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Generic delimitation of *Bionectria* (*Bionectriaceae, Hypocreales*) based on holomorph characters and rDNA sequences

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Abstract: The holomorphic genus *Bionectria,* with anamorphs classified in *Clonostachys,* is characterized and compared to related taxa of the *Hypocreales*. *Bionectria* species form penicillate, solitary or sporodochial conidiophores and imbricately arranged conidia held in chains or columns that may collapse into slimy masses. The superficially free ascomata often occur on other fungi, mainly ascomycetes, on bark of recently dead trees, or on decaying leaves. Anamorphs of *Bionectria* species, such as *Clonostachys rosea* (= *Gliocladium roseum*), are often encountered in soil and are known as destructive mycoparasites. Based mainly on characters of the teleomorph, such as occurrence of a supporting stroma, the interface between the stroma and the perithecial wall, anatomy of the perithecial wall, ascospore morphology, habit on and type of the natural substratum, six infrageneric subgroups are distinguished. Characters of the anamorph, such as the tendency to form dimorphic conidiophores and/or sporodochia, occurrence of intercalary phialides or setae, conidial shape, and pigmentation of conidial masses, partly support the subgroups delimited using teleomorph characters, but all subgroups can be linked with each other by intermediate patterns of anamorphs. Based on the general occurrence of penicillate conidiophores and suspected similarities in their life-styles, classification of all species in one genus is suggested. To address differences found in the anamorphs, the terms dendrodochium-, sesquicillium-, myrothecium-, and gliocladium-like are used. Analyses of rDNA sequences suggest monophyly of all taxa considered, while certain phenotypic characters appear in paraphyletic positions.

Key words: *Clonostachys*, *Gliocladium*, *Sesquicillium*, *Myrothecium*, ITS rDNA, large subunit rDNA, phylogeny, mycoparasitism.

Introduction

The family *Bionectriaceae* (*Hypocreales*) is characterized by pallid, light-coloured, white to brownish, generally solitary perithecia or rarely cleistothecia that mostly lack a stroma and do not change colour in KOH or lactic acid (Rossman *et al.*, 1999). While plant parasitism is more common in the *Nectriaceae* (perithecia in hues of red or purple that change colour in KOH), taxa of the *Bionectriaceae* frequently are fungicolous, myxomyceticolous, or saprotrophic on plant material. Subdivision of the *Hypocreales* based on molecular data was indicated first by Rehner & Samuels (1995). They distinguished the *Bionectria*-, *Nectria*-, *Claviceps*-, and *Hypocrea*-clades, which comprised representatives of taxa that today are classified in different families (Rossman *et al.*, 1999). Although not tested yet by molecular means, the *Niessliaceae* are suspected to represent a further family of the *Hypocreales*. The distinction of the families also receives strong support from phenotypic patterns of characters. Hypocrealean fungi have a *Nectria*-type perithecium development [Luttrell, 1951; Hanlin, 1961, for *B. ochroleuca* (Schw.) Schroers & Samuels sub *N. gliocladioides* Smalley & Hansen], which is characterized by downward directed apical paraphyses and asci arising mainly from the base of the perithecia. However, although clavicipitalean fungi cluster with the *Hypocreales* in phylogenetic analysis based on DNA sequence data (Spatafora & Blackwell, 1993), their type of perithecium

development is distinct from the *Nectria*-type (White, 1997).

Genera of the *Bionectriaceae* are delimited by characters of the perithecium–substratum interface, the anatomy of the perithecial wall, the presence of perithecial surface structures such as setae, fasciculate hyphae, enlarged cells, or warts, characters of the cells embedding the perithecia, and ascospore morphology (Samuels, 1976; Rossman *et al.*, 1999). Discrimination of at least several genera of the *Bionectriaceae* is also supported by sequence data of the large subunit ribosomal DNA (LSU rDNA = 28S rDNA) (Rossman *et al.*, 2000).

Apart from *Ochronectria* Rossman & Samuels, all genera of the *Bionectriaceae* accepted by Rossman *et al.* (1999) were described by previous authors. This indicates considerable morphological discontinuities seen in the teleomorphs. However, at least 10 of the 26 genera in the *Bionectriaceae* were originally described based on a single species that was often represented by a single specimen. Therefore, the multigeneric system could be fixed only after delimitation of naturally related species-groups based on numerous characters such as morphology of the holomorph and life-style, and after reinterpretation of certain characters of the type specimen.

Such a case was *Bionectria* Speg. (Spegazzini, 1919), originally based on a single specimen for species of *Nectria* occurring on living parts of plants. Beside the fact that the type species, *B. tonduzii* Speg., has not been recollected again (thus has remained rather unknown), Spegazzini obviously misinterpreted the primary substratum of the perithecia, which was a fungal stroma (Samuels, 1988b; Schroers *et al.*, 1999b). Life-style was, however, subsequently not considered significant for generic delimitation (Müller & von Arx, 1962; Samuels, 1988b).

The type specimen of *B. tonduzii* has numerous characters that are typical for a few species that formerly were classified as the *Nectria ochroleuca*-group (Booth, 1959; Samuels, 1976) and today form the core of *Bionectria* (Schroers & Samuels, 1997; Rossman *et al.*, 1999; Schroers, in preparation). These main characters are, (i) well-developed stromata erumpent through bark of recently dead trees (rarely on leaves), (ii) perithecia formed superficially in groups of up to 100 on such a stroma, (iii) light orange to brownish orange perithecia if smooth, or pale yellow, tan, or almost off-white if warted, (iv) perithecial walls consisting of three regions, of which the outermost is composed of angular cells, the inner of intricately arranged hyphae, and the innermost layers of thin cells that are common also in unrelated hypocrealean species (Schroers *et al.*, 1999b); (v) ascospores that are 1-septate and verrucose; and (vi) light orange vacuoles formed in cells of the outer perithecial wall region and the stroma. Particularly because the species often produce large clusters of perithecia, sometimes reaching 2 mm diam, and well-developed stromata, *Bionectria* in this sense is easily distinguishable from other genera of the *Bionectriaceae* that include species with solitary, sometimes immersed perithecia and lack a stroma. A similar erumpent stroma, however, occurs in *Stephanonectria keithii* Schroers & Samuels (Schroers *et al.*, 1999a) or, for example, in species of *Nectria* (Fr.) Fr. *sensu stricto*.

