Penicillium araracuarensense sp. nov., *Penicillium elleniae* sp. nov., *Penicillium penarojense* sp. nov., *Penicillium vanderhammenii* sp. nov. and *Penicillium wotroi* sp. nov., isolated from leaf litter

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Several species of the genus *Penicillium* were isolated during a survey of the mycobiota of leaf litter and soil in Colombian Amazon forest. Five species, *Penicillium penarojense* sp. nov. (type strain CBS 113178^T=IBT 23262^T), *Penicillium wotroi* sp. nov. (type strain CBS 118171^T=IBT 23253^T), *Penicillium araracuarensense* sp. nov. (type strain CBS 113149^T=IBT 23247^T), *Penicillium elleniae* sp. nov. (type strain CBS 118135^T=IBT 23229^T) and *Penicillium vanderhammenii* sp. nov. (type strain CBS 126216^T=IBT 23203^T) are described here as novel species. Their taxonomic novelty was determined using a polyphasic approach, combining phenotypic, molecular (ITS and partial β -tubulin sequences) and extrolite data. Phylogenetic analyses showed that each novel species formed a unique clade for both loci analysed and that they were most closely related to *Penicillium simplicissimum*, *Penicillium janthinellum*, *Penicillium daleae* and *Penicillium brasilianum*. An overview of the phylogeny of this taxonomically difficult group is presented, and 33 species are accepted. Each of the five novel species had a unique extrolite profile of known and uncharacterized metabolites and various compounds, such as penicillic acid, andrastin A, pulvilloric acid, paxillin, paspaline and janthitrem, were commonly produced by these phylogenetically related species. The novel species had a high growth rate on agar media, but could be distinguished from each other by several macro- and microscopical characteristics.

INTRODUCTION

The vegetation of the tropical lowland forest along the Caquetá River, Colombia, has been extensively studied and many ecological investigations of this area have been

Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony.

The GenBank/EMBL/DDBJ accession numbers for the β -tubulin and ITS gene sequences of the type strains of *P. araracuarensense* sp. nov., *P. elleniae* sp. nov., *P. penarojense* sp. nov., *P. vanderhammenii* sp. nov. and *P. wotroi* sp. nov. are GU981642 and GU981597, GU981663 and GU981612, GU981646 and GU981570, GU981647 and GU981574, and GU981637 and GU981591, respectively.

The Mycobank (<http://www.mycobank.org>) accession numbers for *P. araracuarensense* sp. nov., *P. elleniae* sp. nov., *P. penarojense* sp. nov., *P. vanderhammenii* sp. nov. and *P. wotroi* sp. nov. are MB51825, MB518028, MB518024, MB518027 and MB518026, respectively.

Supplementary figures are available with the online version of this paper.

conducted. These studies show that the soils of these forests are poor in nutrients, but that the diversity of tree species is among the highest in the world (Vester & Cleef, 1998; Duivenvoorden & Lips, 1998; Gentry, 1996; Sáenz, 1997). Micro-organisms play an important role in nutrient cycling and link the roots of trees with their environment, e.g. through the decomposition of leaf litter, such as lignin, cellulose and hemicelluloses, and through the uptake and immobilization of nutrients, nitrogen fixation, protection from pathogens and soil improvement (Killham, 1994; Richards, 1987). Decomposed leaf litter in such forest ecosystems is a major contributor to nutrient cycling, in which a large proportion of the nutrients from net primary production is returned to the forest floor (Maggs, 1985). Fungi play an important role in the decomposition of leaf litter due to their ability to penetrate dense substrates and enzymically break down macromolecular plant-derived

material and they therefore tend to control the rate of decomposition (Osono & Takeda, 2002; Wardle *et al.*, 2004).

Numerous microfungi were isolated during a project to investigate the role of fungi during the regeneration processes of tropical lowland forests in the Caquetá area of the Colombian Amazon. During this study, microfungi were isolated from decomposing leaf litter, which had been incubated in leaf litter bags and placed on the forest floor for several months. A large number of different microfungi was isolated and the *Penicillia* were tentatively identified based on phenotype and extrolite data. Among these *Penicillium* isolates, several were not assignable to any described species. Many of these undescribed species were related to *Penicillium janthinellum* and *Penicillium simplicissimum*. Both of these species are phenotypically broadly circumscribed taxa and are often encountered in grassland and forest soil (Christensen *et al.*, 2000). Raper & Thom (1949) included both of these species in the *P. janthinellum* series, together with *Penicillium daleae*, *Penicillium ochrochloron*, *Penicillium piscarium* and *Penicillium miczynskii*. Members of this series share a fast growth rate and are variously branched, having monoverticillate to biverticillate asymmetrically and/or symmetrically branched conidiophores. Thirty years later, Pitt (1979) maintained *P. janthinellum* and *P. simplicissimum* as two separate species and placed nine and ten species, respectively, as synonyms with them. He noted that *P. simplicissimum* had an affinity with *P. janthinellum* and strains with intermediate properties occurred. Stolk & Samson (1983) synonymized *P. janthinellum* with *P. simplicissimum*, made a connection with the teleomorphic species *Eupenicillium javanicum* and placed 26 species in synonymy. However, molecular data do not support the combination of all these species (Peterson, 2000). Furthermore, Tuthill *et al.* (2001) also showed that *P. piscarium*, *Penicillium brasilianum* and *Penicillium pulvillorum* are related, but distinct from *P. simplicissimum*, and that at least three undescribed species belong to this clade. Recently, another related species named *Penicillium svalbardense* was described from Arctic environments (Sonjak *et al.*, 2007).

Combining phenotypic, molecular (ITS and partial β -tubulin gene sequences) and extrolite data, five novel species of the genus *Penicillium* are described here. In this study, species descriptions are provided, extrolite patterns are detailed and estimates for the phylogenetic relationships of these novel species are presented. Furthermore, using a phylogenetic approach, the relationship between *P. simplicissimum* and *P. janthinellum* and related species of the genera *Penicillium* and *Eupenicillium* is examined.

