Phlyctema vagabunda*, teleomorph *N. alba*) and 140.22 (*P. corticola*) agree with this group in the profiles of five enzymes, but the *Hha* I profile is unknown. Four additional strains of the *N. malicorticis/perennans* complex and 249.97 (*P. corylina*) differed from NS-group II only by the *Msp* I profile 'A'. The remaining strains with amplicons of 1800 bp diverge in their restriction profiles. CBS 285.39 (*P. corni*) can be distinguished by unique NS1/NS24 profiles of six enzymes. The patterns of the strains with amplicons of 2300 bp representing the species *P. acericola*, *P. rubi* and *Scleropezicula alnicola* diverge without variation within the species. CBS 136.46 *Dermea cerasi*, representing the type species of the genus *Dermea*, agrees in the profiles of *Dde* I, *Rsa* I, *Hha* I and *Taq* I with strains of the genus *Pezicula*. The *Msp* I profile agrees with that of CBS 109.85 (*Fusicoccum* sp.), the *Hae* III profile with that of 299.58 (identity uncertain).

ITS1/ITS4.— An electrophoresis gel of the ITS1/ITS4 amplicon of eight strains of the *P. cinnamomea* complex digested with *Hha* I, *Rsa* I and *Dde* I is shown in Fig. 2. Strains of the *Pezicula cinnamomea* complex were originally identified according to the host, e.g., *P. cinnamomea* was applied to forms on various broad-leaved trees, and *P. livida* to those on conifers. These strains, assembled in the above-mentioned 'NS core-group', can be arranged into five groups according to ITS polymorphisms, correlating with the morphological features on the natural substratum and in culture. The *Msp* I profiles are taxonomically uninformative.

The first and largest ITS group comprises 21 strains of *P. cinnamomea* from broad-leaved trees and conifers, including the reference strain CBS 625.96 from *Quercus*, and the type strains of *P. plantarium* (290.39) and *Cryptosporiopsis balsamea* (184.50). Wollenweber (1939) considered *P. plantarium* from *Prunus avium* closely related to *P. cinnamomea*, but distinct by larger apothecia, asci, ascospores, and conidia. The variability of *P. cinnamomea* is now better known and the measurements of these structures fall within the range of this species. No genetic variation was detected between material of *P. cinnamomea* collected in North America and Europe. The original description of the morphology *in vitro* of *C. balsamea* is insufficient, but the results of

RFLP of the original strain deposited by Robak indicate that it is conspecific with *P. cinnamomea* (anamorph *C. grisea*), and that therefore Robak's *C. balsamea* is to be regarded a synonym of *C. grisea*.

The morphology of dried cultures of CBS 939.70 and 203.82 from *Abies* and *Chamaecyparis*, respectively, points to *P. cinnamomea*, and this is confirmed by RFLP. Furthermore, CBS 355.51, originally identified as *P. betulae*, belongs to *P. cinnamomea*. Two strains isolated from apothecia on necrotic bark in Germany, CBS 315.96 on *Abies procera* and 778.95 on *Larix decidua*, were difficult to place by morphological features alone but genetically agree with *P. cinnamomea*. It should be noted that two German isolates of *P. carpinea* (921.96, 923.96) have identical ITS-profiles, but they can be distinguished by the profiles of *Rsa* I, *Msp* I, and *Hae* III on NS1/NS24.

A second ITS group comprises eight strains of *P. eucrita*, including the reference strain CBS 664.96 from *Pinus*. *Pezicula eucrita* is only known to sporulate on conifers. CBS 230.79 was isolated from a branch of *Acer platanoides*. There is one earlier confirmed report of this species occurring endophytically in a broad-leaved host, viz. *Carpinus betulus* (Kowalski & Kehr, 1992; as *P. livida*, pers. comm. R. Kehr). *Pezicula corni*, represented by CBS 285.39, can also not be distinguished from *P. eucrita* by ITS profiles, but the NS patterns are highly diagnostic.