The anamorphs of the narrowly defined genus *Bionectria* are classified in *Clonostachys* Corda (former *Gliocladium roseum* Bainier complex). They are characterized by light pigments in cultures and dimorphic conidiophores. The so-called primary conidiophores are either verticillium-like or narrowly penicillate, forming conidia in watery drops. The secondary conidiophores are loosely to appressed penicillate, forming imbricately arranged conidia in chains or columns that frequently collapse into slimy masses (Domsch *et al.*, 1980; Schroers *et al.*, 1999b). The colour of the conidial masses is generally white, pale yellow to pale orange, or in rare cases greenish. Of the two kinds of conidiophores, it is the secondary type that in some species forms robust sporodochia, particularly on the natural substratum and from erumpent stromata like those beneath the perithecial clusters. Although the occurrence of more than one kind of conidiophore is common in ascomycetes, the combination of characters seen in *Clonostachys* is unknown in any other hypocrealean taxon.

Since Booth (1959) and Samuels (1976) recognized the *Nectria ochroleuca*-group, three other species-groups have been included or discussed in the context of *Bionectria*. These are, (i) species with striate ascospores and dimorphic or monomorphic conidiophores (Samuels, 1988a; here referred to as the '*N.*' *grammicospora* Ferdinandsen & Winge-group[1]), (ii) species forming green conidial masses on well-developed sporodochia (Samuels, 1976; Samuels *et al.*, 1990; here referred to as myrothecium-like anamorphs of '*N.*' *pityrodes* Montagne and '*N.*' *ralfsii* Berk. & Broome), and (iii) species mostly with superficially formed stromata and sesquicillium-like conidiophores (Samuels, 1989; here referred to as the '*N.*' *sesquicillii* Samuels-group). The perithecial characters of these species differ considerably

[1] Pending publication of the necessary nomenclatural changes (Schroers, in prep.), the original binomials in *Nectria* and other genera are used between inverted commas.

from the patterns found in the *B. ochroleuca*-group and their patterns may overlap with those of other genera of the *Bionectriaceae*.

This paper analyses the relationships of these species-groups, viewed from aspects of the teleomorphs, the anamorphs, and inferences from sequences of the internal transcribed spacers (ITS) and partial sequences of the LSU rDNA. Two further taxa (groups 4 and 5) are included in the discussion, namely (iv) the '*N.*' *epichloë* Speg.-group, characterized by sporodochial anamorphs with green-coloured conidial masses (myrothecium-like) and perithecia formed directly on fungal substrata and lacking a stroma, and (v) '*Nectriella*' *coronata* Juel, which differs conspicuously in characters of the perithecial wall and ascospore morphology, but forming conidiophores typical of *Sesquicillium* W. Gams that are almost indistinguishable from those found in the '*N.*' *sesquicillii*-group.

Material and methods

Morphology.— Morphological data were collected as described elsewhere (Schroers *et al.*, 1999b). The illustrations and drawings are extracted from a monographic treatment of *Bionectria* (Schroers, unpubl.). Characters of the teleomorph were studied from specimens collected in nature and deposited in the herbaria BPI, FH, NY, K, IMI, NY, and PDD (Holmgren *et al.*, 1990). Ascospores were isolated by micromanipulation (mostly by Dr G.J. Samuels, USDA, Beltsville). Conidial isolates were obtained mainly from *Centraalbureau voor Schimmelcultures* (1996), originating from numerous researchers. Microscopic structures of anamorphs were studied using living strains on oatmeal agar (Gams *et al.*, 1998) or cornmeal agar (Difco) and drawn using a camera lucida.

Molecular methods.— Strains used for the molecular study are listed in Table 1. The mycelium was grown as described by Rehner & Samuels (1994). Pieces of mycelial mats *ca* 2.5 cm^2 were harvested. The DNA was extracted using a CTAB procedure adopted from Weising *et al.* (1995) and Gerrits van den Ende & de Hoog (1999). DNA concentration was estimated spectroscopically (Sambrook *et al.*, 1989). For PCR, the genomic DNA was diluted with deionized water to a concentration of *ca* 10 ng/µl or used directly.

Ribosomal DNA (rDNA) was amplified using the primer pairs NS7/ITS4 (White *et al.*, 1990) or ITS5/NL4 (White *et al.*, 1990; O'Donnell, 1993). Primers were from Isogen, Bioscience B.V., Eurogentec or Gibco BRL. The PCR mixtures of 50 or 65 µl contained 1–3 µl DNA extract (diluted or not), 0.2 mM of each dNTP (Pharmacia Biotech 27-2035-01), 3–6 pmol of each of the primers, 1.5 mM $MgCl_2$ (as part of a standard PCR buffer provided together with the DNA polymerase), 1–2 U DNA polymerase (Amplitherm, ITK Diagnostics or Super Taq, HT Biotechnology Ltd.), and deionized water. The reaction mix was pipetted on ice and overlaid with oil (Sigma, M-5904 or M-3526) in 0.2 ml PCR-tubes (Biozym BV). PCR was performed in a Amplitron II Thermolyne using shortest ramp times and a jump start at 90°C. One of 35 cycles was as follows: 35–45 s at 94°C, 55 s at 52–58°C, 120 s at 72°C, a final extension period of 6 min at 72°C, and followed by a chill to 4°C. Size and concentration of the PCR products were estimated on 1% agarose gel (Amresco 0710-500G) after ethidium bromide staining (Serva 21238) compared to a DNA standard (Eurogentec MW-1700-02). The PCR product was cleaned using microspin columns S-300 or S-400 (Amersham Pharmacia Biotech, general protocol) and again checked on gels to estimate the volume for the sequence reaction. For sequencing reactions, the following primers were used: ITS5 and ITS2 (White *et al.*, 1990) for the ITS regions and the 5.8S rDNA, and NL1 and NL4 (O'Donnell, 1993) for the 5' part of the LSU rDNA. Sequence reactions were performed with the dRhodamine Dye Terminator- or the ABI Prism Big Dye™ Terminator Cycle Sequencing Kit, following the instructions of the supplier. 2–4 µl PCR-product (*ca* 15–45 ng) were used depending on its concentration. The PCR of the DNA fragments, washing, precipitation, and sequencing followed the protocols provided with the sequencing kits. An automated ABI PRISM 377 DNA sequencer (Perkin Elmer) was used.