MATERIAL AND METHODS

Strains. Most strains were isolated from four collection sites: mature, 36-year-old and 48-year-old forest in Araracuara, Colombia, and from a dipterocarp forest in Peña Roja, Colombia. To study the microfungal community

during decomposition, leaf litter bags filled with leaves from each collection site were deposited on the forest floor according to the method described by Aerts (1997), and the microfungal composition was analysed after 3 and 6 months of decomposition. To isolate the microfungal colonists, the strongly decayed litter was processed using the soil-washing method protocol of Gams & Domsch (1967). A 4–6 g sample of litter fragments was washed three times with 500 ml sterile distilled water by manual agitation for 5 min. Small litter particles were placed onto 2% water agar and incubated for 7 days at 25 °C. After incubation, subcultures were made on cornmeal agar (CMA, Difco) and pure cultures were maintained on slants of malt extract agar (MEA, Oxoid). The geographical and substrate origins of each isolate, together with the CBS-Fungal Biodiversity Centre and IBT (Technical University Denmark) accession numbers, are given in Table 1. Ex-type and additional cultures are maintained in the culture collection of the CBS-Fungal Biodiversity Centre, Utrecht, the Netherlands.

Morphology and extrolite profiles. For macroscopic analysis, isolates were inoculated in a three point pattern on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), yeast extract sucrose agar (YES), oatmeal agar (OA), Dichloran 18% glycerol agar (DG18) and creatine sucrose agar (CREA). Colony diameters and other macroscopical features were recorded after 7 days of incubation at 25 °C in darkness. The isolates on OA were incubated longer and followed in time for potential ascospore formation. Furthermore, growth curves were made on CYA agar incubated for 7 days at 27, 30, 33, 36, 37 and 40 °C. The isolates were examined for production of alkaloids reacting with Ehrlich reagent using a filter paper method (Lund, 1995). For microscopic examination, mounts of the strains were made in lactic acid from the MEA colonies. Minimum and maximum size values are presented for the conidia and ascospores. The mean and the standard deviation of these values were calculated (given in parentheses).

Extrolites. Isolates were grown on CYA and YES for 7–14 days and three or more plugs were cut out of a colony for extraction. Extractions were performed as described by Smedsgaard (1997). Extracts were analysed by HPLC with diode array UV-VIS detection using alkylphenone retention indices (Frisvad & Thrane, 1987). Identification of the extrolites was conducted by comparison of the retention times and UV spectra with those of the analysis of standards or literature reports. Unknown metabolites were recorded and preliminary names were given to them.

Molecular studies: DNA extraction, PCR and sequencing.

Mycelium and conidia were scraped from agar plates and transferred to 2 ml collection tubes from the UltraClean Microbial DNA kit (MoBio Laboratories Inc.) and processed according to the manufacturer's instructions. Partial β -tubulin fragments were amplified using the primer combinations T10 and Bt2b or Bt2a and Bt2b (Glass & Donaldson, 1995). The ITS regions including the 5.8S were

Table 1. *Penicillium* isolates used in this study

CBS accession no.	Name	Other collections	Origin
CBS 246.67 ^{HT}	<i>E. abidjanum</i>	ATCC 18385; FRR 1156; IMI 136244	Savannah soil, Ivory Coast
CBS 513.74 ^T	<i>E. arvense</i>	DSM 2207; IFO 9712; NHL 6471	Soil, Japan
CBS 233.81 ^{NT}	<i>E. brefeldianum</i>	FRR 71; IFO 31730; IMI 216895	Soil, Murrumbidgee Irrigation Area, NSW, Australia; neotype fide Pitt (1979), p. 119
CBS 443.75 ^T	<i>E. caperatum</i>	ATCC 28046; DSM 2209; NHL 6465	Soil, Papua New Guinea
CBS 324.48 ^{HT}	<i>E. ehrlichii</i>	ATCC 10442; IMI 039737; NRRL 708; VKM F-273	Poland
CBS 341.48 ^{HT}	<i>E. javanicum</i>	ATCC 9099; CSIR 831; FRR 707; IFO 31735; IMI 039733; MUCL 29099; NRRL 707	Root of <i>Camellia sinensis</i> , Indonesia, Java
CBS 345.48 ^{NT}	<i>E. levitum</i>	ATCC 10464; IFO 6101; IMI 039735; NRRL 705	Modelling clay, USA
CBS 339.97	<i>E. limosum</i>		Marine sediment, Nagasaki prefecture, Japan
CBS 188.77 ^{HT}	<i>E. lineolatum</i>	NHL 2776	Soil from copse, Japan
CBS 445.74 ^{HT}	<i>E. meloforme</i>	ATCC 28049; IMI 216903; NHL 6468	Soil, Papua New Guinea
CBS 121.68 ^{AUT}	<i>E. reticulispurum</i>	ATCC 18565; IMI 136699; NHL 6102; NRRL 3446	Soil, Japan
CBS 992.72 ^{HT}	<i>E. zonatum</i>	ATCC 24353	Coastal marsh soil, USA, North Carolina
CBS 113146	<i>P. araracuarensense</i>	IBT 23245	Leaf litter exposed for 6 months, 48-year-old forest, Araracuara, Colombia
CBS 113147	<i>P. araracuarensense</i>	IBT 23246	Leaf litter exposed for 6 months, 36-year-old forest, Araracuara, Colombia
CBS 113148	<i>P. araracuarensense</i>	IBT 23243	Leaf litter exposed for 6 months, 36-year-old forest, Araracuara, Colombia
CBS 113149 ^T	<i>P. araracuarensense</i>	IBT 23247	Leaf litter exposed for 6 months, 36-year-old forest, Araracuara, Colombia
CBS 113150	<i>P. araracuarensense</i>	IBT 23244	Leaf litter exposed for 6 months, 36-year-old forest, Araracuara, Colombia
CBS 253.55 ^{HT}	<i>P. brasilianum</i>	ATCC 12072; FRR 3466; QM 6947	Herbarium specimen, Recife, Brazil
CBS 235.81 ^T	<i>P. brefeldianum</i> and <i>P. dodgei</i>	FRR 710; IFO 31731; IMI 216896; NRRL 710	Human alimentary tract
CBS 275.83 ^T	<i>P. cieglei</i>	CECT 2747; IMI 257691	Rye grain, Spain
CBS 141.45	<i>P. coeruleum</i>	NCTC 6595	As <i>Citreomyces coeruleus</i> ; unknown source
CBS 223.66 ^{NT}	<i>P. cremeogriseum</i>	ATCC 18323; FRR 1734; IJFM 5011; IMI 197492; NRRL 3389	Forest soil, Kiev, Ukraine
CBS 211.28 ^T	<i>P. daleae</i>	ATCC 10435; DSM 2449; FRR 2025; IFO 6087; IFO 9072; IMI 034910; MUCL 29234; NRRL 2025	Soil under conifer, Poland
CBS 328.59 ^T	<i>P. echinulonalgiovense</i>	ATCC 18314; FRR 638; IFO 6229; IMI 068213	Soil, Japan
CBS 118134	<i>P. elleniae</i>	IBT 23201	Leaf litter exposed for 6 months, 48-year-old forest, Araracuara, Colombia
CBS 118135 ^T	<i>P. elleniae</i>	IBT 23229	Leaf litter exposed for 6 months, mature forest, Araracuara, Colombia
CBS 118136	<i>P. elleniae</i>	IBT 23230	Leaf litter exposed for 6 months, mature forest, Peña Roja, Colombia
CBS 126215	<i>P. elleniae</i>	DTO 85B8; IBT 30008	Soil, <i>Ranoma fana</i> , 2 m from road, Madagascar
CBS 340.48 ^{NT}	<i>P. janthinellum</i>	ATCC 10455; IMI 040238; NRRL 2016	Soil, Nicaragua
CBS 271.83 ^T	<i>P. mariaecrucis</i>	CECT 2742; IMI 256075	<i>Secale cereale</i> , Spain
CBS 357.48 ^{NT}	<i>P. ochrochloron</i>	ATCC 10540; IMI 039806; NRRL 926	Copper sulphate solution, Washington, USA
CBS 349.51 ^T	<i>P. oligosporum</i>		Japan
CBS 174.81 ^{NT}	<i>P. onobense</i>	ATCC 42225; IJFM 3026; VKM F-2183	Soil, Navarra, Spain
CBS 430.65 ^{AUT}	<i>P. paraherquei</i>	FAT 824; IAM 7288	Soil, Japan