For the third ITS group, the new species *P. sporulosa* is here proposed. The ex-type strain, CBS 224.96, and a further six isolates from the NS core group belong here. Two of these originated from Gregor-Wilson's study of '*Dermatea livida*' from various conifers in the UK (Gregor-Wilson, 1931). She distinguished three groups by colony characters and mean ascospore length (*in vitro*). She did not report ascospore width or ascospore number per ascus, which hampers a comparison with more recent material. The strains from her groups 'II' and 'III' that were deposited, 261.31 and 262.31 respectively, belong to *P. sporulosa*. The isolates from her group 'I', which included four from *Pinus*, were not deposited but data on sporulation and ascospore length suggest that they were *P. eucrita*.

Two further ITS groups comprise rather heterogeneous material. One includes CBS 101.96, which is associated with necrosis of *Abies alba* and is morphologically very close to *P. sporulosa*, but also the morphologically distinct *P. aurantiaca* (201.46) and *P. subcarnea* (203.46), and one of the two strains of *P. frangulae* (286.39). Strains differing from this group only by NS profiles are *P. heterochroma* (199.46, in NS-group II), and the root endophyte *C. melanogena* (898.97). The identity of CBS 242.60 (sub *P. cinnamomea*) is uncertain due to lack of morphological data. A fifth ITS group comprises 350.52, 257.32, and 100248, which are morphologically closer to *P. cinnamomea*. As derived from ITS sequences (Abeln *et al.*, in prep.), CBS 949.97, a strain of *P. ocellata*, would give similar profiles, differing from 267.39 of the same species only in the *Hae* III profile. *Cryptosporiopsis radicicola* strains CBS 681.83 and 640.94 agree with each other in the patterns of all enzymes, and only that of *Dde* I is unique to this pair, while the type strain of a second species described from roots of *Quercus*, *C. melanogena*, differs in profiles of three enzymes. The results indicate that these anamorphs are specifically distinct and, despite their unusual habitat and morphology, are closely related to the species of the *P. cinnamomea* complex.

The profiles 'E' of *Hae* III and 'H' of *Hha* I are diagnostic of the species complex of *Neofabraea* pathogenic to *Malus* spp. and related hosts, but the anthracnose canker fungus *N. malicorticis* and perennial canker fungus *N. perennans* are not distinguished. These profiles differ from those in *Phlyctema vagabunda*, which, on the basis of morphology, is regarded to belong to *Neofabraea* too.

The *Dde* I profile 'F' is diagnostic of *Pezicula acericola*, although some variation is recorded between the strains. Correlating with the morphological data, particularly *in vitro*, the results of RFLP strongly support a specific status distinct from *P. cinnamomea* and other species of the *P. cinnamomea* complex. Wollenweber (1939) considered *P. acericola* a synonym of *P. cinnamomea*, while Seaver (1951) reversed the synonymy.

The strains representing *Scleropezicula alnicola* show unique profiles 'E' of *Msp* I and 'B' of *Taq* I.

P. rubi and *P. carpinea* show intraspecific variation for ITS1/ITS4, but the strains of each species are morphologically homogeneous.

The ITS digestions of CBS 249.97 and 243.38 (*Pezicula corylina*) from *Corylus* and 140.22 from *Malus* gave the same results. The identity of the latter is, however, uncertain. CBS 450.68, a European strain formerly kept as *P. corylina*, has a longer NS1/NS24 amplicon and, due to lack of morphological data, the relation with the Canadian isolates remains obscure. The remaining strains are rather heterogeneous and most of them are difficult to assess because their morphological features are incompletely known.