Data analysis.— Sequence chromatographs were assembled and edited using SeqmanII (DNAStar, Inc.). The alignment of sequences was initially performed using Clustal X (v. 1.8; ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX). The alignment was adjusted using Megalign (DNAStar, Inc.). According to a predicted secondary structure of the ITS regions, certain variable areas were identified as terminal loops and delimited from flanking stem regions (cf. Takamatsu *et al.*, 1998). Loops and flanking stem area were initially aligned separately. Because in some taxa the stem areas were longer by few bases, mostly C or G, several gaps had to be introduced in taxa with slightly shorter stems, which resulted in several parsimony-uninformative positions. To avoid loss of information, the slightly longer stem areas were realigned with bases in other sequences that initially were identified as loops. A few gap-rich positions were excluded from the data set. Unknown data of the LSU rDNA were coded as such.

The sequences of *Nectriopsis sporangiicola* (Samuels) Samuels (*Bionectriaceae*) and *Nectria cinnabarina* (Tode) Fr., the type species of *Nectria sensu stricto* (*Nectriaceae*), were chosen as outgroup taxa according to available phylogenetic inferences (Rehner & Samuels, 1995; Rossman *et al.*, 2000) based on partial LSU rDNA sequences. While *Nectria cinnabarina* is unrelated, *N. sporangiicola*, together with *Nectriopsis violacea* (Fr.) Maire and other taxa such as *Hydropisphaera erubescens* (Desm.) Rossman & Samuels, appear to be the closest sisters to the monophyletic group that comprises *Bionectria*.

All phylogenetic analyses were done using PAUP 4.0b2 (Swofford, 1998). The data were analysed with gaps coded as missing data. Characters were defined as unordered and equally weighted. After exclusion of uninformative characters, heuristic searches of parsimonious trees were performed on all sequences with random sequence addition and 1000 replicates, using starting trees from stepwise addition, with tree bisection-reconnection (TBR) as the swapping algorithm, and all optimal trees for the next swapping round (Fig. 1). Branch robust-

ness was tested by 1000 replications of such searches based on bootstrapped data sets with random sequence addition and 10 replicates per search. Congruence of the data was tested using the partition homogeneity test (PHT) by heuristic searches and 1000 replicates, comparing parsimony-informative characters of, (i) both spacers (non-functional rDNA) with those from LSU rDNA and 5.8S rDNA (functional rDNA) after exclusion of 11 sequences, (ii) both spacers with each other, based on all sequences, and (iii) the first half of the ITS-1 with the other half, also based on all sequences. Heuristic searches of maximum likelihood trees using 19 sequences and including parsimony-uninformative data were performed with random sequence addition and 100 replicates, a transition/transversion ratio of 2, use of empirical base frequencies, and equal rates for variable sites. Branch robustness was tested by 1000 replications of such searches based on bootstrapped data sets but with sequence addition 'as is' (Fig. 2 a). A neighbor-joining tree based on a dissimilarity matrix using the Jukes Cantor correction for multiple mutations was performed on the same data used in the maximum likelihood analyses. Its branch support was tested by 1000 replications based on bootstrapped data sets (Fig. 2 b). All trees were rooted with a basal polytomy. The most distant taxon was inferred from cluster analyses (UPGMA).

Results

Molecular Phylogeny

Of the 1123 alignment positions and after exclusion of 15 positions, 168 characters were informative in parsimony analyses. The lengths of the internal transcribed spacers (ITS-1 and ITS-2) were similar in most sequences (ITS-1: *ca* 157 bp; ITS-2: *ca* 167 bp). Indels were found in *S. keithii* (ITS-1: 162 bp; ITS-2: 171 bp), '*N.*' *grammicosporopsis* Samuels (ITS-1: 137 bp, the short length caused by one predicted deletion event; ITS-2: 169 bp), *Sesquicillium* sp. 7 (ITS-1: 161 bp; ITS-2: 173 bp), and '*N.*' *lucifer* (ITS-1: 164 bp; ITS-2: 176 bp). A variable area was found at the 3'-end of the ITS-1. The most distant sequence was that of '*N.*' *lucifer* Samuels (GenBank AF210683), which was placed at the root in trees inferred by UPGMA (not shown). In parsimony analyses, '*N.*' *lucifer* clustered with a very long branch in the '*N.*' *grammicospora*-group. Its sequence, however, was excluded from the data set. The partition homogeneity test (PHT) did not indicate data conflict when both spacers were compared (P value < 0.1), while it concluded significant conflict when the non-functional rDNA was compared with the functional (P value = 0.325). The result of congruency tests, however, is difficult to interpret, because conflict was also measured when the first half of the ITS-1 was tested against its second half (P value = 0.538). The choice of these two fragments for this third PHT was arbitrary. Based on secondary structure approaches and to test consistency, consequently different stem regions and different loop regions would have to be tested against each other and against those from different regions of the rDNA. Furthermore, because a character change in the stem regions may be less likely than in the more variable terminal loops, reweighting of sequence characters possibly is necessary to reach congruent data sets that without such weighting are incongruent by nature.

The relatedness of genera of the *Bionectriaceae* was analysed based on phylogenetic inferences of LSU rDNA sequence data (Rossman *et al.*, 2000). Five of the species studied here (*B. ochroleuca*, *Bionectria* sp. 12, '*N.*' *grammicospora*, '*N.*' *sesquicillii*, and '*N.*' *pityrodes*) were found to form a monophyletic group, which is here referred to as the *Bionectria*-clade [note that the *Bionectria*-clade in the sense of Rehner & Samuels (1995) comprises roughly all of the *Bionectriaceae* as it is understood today]. The sister-group to the *Bionectria*-clade contains myxomyceticolous species of *Nectriopsis* Maire and species of *Hydropisphaera* Dumort., *Roumegueriella* Speg., and others (Rehner & Samuels, 1995; Rossman *et al.*, 2000). One of the myxomyceticolous species, *N. sporangiicola*, is used here as an outgroup taxon together with *N. cinnabarina*. The *Bionectria*-clade was analysed after inclusion of several other species using data from the internal transcribed spacers and partial LSU rDNA (*ca* 500 bp from the 5' end).

Six most parsimonious trees were found. They differed in the relative positions of four species, viz. *Clonostachys* sp. 14, *Bionectria* sp. 13 [anamorph = *Clonostachys compactiuscula* (Sacc.) D. Hawksw. & W. Gams], *Bionectria* sp. 12 (anamorph dendrodochium-like), and *Clonostachys* sp. 5 (myrothecium-like that fell outside the supported subclades A–C. Using maximum likelihood analysis, a single tree was found (Fig. 2 a). The trees from all different analyses showed similar branching patterns. The bootstrap support of the main clades is similar in the trees from parsimony and maximum likelihood analyses (Figs 1, 2 a), but sometimes higher in the neighbor-joining tree (Fig. 2 b).