Table 1. cont.

CBS accession no.	Name	Other collections	Origin
CBS 113132	<i>P. penarojense</i>	IBT 23259	Leaf litter exposed 6 months, mature forest, Peña Roja, Colombia
CBS 113133	<i>P. penarojense</i>	IBT 23260	Leaf litter exposed 6 months, 36-year-old forest, Araracuara, Colombia
CBS 113144	<i>P. penarojense</i>	IBT 23261	Leaf litter exposed 6 months, mature forest, Araracuara, Colombia
CBS 113178 ^T	<i>P. penarojense</i>	IBT 23262	Leaf litter exposed 6 months, mature forest, Peña Roja, Colombia
CBS 362.48 ^T	<i>P. piscarium</i>	ATCC 10482; FRR 1075; IMI 040032; NRRL 1075	Cod-liver oil emulsion, Norway
CBS 280.39 ^{NT}	<i>P. pulvillorum</i>	IFO 7763; NRRL 2026	Acidic soil, UK
CBS 281.58 ^{NT}	<i>P. raperi</i>	ATCC 22355; IFO 8179; IMI 071625; NRRL 2674	Soil, Bedford, UK
CBS 368.48 ^T	<i>P. rolfsii</i>	ATCC 10491; FRR 1078; IFO 7735; IMI 040029; MUCL 29229; NRRL 1078	Pineapple, Florida, USA
CBS 372.48 ^{NT}	<i>P. simplicissimum</i>	ATCC 10495; FRR 902; IFO 5762; IMI 039816	Flannel bag, South Africa
CBS 439.75 ^{NT}	<i>P. skrjabinii</i>	FRR 1945; IMI 196528; VKM F-1940	Soil, Russia (far East)
CBS 122416 ^T	<i>P. svalbardense</i>	IBT 23856; EX-F 1307	Glacial ice, Svalbard, Greenland
CBS 116296	<i>P. vanderhammenii</i>	IBT 23232	Leaf litter exposed for 6 months, mature forest, Araracuara, Colombia
CBS 126216 ^T	<i>P. vanderhammenii</i>	DTO 97A3; IBT 23203	Leaf litter exposed for 6 months, mature forest, Araracuara, Colombia
CBS 339.79 ^T	<i>P. vasconiae</i>	CBS 175.81; IJFM 3008	Acid-washed brown soil, Spain
CBS 116294	<i>P. wotroi</i>	IBT 23252	Leaf litter exposed for 6 months, mature forest, Peña Roja, Colombia
CBS 116295	<i>P. wotroi</i>	IBT 23255	Leaf litter exposed for 6 months, mature forest, Araracuara, Colombia
CBS 118138	<i>P. wotroi</i>	IBT 23254	Leaf litter exposed for 6 months, mature forest, Araracuara, Colombia
CBS 118171 ^T	<i>P. wotroi</i>	IBT 23253	Leaf litter exposed for 6 months, mature forest, Araracuara, Colombia
CBS 126217	<i>P. wotroi</i>	DTO 8F3	<i>Gladiolus</i> corm, Argentina

amplified with the primers V9G and LS266 (Gerrits van den Ende & de Hoog, 1999). Both strands were sequenced using the ABI Prism BigDye Terminator v. 3.0 Ready Reaction Cycle Sequencing kit (Applied Biosystems). Sequence products were purified using a 96-well multiscreen HV plate (Millipore) and Sephadex G-50 superfine columns (Amersham Biosciences). Samples were analysed with a ABI Prism 3700 Genetic Analyzer (Applied Biosystems) and contigs were assembled for the forward and reverse sequences with the SeqMan program from the LaserGene package (DNASTAR).