After successful induction of sporulation, CBS 109.85, originally identified as *Cryptosporiopsis* sp., could be recognized as a *Fusicoccum* sp (CBS 109.85). CBS 136.46 (*Dermea cerasi*) shows unique *Rsa* I and *Hha* I profiles, but agrees in the *Dde* I profile with the strains of *Scleropezicula alnicola* and *Fusicoccum* sp., and in the *Hae* III profile only with the former. The *Taq* I and *Msp* I profiles of *Dermea cerasi* agree with vari-

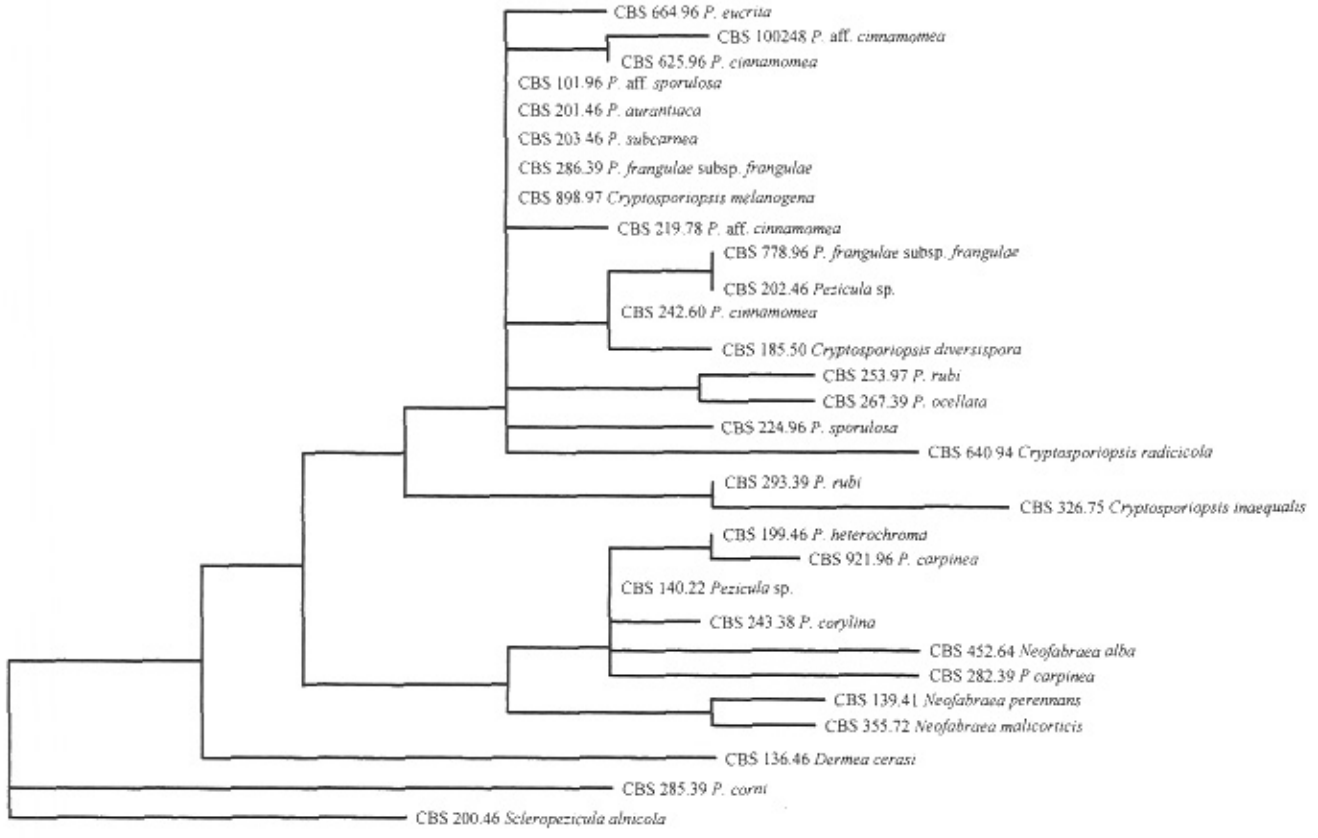

Fig. 3. Dendrogram of combined NS and ITS RFLP data of selected reference strains, using UPGMA cluster analysis (PAUP version 4.0 d64; Swofford, 1998).

ous species of *Pezicula* and *Neofabraea*. Strains with aberrant amplicon lengths are incomparable.