Stephanonectria keithii is the closest sister to the *Bionectria*-clade. Both clades together form a statistically highly supported clade, contrasting with the outgroup taxa *N. cinnabarina* and *Nectriopsis sporangiicola*. The *Bionectria*-clade is statistically highly supported. Within the *Bionectria*-clade, a supported clade comprises species with *Sesquicillium* anamorphs, which are members of the '*N.*' *sesquicillii*-group (E in Figs 1, 2), and '*Nectriella*' *coronata* (F). Another *Sesquicillium*

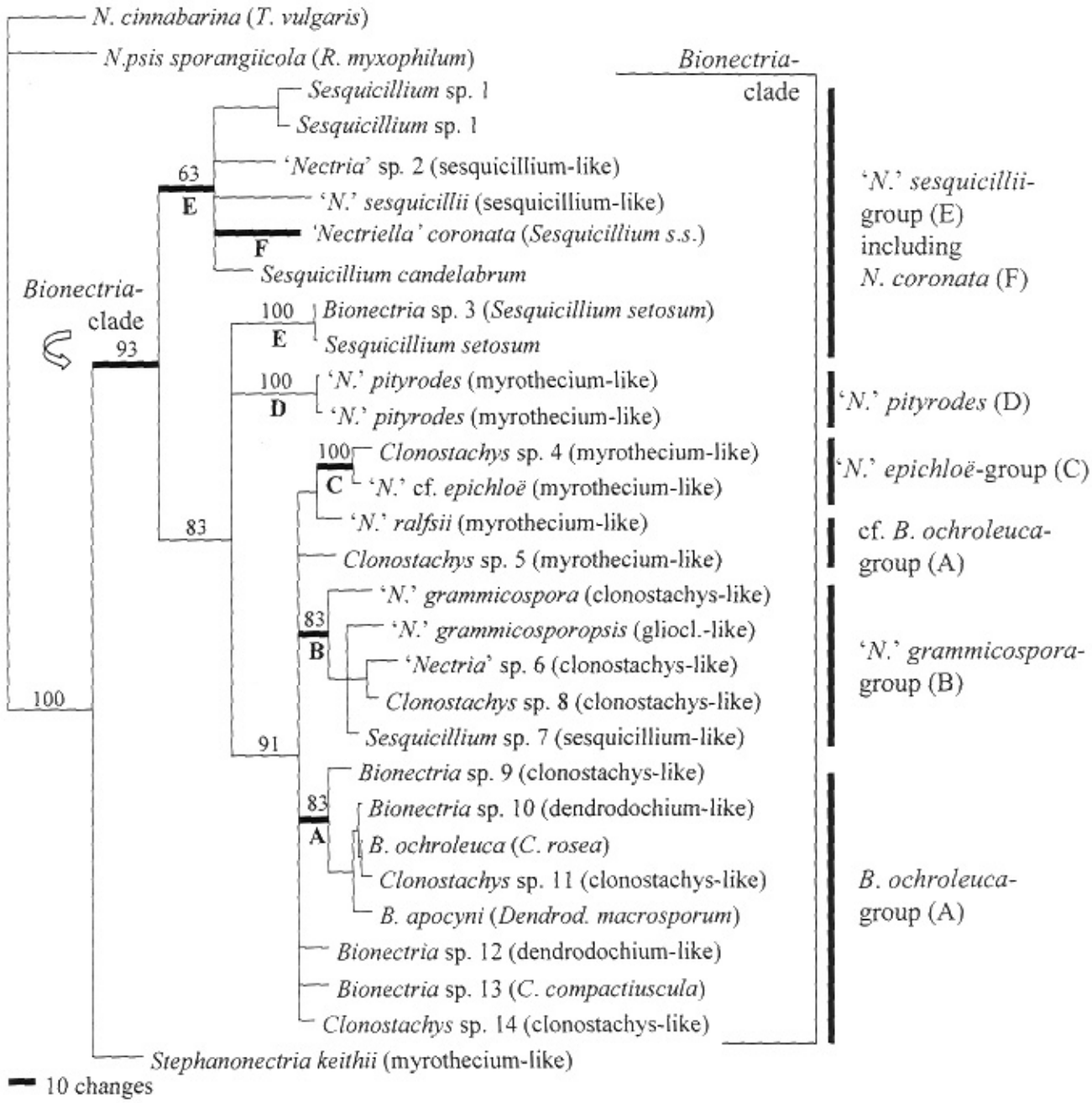

Fig. 1. Phylogram showing relationships between species and species groups within *Bionectria*. One of six most parsimonious trees obtained by heuristic searches (560 steps, CI = 0.470, RI = 0.608) based on parsimony-informative data of rDNA sequences (ITS1–5.8S–ITS2–LSU, *ca* 500 bp). Bootstrap values were derived from 1000 resampled data sets.

species, *S. setosum* Vittal, falls outside this clade. Therefore, species with *Sesquicillium* anamorphs appear to be paraphyletic. '*Nectria*' *pityrodes*, forming a myrothecium-like anamorph, also occupies an isolated position within the *Bionectria*-clade (D). The remaining species and species-groups form a highly supported clade comprising the *B. ochroleuca*-group (A), the '*N.*' *grammicospora*-group (B), and '*N.*' *epichloë*-group. The clade comprises four myrothecium-like species, of which those of the '*N.*' *epichloë*-group (two species) are considered monophyletic. The placement of '*N.*' *ralfsii* in a weakly supported clade together with the '*N.*' *epichloë*-group is considered insignificant. Similarly, the myrothecium-like *Clonostachys* sp. 5 is not included in a supported subclade. Both '*N.*' *ralfsii* and *Clonostachys* sp. 5 are included in the morphologically characterized *B. ochroleuca*-group. The '*N.*' *grammicospora*-group forms a monophyletic subclade (B), which also includes '*N.*' *lucifer* (not shown). The anamorphs of this group include species with dimorphic conidiophores (indicating relatedness to the *B. ochroleuca*-group)