Phylogenetic analysis. The consensus sequences were aligned using MUSCLE software (Edgar, 2004) and adjusted manually with the CLUSTAL W program in MEGA4 (Thompson *et al.*, 1994; Tamura *et al.*, 2007). Phylogenetic trees were computed with the MEGA4 software, using the maximum-parsimony (MP) analysis method. To determine the branch support for each clade, a bootstrap analysis was performed with 1000 replicates. The MP tree was obtained using the Close-Neighbor-Interchange algorithm (Nei & Kumar, 2000) with search level 3 (Felsenstein, 1985; Nei & Kumar, 2000) in which the initial trees were obtained with the

random addition of sequences (ten replicates). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). Maximum-likelihood (ML) analysis was conducted using RAXML (randomized accelerated maximum-likelihood) software (Stamatakis *et al.*, 2005) through the CIPRES portal (www.phylo.org). All identical sequences were deleted before the analysis. Newly generated sequences were deposited in GenBank under the accession numbers listed in Fig. 1 and Supplementary Fig. S1 (available in IJSEM Online). In order to get higher bootstrap supports at the nodes, the ITS and tubulin datasets were combined. Before combining these datasets, incongruence between the datasets was detected by comparing reciprocal 70 % bootstrap values obtained by the ML analysis for all gene combinations, using the program COMPAT.PY (Kauff & Lutzoni, 2002; <http://www.lutzonilab.net>). Conflicts were considered significant when a taxon was differentially resolved between two gene trees with >70 % bootstrap support. Finally, the combined dataset was analysed using RAXML, with individual branch length optimization per partition and automatic determination of the number of bootstrap replicates.

Fig. 1. One of the 25 most parsimonious trees (length 455) based on partial β -tubulin sequences and depicting the relationship between the novel species and various taxa related to *P. simplicissimum* and *P. janthinellum*. The bootstrap percentages of the MP analysis (first number) and ML (second number) analysis are given at the nodes. Only bootstrap values $>50\%$ are shown, and branches supported $<50\%$ in either the MP or ML analysis are indicated with a hyphen. The novel species are shown in bold and the tree is rooted with *P. glabrum*. Bar, 10 changes over whole sequence (genetic distance).

RESULTS AND DISCUSSION

Morphology

Many *Penicillium* isolates were obtained during the investigation of different decomposition stages of leaf litter of tropical rain forests in the area along the Caquetá River, Colombia. Combined extrolite, morphological and

molecular data showed that many of the *Penicillium* isolates were related to *P. simplicissimum* and *P. janthinellum*, and represented novel species that are described here. The five novel species shared a high growth rate on CYA and MEA and attained a diameter of >40 mm in 7 days. All five novel species grew rather slowly on DG18 medium (15–25 mm). Growth at 37°C was a useful characteristic for differentiating the five novel species.

Penicillium wotroi sp. nov. grew well at 37 °C (20–30 mm), *Penicillium elleniae* sp. nov. grew restrictedly (0–10 mm) or not at all, while the other three novel species did not grow at 37 °C. Pitt (1979) also observed variation in growth at 37 °C, but he did not find sufficient evidence to use this as a diagnostic feature. In his description of *P. simplicissimum*, he stated that his isolates from New Zealand and Australian sources did not grow at 37 °C and that this difference was not sufficient to transfer these isolates to a novel species. Furthermore, growth and pH reactions on creatine agar were useful characteristics and appeared to be species-specific. With the exception of *P. wotroi* sp. nov., all of the other novel species grew moderately to well on creatine agar. Most isolates had weak acid production on creatine agar and acid production was followed by base production in *Penicillium penarajense* sp. nov., *Penicillium vanderhammenii* sp. nov. and *P. elleniae* sp. nov. after prolonged incubation for 14 days. Large interspecies variation was observed in conidial morphology and branching patterns. *P. elleniae* sp. nov. formed distinct globose, spinose conidia. The conidia of *P. penarajense* sp. nov. and *Penicillium araracuarensense* sp. nov. were rough-walled, while those of *P. wotroi* sp. nov. and *P. vanderhammenii* sp. nov. were smooth or nearly so. In addition, differences in branching patterns and stipe ornamentation were observed among the five novel species. *P. araracuarensense* sp. nov., *P. vanderhammenii* sp. nov. and *P. elleniae* sp. nov. formed sclerotoid structures on oatmeal agar, and ascospores were detected in the latter two species after an incubation period of eight weeks. The ascospores of *P. elleniae* sp. nov. were broadly ellipsoidal with spinose valves and the conidia were distinctly spinose and darkly pigmented. Morphologically, this species resembled *Eupenicillium zonatum* in many aspects, such as smooth-walled stipes, conidia and ascospore size and ornamentation. However, *E. zonatum* grows rapidly at 37 °C, whereas strains of *P. elleniae* sp. nov. did not grow or had restricted growth at this temperature. The formation of spinose conidia and monoverticillate conidiophores also suggested affinity with *Eupenicillium* section *Pinetora*, but members of this section are characterized by the production of flanged ascospores. The colony colour and ascospore size and shape of *P. vanderhammenii* sp. nov. resembled those of *Eupenicillium javanicum*. However, the stipes of *P. vanderhammenii* sp. nov. were smooth, whereas those of *E. javanicum* are rough-walled.

Molecular analysis

Partial β -tubulin and ITS 1 and 2 regions (including the 5.8S rDNA) were analysed. Phylogenetic analyses of the data were carried out using MP and ML. The phylograms of the two analyses were similar if branches with bootstrap values <70 % were disregarded as weak or unreliable. The β -tubulin gene dataset contained 514 characters, of which 178 were parsimony-informative (25 MP trees; CI: 0.4723, RI: 0.7587). The ITS dataset included 559 characters, with 49 parsimony-informative (84 MP trees; CI: 0.4124, RI:

0.7979). The majority of the bootstrap values of the branches of the ITS phylogram were low, indicating that the tree was poorly resolved (see Supplementary Fig. S1 in IJSEM Online).