Cluster analyses.— Because of the limited number of data per taxon, only a joint cluster analysis for the NS and ITS regions was performed. *Pezicula acericola* was omitted because of the longer NS1/NS24 amplicon. The results obtained with UPGMA are shown in Fig. 3, those with Neighbor Joining in Fig. 4. As already suspected by the raw data, the results do not comply with the genera as defined by morphology. In the UPGMA tree, all strains of *Neofabraea* spp. cluster in a single clade with *P. heterochroma*, *P. carpinea*, and *P. corylina*, while in the Neighbor Joining tree the strains CBS 139.41 (*N. perennans*) and 355.72 (*N. malicorticis*) are nested within the major group of *Pezicula* spp. Thus it seems that with UPGMA the similarity in NS1/NS24 profiles between the strains of *P. carpinea* and those of the genus *Neofabraea* weigh more than in Neighbor Joining.

Most of the species recognized by distinct morphological and ecological traits could also be sufficiently distinguished with RFLP, except *Pezicula subcarnea* and *P. aurantiaca* that gave identical results. Similarly, the strains originally identified as *P. perennans* and *P. malicorticis* could not be discriminated. Pending the above-mentioned sequencing study (Abeln *et al.*, in prep.), no further enzymes were tested. It can be concluded that, for purposes of identification, the data obtained with RFLP provide a reliable set of markers, when applied in combination with morphological analyses.

Biology and ecology

Life-style

In the colonization of standing logs most species of *Pezicula* are pioneers. Indeed, most are the first to open the bark of the host. This indicates that these fungi are already present in living tissues, and can be regarded as endophytes. When the host tissue dies, they colonize the substratum and sporulate more rapidly than secondary invaders. In studies of endophytic fungi colonizing healthy bark and wood of trees and shrubs, species of *Pezicula* or *Cryptosporiopsis* anamorphs have been isolated repeatedly (Carroll *et al.*, 1977; Petrini *et al.*, 1982; Petrini & Fisher, 1988; Espinosa-García & Langenheim, 1990; Fisher & Petrini, 1990; Sieber *et al.*, 1991; Rollinger & Langenheim, 1993; Redlin & Carris, 1996; Stone *et al.*, 1996). Kowalski & Kehr (1992) screened the endophytic fungal coloniza-