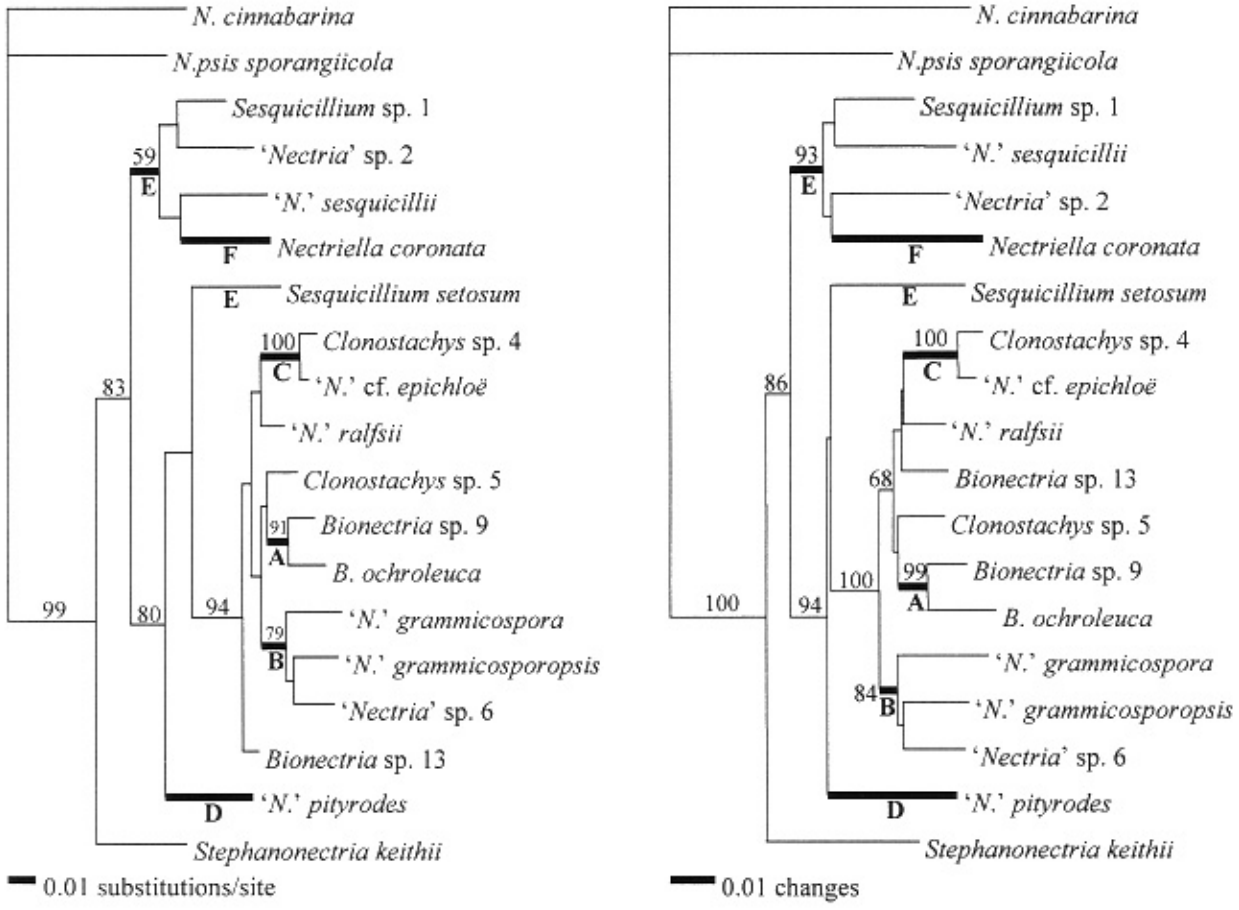

Fig. 2. Phylograms showing relationships between species and species groups in *Bionectria*. **a.** Single tree obtained by maximum likelihood analysis and heuristic searches (–Ln likelihood = 4929.79664) based on the same data as Fig. 1 but including parsimony-uninformative data and excluding 11 sequences. Bootstrap values were derived from 1000 resampled data sets. **b.** Neighbor-joining tree based on a dissimilarity matrix using the Jukes Cantor correction of multiple mutations. Bootstrap values were derived from 1000 resampled data sets.

and species with intercalary phialides in their conidiophores (sesquicillium-like), again indicating the paraphyletic distribution of this phenotype. Several species close to *B. ochroleuca* form a supported subclade, all of which have dimorphic conidiophores (A). However, two species with such an anamorph (*Bionectria* sp. 13 and *Clonostachys* sp. 14) and *Bionectria* sp. 12, forming sporodochia exclusively, fall outside that group. Because of morphological evidence they are included in the *B. ochroleuca*-group. Therefore, the *B. ochroleuca*-group in its narrow sense is paraphyletic.

MORPHOLOGY

The teleomorphs of the *Bionectria*-clade (Figs 3 a–f, 4 a–h) differ in several characters, mainly of their perithecial walls. Six main groups (A–F in Figs 1, 2) can be segregated. Their anamorphs (Figs 5 a–j, 6 a–h, 7 a–d) are characterized by penicillate conidiophores, which mostly form chains or columns of conidia, but differ by occurrence of primary conidiophores, sporodochium formation, conidial shape, and cultural characters. Descriptions of the groups follow:

The ***Bionectria ochroleuca*-group** (A in Figs 1, 2) is characterized by perithecia with mostly three wall regions (Fig. 4 c–e), of which the middle region consists of hyphae (Fig. 4 d). Ascospores are irregularly warted (Fig. 5 i, 6 b, c) but warts are arranged in rows in one species (not shown). Perithecia form on an erumpent stroma consisting of angular cells (Fig. 4 a) that do not differ from the cells of the outer perithecial wall region (Fig. 4 a, b; arrow in b marks the continuous interface between the cells of the outer wall region and the stroma). Conidiophores are dimorphic (Fig. 5 i, j) and the secondary conidiophores frequently form sporodochia or are entirely sporodochial (Fig. 7 a). Intercalary phialides are lacking or formed below whorls of terminal phialides (not shown). Conidia are ellipsoidal but slightly curved with one somewhat flattened side and have a