The ITS and partial β -tubulin data had sufficient discriminatory power to differentiate the novel taxa from other recognized species. Partial β -tubulin and combined ITS and β -tubulin data showed that *P. vanderhammenii* sp. nov. and *P. penarajense* sp. nov. were related to each other with high bootstrap support, as were *P. wotroi* sp. nov. and *P. araracuarensense* sp. nov. (Fig. 1 and Supplementary Fig. S2). Phylogenetically, *P. elleniae* sp. nov. was related to *Eupenicillium caperatum*, *Eupenicillium reticulispurum* and *E. javanicum* and not to any of the anamorphic *Penicillia*. This suggests that this clade only comprises sexually reproducing species. Both *P. elleniae* sp. nov. and *P. vanderhammenii* sp. nov. formed ascospores after prolonged incubation. Peterson's data (Peterson, 2000) showed that the genera *Penicillium* and *Eupenicillium* are a homogeneous monophyletic group and that species related to *P. simplicissimum* and *P. janthinellum* are placed, together with other *Penicillia* and *Eupenicillia*, in a clade with 90 % bootstrap value (i.e. Peterson's group 5). Applying the concept of one name for one fungus (Reynolds & Taylor, 1991), we have chosen to describe these two novel species under their anamorphic names. Although the teleomorph name has precedence in the ICBN, it is more pragmatic to use the genus name *Penicillium*, since this name is well known and commonly used. It is suggested here that the name *Penicillium* should be refined to include the perfect state(s) and that only that name should be used in all cases.

P. simplicissimum and *P. janthinellum* represent a species-rich complex of at least 33 recognized species of the genera *Penicillium* and *Eupenicillium*, including the five novel species described here. The majority of these *Penicillium* species were synonymized with *P. simplicissimum* and/or *P. janthinellum* by Pitt (1979) and Stolk & Samson (1983), but, after molecular studies, appear to be distinct species and should be retained or reinstated. However, there are many other tentative novel species belonging to this complex. In the study of Tuthill *et al.* (2001), which combined extrolite, morphological and molecular data, three other novel species were shown to belong to this complex. These species were not named and were labelled as 'I', 'II' and 'III'. Representative ITS sequences were included in our ITS phylogram; none of these species resembled our newly described taxa. Furthermore, an ITS sequence of *Paecilomyces parvosporus* (GenBank accession no. DQ187954) was included in the ITS analysis and our data indicated that this species should be transferred to the genus *Penicillium*. Although no material of this species was examined, the figure in the original description (Han *et al.*, 2005) shows Furcatum-like *Penicillium* conidiophores and phialides with long slender necks, which are also typically formed by *P. janthinellum* (Pitt, 1979). The ITS sequence of an undescribed species, *Penicillium gastrivorus* (S. W.

Peterson, unpublished results, GenBank accession no. AF125941), was also included in the phylogram. This sequence was identical to the ITS sequence for the type strain of *P. brasilianum* CBS 253.55. A large number of *Eupenicillium* belong to this species-rich group. All members of *Eupenicillium* section *Javanica* (i.e. *E. javanicum*, *E. brefeldianum*, *E. levitum*, *E. ehrlichii*, *E. zonatum* and *E. ludwigii*) are linked to this complex. This is unremarkable, because members of this section have, like many related *Penicillia*, a high growth rate on agar media. However, *Eupenicillia* from other sections are also related, including *E. caperatum*, *E. reticulisporeum* (syn. *E. arvense*), *E. meloforme*, *E. lineolatum*, *E. limosum*, *E. meloforme*, *P. vanderhammenii* sp. nov. and *P. elleniae* sp. nov. A detailed taxonomic study of this group of *Penicillia* is necessary to further delimit species boundaries, determine relationships among various species, and reinstate species, such as *Penicillium echinulonalgoense*.

Extrolite analysis

Species of the genus *Penicillium* belonging to the subgenus *Furcatum* are efficient producers of metabolites and the extrolite patterns are effective markers for species recognition (Frisvad & Filtenborg, 1990; Tuthill *et al.*, 2001; Sonjak *et al.*, 2007). We found that each novel species produced unique and characteristic patterns of known and uncharacterized metabolites; an overview is given in Table 2. *P. vanderhammenii* sp. nov. and *P. penarojense* sp. nov. were closely related and both produced paxillin, paspaline, shaerinin/janthitrem and a number of uncharacterized metabolites. This close relationship was also observed in the phylogenetic study. These species could be differentiated by the ability of *P. vanderhammenii* sp. nov. to produce penicillic acid. Penicillic acid is also produced by *P. ochrochloron* and *P. pulvillorum* (Frisvad & Filtenborg, 1990). Pulvilloric acid was produced by *P. wotroi* sp. nov. and this metabolite is also produced by *P. pulvillorum* (syn. *P. cieglei*) and *P. piscarium* (Tuthill *et al.*, 2001). The extrolite data indicate that several metabolites are shared by various species related to *P. simplicissimum*. In this case, it is not the presence of a single extrolite, but rather the pattern of extrolites that is characteristic for a species. Paxillin, paraherquamide, penicillic acid, pulvilloric acid, janthitrems, penitrem A, xanthoepocin, xanthomegnin and viomellein are formed by several species in this clade. This

indicates that the biosynthetic genes for the formation of these metabolites are shared by these closely related species.

Ecology

The new species described in this study were predominantly present in decomposed leaf litter in Amazonia Colombia. However, BLAST searches on GenBank and the internal databases of the CBS-Fungal Biodiversity Centre showed that several of these species were not restricted to Colombian leaf litter. *Penicillium wotroi* sp. nov. has also been isolated from stored corms of *Gladiolus grandiflorus* in Mexico (González-Peréz *et al.*, 2009; GenBank accession no. DQ279802) and seeds of *Amaranthus hypochondriacus* in Mexico (Moreno-Velazquez *et al.*, 2005; DQ026013). *P. elleniae* sp. nov. has also been isolated from soil in (sub)tropical rainforests of Madagascar and Malaysia. On the other hand, no definitive similarity matches were found for *P. penarojense* sp. nov., *P. vanderhammenii* sp. nov. or *P. araracuarensis* sp. nov. The distribution of these species remains unknown, but it is probable that they are not restricted only to Colombian leaf litter.