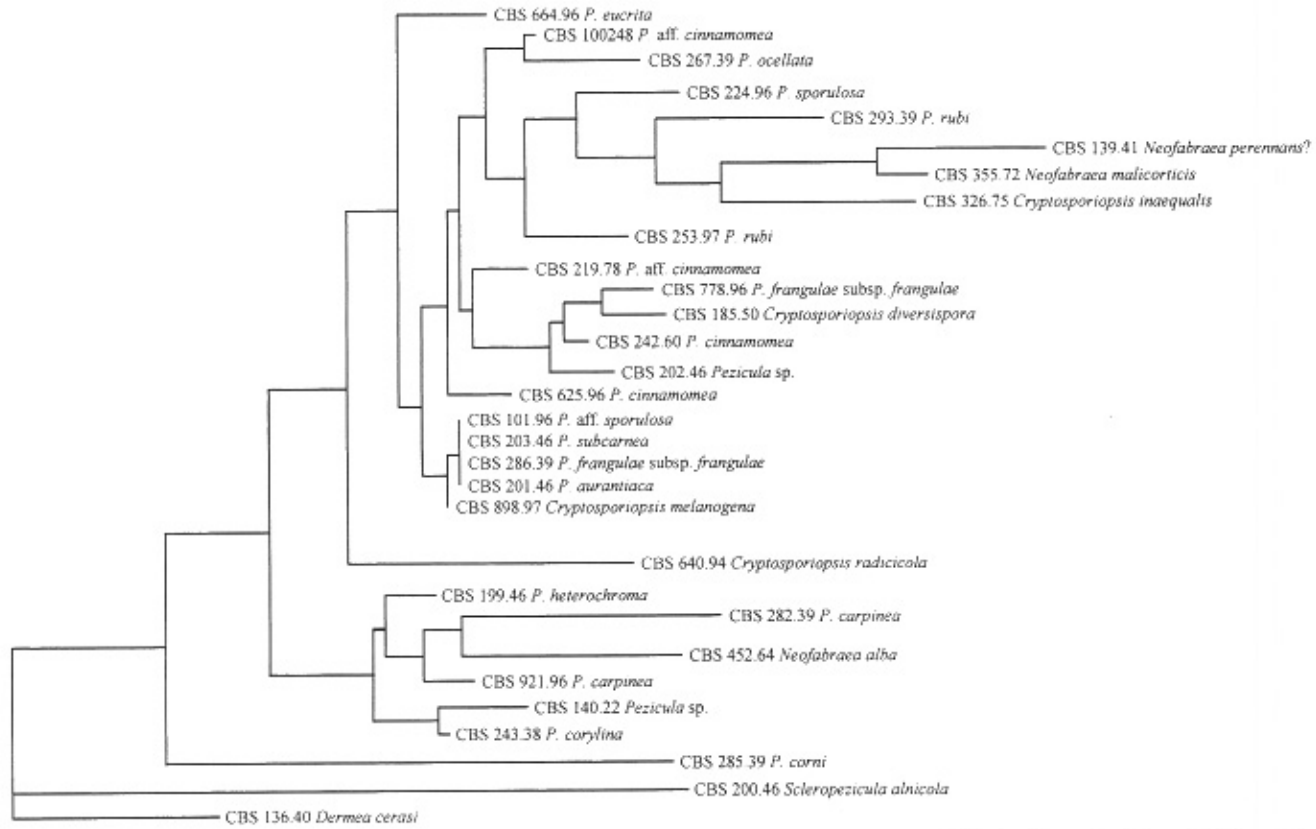

Fig. 4. Dendrogram of combined NS and ITS RFLP data of selected reference strains, using the Neighbor Joining algorithm (PAUP version 4.0 d64; Swofford, 1998).

tion of branch bases in several European forest tree species, and found *Pezicula* in the peridermal bark of 6–71 % of living branches, and occasionally also in subperidermal bark and wood. As the presence of most species of *Pezicula* normally does not inflict any symptoms of disease, the genus is generally regarded as endophytic. However, some strains of *Pezicula* are weakly pathogenic, particularly to hosts under stress (Boerema & Gremmen, 1959; Domański, 1978; Kowalski, 1982; Pratt, 1982; Domański & Kowalski, 1987; Kobayashi *et al.*, 1990; Kehr, 1991, 1992). Some of the strains of *P. cinnamomea* and *P. sporulosa* were found in association with a bark necrosis fatal to young trees (this study). Further work is needed to assess whether the strains that behave as pathogens are genetically distinct entities. The species of the related genus *Neofabraea* generally have adopted a more pathogenic lifestyle, but also occur as saprophytes (and perhaps endophytes).

Species of *Pezicula* produce a number of antibiotics. An echinocandin with antimicrobial properties against certain yeasts was isolated from endophytic *Cryptosporiopsis* sp. and *Pezicula* sp. in *Pinus sylvestris* and *Fagus sylvatica* (Noble *et al.*, 1991). From several species of *Pezicula*, Schulz *et al.* (1995) isolated and described five metabolites with strong fungicidal and herbicidal activity, including (R)-mellein, mycorrhizin, and cryptosporiopsin. It has been suggested that the endophytes protect the host against infection by parasites via twigs and branches that remain attached to the plant for some time after their natural death.

Fructifications of *Pezicula* are predominantly found on the bark of woody angiosperms and gymnosperms, and also cone scales of conifers. Fructifications of *Neofabraea malicorticis* and *N. perennans* also occur on fruits of the host, and those of *N. alba* even on leaves of a wide variety of herbaceous plants. *Cryptosporiopsis citri* and *C. eucalypti* cause leaf spots on their hosts. Although ecologically deviating, the two species isolated from roots of *Quercus*, viz. *Cryptosporiopsis radicicola* and *C. melanogena*, can be assigned to *Cryptosporiopsis* based on conidiogenesis, conidial contents, and RFLP of the nSSU and ITS regions. Possibly more specialized root-colonizing species can still be discovered. To date, no such species have been found sporulating *in vivo*.