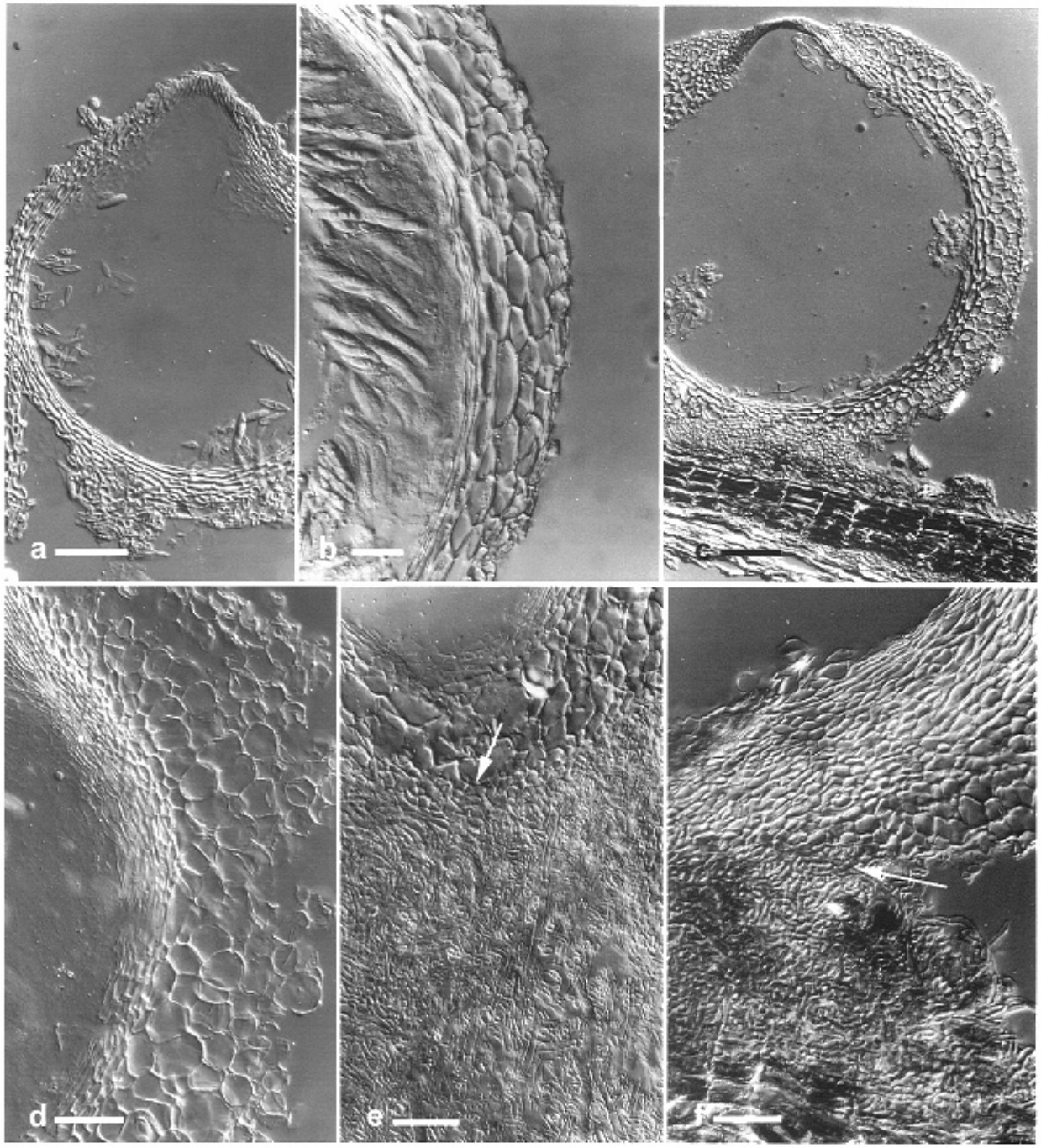

Fig. 3. Sections through perithecia and stromata of selected species of the *Bionectria*-clade. **a**. '*Nectriella*' *coronata*; perithecial wall consisting of a single region. **b–c.** '*N.*' *sesquicillii*-group. b. Perithecial wall consisting of 2 regions. c. Perithecium seated on a superficial, reduced, hyphal stroma. **d–f.** '*Nectria*' *grammicospora*-group. d. Lateral perithecial wall consisting of 2 regions. e. Erumpent, hyphal stroma; cells of stroma and perithecial wall discontinuous (arrow). f. '*Nectria*' *lucifer*, perithecium base and prosenchymatous to hyphal stroma; cells discontinuous with the cells of the perithecial wall (arrow). All from nature. Scale bars: a, d–f: 30 μm; b: 15 μm; c: 50 μm. a: BPI 802521; b: G.J.S. 6322; c: GJS 8971; d: Raunkier 3103; e: W.G.B. 809; f: H.J.S. 47B.

laterally displaced hilum (Fig. 5 i, j, 6 a, b, d); in some species, the conidia are almost straight (Fig. 6 c). Conidial masses are either white to pale orange or greenish (myrothecium-like).

The **'*Nectria*' *grammicospora*-group** (B in Figs 1, 2) is characterized by perithecia with two wall regions (Fig. 3 d). The middle region, described for the *B. ochroleuca*-group above, is lacking. Ascospores are conspicuously striate (Fig. 5 f, g) but in one case smooth (Fig. 6 h); the striae extend over the entire length of the spore. The stroma is well-developed (Fig. 3 e, f), mostly erumpent (Fig. 3 e) and of