The presence of a high number of *Penicillium* species in the decomposed leaf litter suggested that these species may have remarkable properties. In forest soils, where large amounts of plant materials are degraded, *Penicillium* species are usually detected abundantly, especially members of the *Penicillium* subgenus *Furcatum*. This subgenus includes many taxa related to *P. simplicissimum*, which are well-represented and common in hardwood forest soils and grasslands (Christensen *et al.*, 2000). Enzyme mixtures from *Penicillia*, including those of *P. simplicissimum* and related species (e.g. *P. brasilianum*), perform well in the hydrolysis of different kinds of lignocellulosic compounds (van Wyk, 1999; Thygesen *et al.*, 2003; Krogh *et al.*, 2004; Song *et al.*, 2010). The novel species described here might also be efficient lignocellulose degraders and represent a new source for cellulolytic and xylanolytic enzymes.

Nutrient analysis of the leaf litter and soil showed that the manganese, copper, zinc, iron and boron contents of the decomposing leaves were relatively high in comparison with the surrounding soils. These cations have also been shown to accumulate during decomposition (unpublished results, C. Lopez-Quintero & T. Boekhout). *P.*

Table 2. Overview of extrolites produced by the novel species

Species	Extrolites
<i>P. araracuarensis</i> sp. nov.	Pulvilloric acid and several other uncharacterized extrolites
<i>P. elleniae</i> sp. nov.	Paxillin, anthraquinones, sorbicillins and several other uncharacterized extrolites
<i>P. penarojense</i> sp. nov.	Paxillin, paspaline, shaerinins/janthitrems and several uncharacterized extrolites
<i>P. vanderhammenii</i> sp. nov.	Paxillin, paspaline, penicillic acid, shaerinins/janthitrems and several other uncharacterized extrolites
<i>P. wotroi</i> sp. nov.	Pulvilloric acid and several other uncharacterized extrolites

simplicissimum is tolerant of heavy metals and this species has been proposed as an efficient biosorbent agent in the bioleaching of zinc oxide, copper, lead and nickel (Burgstaller *et al.*, 1992; Li *et al.*, 2008; Valix *et al.*, 2001). Pulvilloric acid and penicillic acid, two extrolites produced by the novel species, can also chelate cations (Weinberg, 1957) and may contribute to this accumulation. Currently, no biochemical studies have been performed on the metabolome of leaf litter and it is unknown whether these chelating extrolites are produced in litter.

TAXONOMY

Latin diagnosis of *Penicillium araracuarens* Houbraken, C. López-Q, Frisvad *et* Samson *sp. nov.*

Penicillium simplicissimo affine, sed stipitibus longis, verruculosis, coloniis 37 °C haud crescentibus, conidiis dilute verruculosus, acore pulvillorica producenti. Typus HUA 170334. MB518025.

Description of *Penicillium araracuarens* Houbraken, C. López-Q, Frisvad *et* Samson *sp. nov.* (Fig. 2)

Penicillium araracuarens (a.ra.ra.cu.a.ren'se. N.L. neut. adj. *araracuarens* of or belonging to Araracuara, department of Caquetá, Colombia, the collection site of the holotype).

Colony diameters, 7 days, in mm: CYA25 °C 38–46; CYA30 °C 41–48; CYA37 °C 0; MEA 40–51; YES 51–57; DG18 15–22; creatine sucrose agar 23–31, moderate to good growth, weak acid and no base production. No sporulation on CYA after 7 days of incubation, sparse sporulation after 18 days, conidia light grey–green; exudates and soluble pigments absent, colonies moderately sulcate, reverse orange–brown. No or weak sporulation on YES, soluble pigments absent, inconspicuous mycelium, white, reverse orange–red with red centre. Weak sporulation on MEA, becoming strongly lanose in time, dull grey–green conidia with medium red coloured reverse. No reaction with Ehrlich test. Sclerotia produced on oatmeal, sclerotoid, globose, 80–120 µm in diameter, buff when

Fig. 2. *Penicillium araracuarens* *sp. nov.* (a) Colonies on CYA, obverse; (b) colonies on CYA reverse; (c) colonies on OA; (d–h) conidiophores; (i) conidia. Bars, 10 µm.

young, becoming yellow–brown in age; no ascospores observed after prolonged incubation.

Conidiophores predominantly arising from mycelial mat, biverticillate, with irregular verticils of metulae and occasionally with a divaricate branch; stipes long ($300\text{--}600 \times 2.0\text{--}3.0\text{ }\mu\text{m}$) with rough walls. Metulae unequal in length, terminating in a whorl of 2–3(–5), bearing 5–8 phialides, $(10\text{--})15\text{--}20\text{--}(25) \times 2.0\text{--}3.0\text{ }\mu\text{m}$. Phialides ampuliform to cylindrical, with a distinct neck, $9.0\text{--}12 \times 2.0\text{--}3.0\text{ }\mu\text{m}$. Conidia subglobose to broadly ellipsoidal, $2.5\text{--}3.5\text{ } (3.0 \pm 0.2) \times 2.4\text{--}3.0\text{ } (2.7 \pm 0.2)\text{ }\mu\text{m}$, with finely roughened walls.

Extrolites: Pulvilloric acid and several other unidentified extrolites.

Holotype: HUA 170334^{HT}; ex-type cultures are CBS 113149^T and IBT 23247^T, isolated from leaf litter in a leaf litter bag, which was deposited on the forest floor for 6 months, Araracuara, department of Caquetá, Colombia. Mycobank accession no. MB518025.

Ecology: Isolated from leaf litter exposed in a litter bag for 6 months. This species was isolated from various plots

of 36-year-old and 48-year-old secondary forests in Araracuara, Colombia (plot4-142; plot3-163b; plot3-164c; plot3-169 and plot 3-186a).

Latin diagnosis of *Penicillium elleniae*
Houbraken, C. López-Q, Frisvad et Samson
sp. nov.

Eupenicillio zonato affine, sed coloniis 37 °C haud vel vix crescentibus, conidiophoris monoverticillatis, conidiis crassitunicatis, spinulosis; paxillinum, anthraquinona et sorbicilina formantur. Typus HUA 170339. MB518028.

Description of *Penicillium elleniae* Houbraken, C. López-Q, Frisvad & Samson sp. nov. (Fig. 3)

Penicillium elleniae (el.le.ni' a.e. N.L. gen. fem. n. *elleniae* of Ellen, named in honour of Ellen Hoekstra, formerly a mycologist in the field of *Penicillium* taxonomy and food mycology).