Host specificity

Species of *Pezicula* diverge in host specificity. *Pezicula cinnamomea* has an extremely wide host range, while other taxa apparently have a narrow one. Several

species are to date only known from a single host species or genus, e.g. *P. corni* on *Cornus* spp., *P. frangulae* on *Rhamnus frangula*, *P. grovesii* on *Rhododendron canadense*, *P. hamamelidis* on *Hamamelis virginiana*, *P. pruinosa* on *Amelanchier* spp., and *Neofabraea krawtzewii* on *Populus* spp. There seems to be a trend for the highly host-specific species of *Pezicula* to inhabit smaller species of deciduous trees that often dominate the understory of temperate forests. However, species with a wider host-range may also occur on these plants.

Kowalski & Kehr (1992) concluded that the host spectrum of *P. cinnamomea* in the endophytic phase included deciduous trees and conifers, which is considerably wider than reported in the literature (as based on sporulating material). The present study has shown that this species sporulates on a large variety of both coniferous and deciduous hosts. On the other hand, *P. eucrita* is only known to sporulate on conifers (especially *Pinus* spp.), but was also isolated as an endophyte from living bark of *Carpinus betulus* at a low percentage (Kowalski & Kehr, 1992; identified as *P. livida*, but conspecific with *P. eucrita*, R. Kehr, pers. comm.). The differences observed so far in the host range of these fungi found either as sporulating 'saprophytes' or as endophytes are difficult to interpret. The infection pathways may differ between species that primarily colonize peridermal bark ('phellophytes', Kowalski & Kehr, 1992), leaves, or roots. Vectors, particularly insects, may be involved (Taylor, 1983).

Life-cycle

The life-cycle of these fungi is still poorly understood. *Pezicula cinnamomea* and *P. eucrita* were proven to be homothallic as single-ascospore isolates produced fertile apothecia. Other species only produce the anamorph in culture and may be heterothallic. No compatibility tests were performed in this study.

Some species produce massive amounts of microconidia *in vivo* and *in vitro* or morphologically often quite similar small conidia from ascospores. It is still unclear what their function is in the life-cycle, or what structures function as nuclear receptors for these propagules.

In Europe the anamorph can be found all the year round, but most were collected in late winter and early spring. Apothecia can also be found the year round, but they optimally develop their diagnostic features only in moist periods without frost. Especially (repeated) desiccation and frost cause sterility of the hymenium, or an irrecognizable deformation of the diagnostic features. Some of the type collections had clearly suffered from such adverse conditions.

As Gregor-Wilson (1931) already observed in heterogeneous material from conifers (as *Dermatea livida*, including the present *P. sporulosa*, and probably also *P. eucrita* and *P. cinnamomea*), ascopores are discharged in the aseptate stage under natural conditions. Baral (1992) reported that septate ascospores never occur inside living asci of *Pezicula*. Liberated ascospores soon form transverse and sometimes also oblique septa. Ascospores that remain behind in asci that fail to discharge, go through the same process and may germinate in the ascus. Small conidia can be formed subsequently; in *P. eucrita* they arise from germination hyphae that penetrate the ascus tip and form conidiogenous cells, basically microconidiophores.

Distribution

Although little can be said about the distribution of the *Pezicula* species in general, the genus has been predominantly collected in temperate and boreal forests of the northern hemisphere. Several species are only known from a relatively small area or a single locality. A few species with a wide host-range are known from several continents and seem to have a more or less world-wide geographical distribution. One of these, *P. cinnamomea*, has also been recorded from a locality in Java (at unknown altitude). However, tropical areas and even large parts of the northern hemisphere have not been explored to date. An additional problem is that almost every record of a species requires revision due to the unreliable identifications that were based on the host and morphological features *in vivo* only. It is hoped that the publication of this monograph will stimulate others to collect more of these fungi and to reidentify previously collected material.