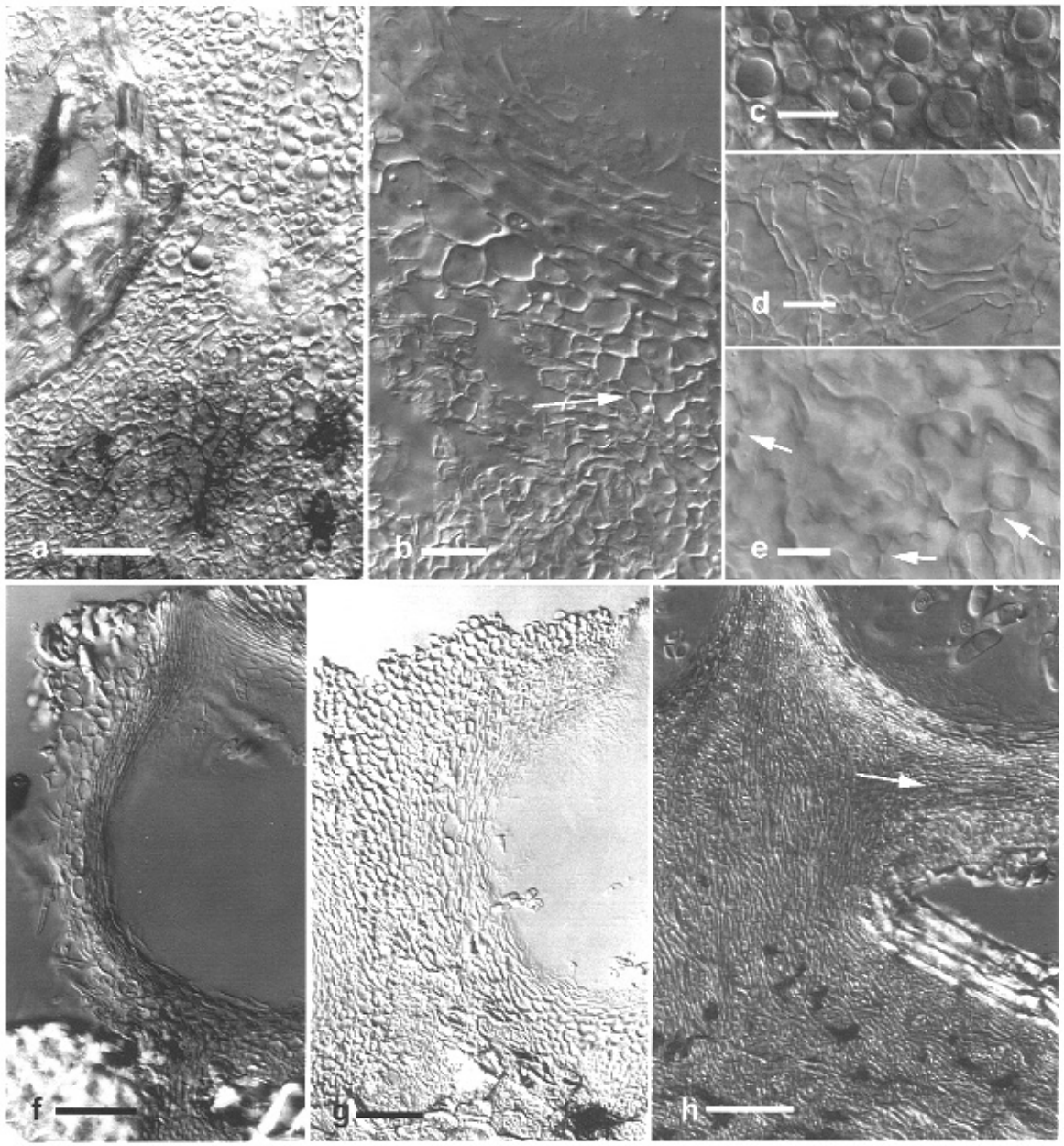

Fig. 4. Sections through perithecia and stromata of selected species of the *Bionectria*-clade. **a–e.** *Bionectria ochroleuca*-group. a. Erumpent pseudoparenchymatous stroma. b. Cells of outer perithecial wall region continuing with cells of stroma (arrow). c–e. Angular cells of the outer (c), hyphal cells of the middle (d), and lobed cells of the inner (e) regions of the perithecial wall being connected by 'pseudopores' in surface or subsurface view. Arrows (e) indicate 'pseudopores'. **f.** '*Nectria*' cf. *epichloë*, perithecium connected by hyphae to the substratum. **g.** '*Nectria*' *ralfsii*, outer perithecial wall region discontinuous with cells of the more or less hyphal stroma. **h.** '*Nectria*' *pityrodes*, erumpent, prosenchymatous stroma with cells continuing into the inner, hyphal region of the perithecial wall (arrow). All from nature. Scale bars: a, f: 30 μm; b: 15 μm; c–e: 10 μm; g, h: 50 μm. a: HJS 206; b: PDD 46486. c: GJS 4518; d, e: Ve 4781; f: PDD 46482; g: PDD 49950; h: Ve 3222.

similar dimensions to stromata of the *B. ochroleuca*-group, but the cells of the stroma are hyphal (Fig. 3 e) and differ from the cells of the angular to subglobose cells of the outer perithecial wall region (Fig. 3 e, f, see arrows pointing to the interface between stroma and perithecial wall). Conidiophores are dimorphic (Fig. 5 g, h), as they are in the *B. ochroleuca*-group, or monomorphic (Fig. 5 f). Intercalary phialides are frequently formed below whorls or solitary terminal phialides (Fig. 5 e). Sporodochia are not formed on the natural substratum or in culture. Conidia are ellipsoidal and generally straight; hila are