Colony diameters, 7 days, in mm: CYA25 °C 33–42; CYA30 °C 40–50; CYA37 °C 0–26; MEA 40–51; YES

Fig. 3. *Penicillium elleniae* sp. nov. (a) Colonies on CYA, obverse; (b) colonies on CYA reverse; (c) colonies on OA; (d) ascomata; (e) conidiophores; (f) conidia; (g) ascospores; (h–i) conidiophores; (j) scanning electron micrograph of ascospores. Bars, 10 μm .

(30)40–51; DG18 20–30; creatine sucrose agar 15–35, good growth, no or weak acid and delayed base production. Cleistothecia production abundant on CYA after 7 days of incubation, conidiogenesis sparse and inconspicuous, dull green conidia, colonies deep radially sulcate, large exudate droplets in the centre of colony, red–brown, clear or light yellow coloured, reverse yellow with yellow–brown or reddish brown centre, some isolates with cream or beige reverse. No or weak sporulation on YES, mycelium white or light yellow in centre, weak soluble pigments produced, yellow, reverse ranging from cream–light yellow to bright yellow. No reaction with Ehrlich test. Cleistothecia on oatmeal agar yellow–brown (ochre to caramel), 100–200 µm, sclerotoid, maturing after 3 weeks or more, asci 8–12 µm in diameter; ascospores (broadly) ellipsoidal, $3.0\text{--}3.5$ (3.2 ± 0.1) $\times$ $2.5\text{--}3.0$ (2.6 ± 0.1) µm (without spines), traces of an inconspicuous equatorial ridge, valves spinose to aculeate, 0.2–0.4 µm long.

Conidiophores borne from aerial hyphae, mono- and biverticillate, occasionally metulae indistinguishable from monoverticillate stipes. Stipe short, $30\text{--}200 \times 2.0\text{--}3.0$ µm, smooth-walled. Metulae variable in size, $10\text{--}40 \times 2.0\text{--}3.0$ µm,

bearing 3–7 phialides. Phialides ampulliform with broad width, $7.0\text{--}9.0 \times 2.5\text{--}3.5$ µm. Conidia globose, $2.5\text{--}4.0$ (3.3 ± 0.3) µm, thick-walled, distinctly spinose.

Extrolites: Paxillin, anthraquinones, sorbicillins and several other unidentified metabolites.

Holotype: HUA 170339^{HT}; cultures ex-type are CBS 118135^T and IBT 23229^T, isolated from leaf litter in a litter bag, which was deposited on the forest floor for 6 months, Araracuara, department of Caquetá, Colombia. Mycobank accession no. MB518028.

Ecology: Isolated from decomposed leaf litter exposed in a litter bag for 6 months. This species was isolated from 48-year-old and mature forests in Araracuara, Colombia (plots 4-121 and 6-43, respectively) and from a mature forest in Peña Roja, Colombia (plot 'PR'-22).

Latin diagnosis of *Penicillium penarajense*
Houbraken, C. López-Q, Frisvad et Samson sp. nov.

Penicillii vanderhammenii et *P. janthinelli* affines, sed coloniis in agaro CREA bene crescentibus, sed 37 °C haud

Fig. 4. *Penicillium penarajense* sp. nov. (a) Colonies on CYA, obverse; (b) colonies on CYA reverse; (c) colonies on OA; (d–h) conidiophores; (i) conidia. Bars, 10 µm.

crescentibus; *paxillinum*, *paspalinum*, *shaerinina/janthitrema formantur*. *Typus* HUA 170335. MB518024.

Description of *Penicillium penarojense*
Houbraken, C. López-Q, Frisvad & Samson
sp. nov. (Fig. 4)

Penicillium penarojense (pe.na.ro.jen'se. N.L. neut. adj. *penarojense* of or belonging to Peña Roja, department of Amazonas, Colombia, the collection site of the holotype).

Colony diameters, 7 days, in mm: CYA25 °C 40–45; CYA30 °C 20–30; CYA37 °C 0; MEA 40–45; YES 35–40; DG18 20–25; creatine sucrose agar 16–25, good growth, variable acid production (absent to moderately present) and base production after prolonged incubation. Good sporulation on CYA after 7 days of incubation, velvety to slightly floccose, conidia grey–green to turquoise–grey, exudates absent, some strains with soluble yellow pigments, radially sulcated colonies, reverse orange–brown or yellow–brown with orange–red centre. Weak sporulation on YES, soluble pigments absent, pale yellow mycelium, reverse orange–red. Colonies on MEA weakly floccose, conidia

grey–green, reverse colour like agar medium or in shade of brown. No reaction with Ehrlich test. Sclerotia absent.

Conidiophores predominantly arising from mycelial mat, monoverticillate and biverticillate structures present; stipes long and slender (200–600 × 1.5–2.5 µm), smooth-walled. Metulae, when present, unequal in length, terminating in a whorl of 2(–3), occasionally indistinguishable from stipes of monoverticillate conidiophores, bearing 4–8 phialides, 15–25(–30) × 1.5–2.5 µm. Phialides ampulliform with a distinct neck, 6.0–9.0 × 2.0–3.0 µm. Conidia subglobose, 2.2–3.0 (2.6 ± 0.2) × 2.0–2.5 (2.3 ± 0.1) µm, rough-walled.

Extrolites: Paxillin, paspaline, shearinins/janthitrems and several unidentified extrolites.

Holotype: HUA 170335^{HT}; cultures ex-type are CBS 113178^T and IBT 23262^T, isolated from leaf litter in a leaf litter bag, which was deposited on the forest floor for 6 months, Peña Roja, department of Amazonas, Colombia. Mycobank accession no. MB518024.

Ecology: Isolated from leaf litter exposed for 6 months in a leaf litter bag. This species was isolated from 36-year-old

Fig. 5. *Penicillium vanderhammenii* sp. nov. (a) Colonies on CYA, obverse; (b) colonies on CYA reverse; (c) colonies on OA; (d, e, f, h) conidiophores; (g) conidia; (j) ascospores. Bars, 10 µm.

and mature forests in Peña Roja and Araracuara, Colombia (plots 'PR'-49b, 3-167a, 6-75, 'PR'-50a).

Latin diagnosis of *Penicillium vanderhammenii* Houbraken, C. López-Q, Frisvad et Samson sp. nov.

Penicillium penarojensi et *P. janthinello* affines, sed cleistotheciis et acore penicillica formantibus. Cleistothecia ochracea; ascospores minutae, $2.2\text{--}2.6 \times 1.8\text{--}2.2\ \mu\text{m}$. Typus HUA 170337. MB518027.

Description of *Penicillium vanderhammenii* Houbraken, C. López-Q, Frisvad & Samson sp. nov. (Fig. 5)

Penicillium vanderhammenii (van.der.ham.me'ni.i. N.L. gen. masc. n. *vanderhammenii* of van der Hammen, named in honour of Professor Thomas van der Hammen, an eminent palaeo-ecologist who studied the biodiversity of Colombia).

Colony diameters, 7 days, in mm: CYA25 °C 42–49; CYA30 °C 40–46; CYA37 °C 0; MEA 45–53; YES 41–47;

DG18 15–20; creatine sucrose agar 16–30, weak, moderate or good growth, weak acid and delayed base production. Conidiogenesis on CYA after 7 days of incubation good, velvety, grey–green conidia, exudate and soluble pigments absent, radially sulcate colonies, reverse beige with dark black–brown centre. Good sporulation on YES, soluble pigments absent, mycelium inconspicuous, white, grey–green conidia, reverse yellow. Colonies on MEA velvety to floccose, grey–green conidia, medium yellow reverse. No reaction with Ehrlich test. Cleistothecia on oatmeal agar ochre, 80–150 μm , often covered with a network of red–brown hyphae, maturing slowly, after 2 months or more, sclerotoid; asci 8–12 μm in diameter; ascospores ellipsoidal, $2.2\text{--}2.6$ (2.4 ± 0.1) $\times$ $1.8\text{--}2.3$ (2.0 ± 0.1) μm (without spines), traces of an inconspicuous equatorial ridge, valves spinose, 0.2–0.3 μm long.

Conidiophores arising from mycelium mat, predominantly monoverticillate, occasionally biverticillate and indistinguishable from stipes of monoverticillate conidiophores. Stipes long, delicate and slender, 200–800 $\times$ 1.5–2.5 μm , smooth-walled. Phialides ampulliform with distinct neck, 7.0–9.0 $\times$ 2.0–2.5 μm . Conidia broadly subglobose to

Fig. 6. *Penicillium wotroi* sp. nov. (a) Colonies on CYA, obverse; (b) colonies on CYA reverse; (c) colonies on OA; (d–h) conidiophores; (i) conidia. Bars, 10 μm .

broadly ellipsoidal, $2.2\text{--}3.0$ (2.6 ± 0.2) $\times$ $2.0\text{--}2.7$ (2.2 ± 0.2) μm , smooth to very finely rough-walled.

Extrolites: Paxillin, paspaline, penicillic acid, shaerinin/janthitrem and several other unidentified metabolites.

Holotype: HUA 170337^{HT}; cultures ex-type are CBS 126216^T and IBT 23203, isolated from leaf litter in a litter bag, which was deposited on the forest floor for 6 months, Araracuara, department of Caquetá, Colombia.

Ecology: Isolated from leaf litter exposed for 6 months in a litter bag (plots 6-91a and 6-95).

Latin diagnosis of *Penicillium wotroi* Houbraken, C. López-Q, Frisvad et Samson sp. nov.

Penicillio simplicissimo affine, sed stipitibus longis, verruculosis, coloniis 37 °C haud crescentibus, conidiis levibus, acore pulvillorica producenti. Typus HUA 170336. MB518026.

Description of *Penicillium wotroi* Houbraken, C. López-Q, Frisvad & Samson sp. nov. (Fig. 6)

Penicillium wotroi (wo.tro'i. N.L. gen. n. *wotroi* of WOTRO, referring to NWO-WOTRO, the funding organization that supported the project 'Role of fungi during regeneration of Colombian Amazonian forests').

Colony diameters, 7 days, in mm: CYA25 °C 38–50; CYA30 °C 50–60; CYA37 °C 20–30; MEA 30–55; YES 35–50; DG18 10–20; creatine sucrose agar (10–)20–30, weak growth, no acid or weak acid production, no base production. Conidiogenesis on CYA after 7 days of incubation moderate to good, absent in some isolates, colony surface velvety to floccose, conidia dull green, clear small exudate droplets, soluble pigments absent, weakly sulcate colonies, reverse cream with cream–orange centre. Weak to moderate sporulation on YES, soluble pigments absent, mycelium inconspicuous, white, dull green conidia, soluble pigments absent, reverse beige to light yellow with occasionally a beige–brown centre. Colonies on MEA velvety, dull grey–green, reverse medium coloured. No reaction with Ehrlich test. Sclerotia absent.

Conidiophores arising from mycelial mat, predominantly biverticillate but also many terverticillate structures present, branches divaricate, stipes long, $300\text{--}800 \times 2.5\text{--}3.5$ μm , coarsely roughened. Metulae unequal in length, terminating in a whorl of (2)3–6, each bearing 3–8 phialides, $10\text{--}20 \times 2.5\text{--}3.5$ μm . Phialides ampulliform with a distinct neck, $8.0\text{--}10 \times 2.3\text{--}3.3$ μm . Conidia subglobose to broadly ellipsoidal, $2.3\text{--}3.5$ (2.8 ± 0.2) $\times$ $2.1\text{--}2.9$ (2.0 ± 0.2) μm , smooth.

Extrolites: Pulvilloric acid and various other unidentified metabolites.

Holotype: HUA 170336^{HT}; cultures ex-type are CBS 118171^T and IBT 23253^T, isolated from leaf litter in a leaf litter bag, which was deposited on the forest floor for

6 months in a mature forest, Araracuara, department of Caquetá, Colombia. Mycobank accession no. MB 518026.

Ecology: Isolated from leaf litter exposed in a leaf litter bag for 6 months. The strains were isolated from mature forests in Peña Roja and Araracuara in Colombia (plots 'PR'-45b, 6-71a, 6-88a and 6-68).

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