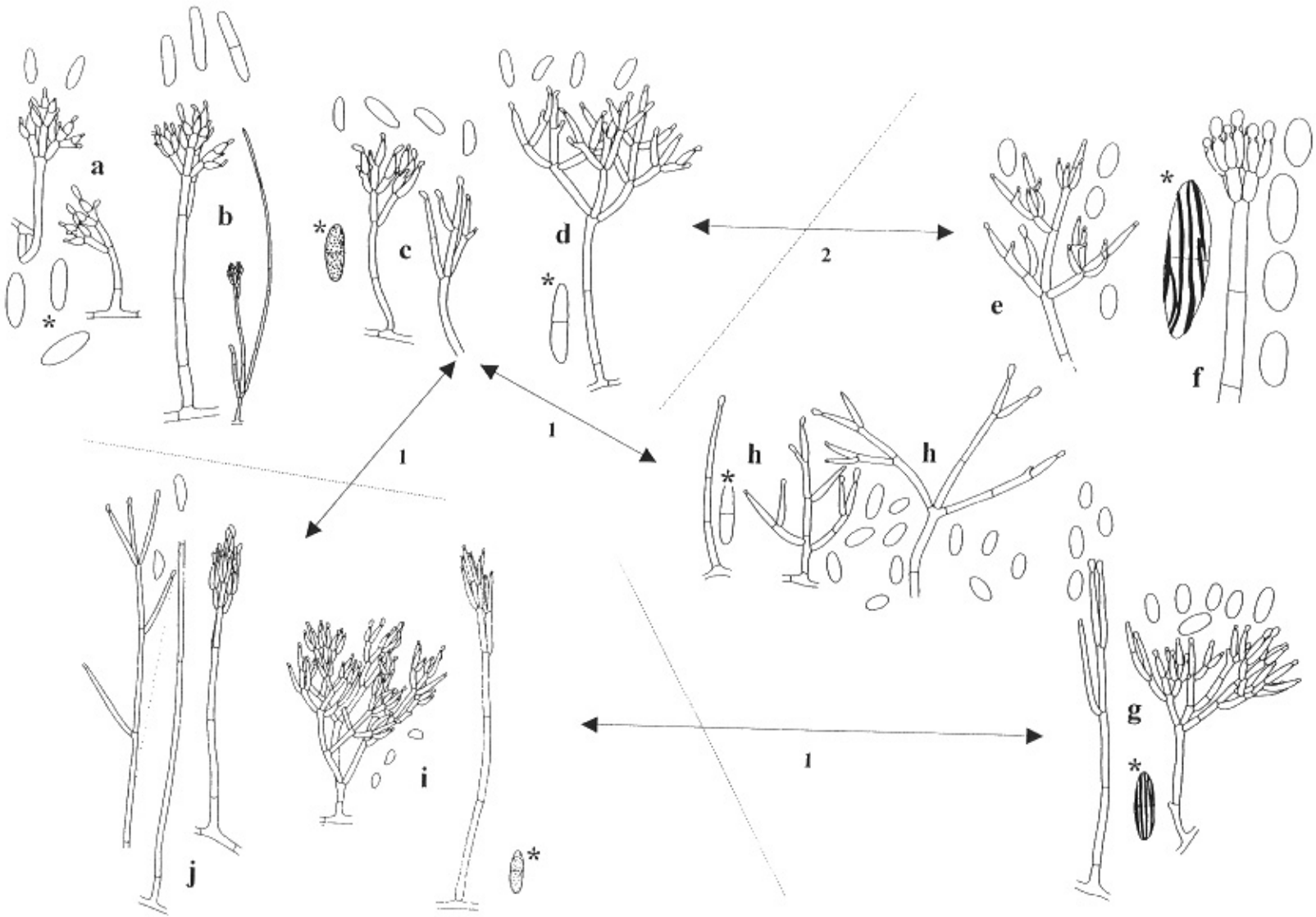

Fig. 5. Mononematous conidiophores, conidia, and ascospores (*) of selected species; dotted lines delimit three teleomorphic species-groups; arrows indicate phenotypic similarities between conidiophores of these groups: dimorphic conidiophores (arrows 1); intercalary phialides (arrow 2). **a–d.** '*Nectria*' species with *Sesquicillium* anamorphs. a. '*Nectriella*' *coronata/Sesquicillium buxi*. b. *Sesquicillium setosum*. c. '*N.*' *sesquicillii/Sesquicillium* cf. *buxi*. d. '*N.*' *lasiacidis/Sesquicillium* cf. *buxi*. **e–h.** '*Nectria*' *grammicospora*-group. e. *Sesquicillium* sp. 7. f. '*N.*' *lucifer/Gliocladium* sp. g. '*N.*' *grammicospora/Clonostachys* sp. h. *Bionectria* sp. 6/*Clonostachys* sp. **i, j.** *Bionectria ochroleuca*-group. i. *Clonostachys* sp. 11. j. *Gliocladium* (*Clonostachys*) *catenulatum*. Penicillate conidiophores as in Fig. 5 i also occur in sporodochia (Figs 6, 7). All from culture.

not visible, median, or almost median (Fig. 5 e–h). Conidial masses are either white to pale orange, arranged in columns or, on monomorphic conidiophores, in watery heads (e.g. '*N.*' *lucifer*).

The **'*Nectria*' *epichloë*-group** (C in Figs 1, 2) is characterized by perithecia with two wall regions (Fig. 4 f). The middle region, described for the *B. ochroleuca*-group above, is lacking. Ascospores are warted (Fig. 6 f), as they are in species of the *B. ochroleuca*-group. A stroma is more or less lacking and perithecia are loosely attached, mostly to a fungal host (Fig. 4 f). The conidiophores are almost entirely sporodochial (Fig. 6 f, 7 b). In one species, *Clonostachys* sp. 4, the conidiophores are rather irregularly penicillate. Conidia are slightly curved, possess a somewhat protruding hilum, which results in a somewhat clavate shape (Fig. 6 f, g), and are green in masses (myrothecium-like).

'*Nectria*' *pityrodes* (D in Figs 1, 2) has a perithecial wall similar to that found in the *B. ochroleuca*-group (3 regions), but the relatively thick hyphal region appears to merge with the cells of the prosenchymatous stroma (arrow in Fig. 4 h). Ascospores are smooth and frequently somewhat bean-shaped (Fig. 6 h). The stroma is erumpent, as it is in species of the *B. ochroleuca*- and '*N.*' *grammicospora*-groups (Fig. 4 h). Conidiophores are entirely sporodochial (Fig. 6 h, left), although in culture mononematous conidiophores also occur. The sporodochia are particularly characterized by a differentiated margin of sterile and conidiogenous hyphae (Fig. 6 h, right) and appear cup-shaped (Fig. 7 d). Intercalary phialides are absent. Conidial masses are green (myrothecium-like). Conidia are similar to those of species of the '*N.*' *grammicospora*-group, almost ovoidal (Fig. 6 h).

The **'*Nectria*' *sesquicillii*-group** (E in Figs 1, 2), inclusive of *Bionectria* sp. 3/*Sesquicillium setosum*, is characterized by perithecia with two wall regions (Fig. 3 b). The middle region, described above for the *B. ochroleuca*-group, is lacking. Ascospores are warted (Fig. 5 c), inconspicuously striate (not shown), or almost smooth (Fig. 5 d). The stroma is reduced to a small perithecial base that superficially covers the substratum (Fig. 3 c), but one species has an erumpent stroma similar to that produced by species of the *B. ochroleuca*-group (not shown); the dense, almost hyphal cells of the stroma do not continue into the outer perithecial wall region (but possibly into the outermost cell layer of the perithecia, with cells that are of different morphology from the main outer region). Conidiophores are monomorphic (Fig. 5 d), but one species is known with somewhat dimorphic conidiophores (Fig. 5 c). Intercalary phialides are consistently formed, almost always below terminal phialides (Fig. 5 b–d). Sporodochia are not formed on the natural substratum or in culture. Conidia are similar to those produced by species of the *B. ochroleuca*-group, slightly curved, with a laterally displaced hilum (Fig. 5 b–d), and are held in imbricate chains or columns that are whitish to pale orange, not greenish.

'*Nectriella*' *coronata* (F in Figs 1, 2) has perithecia with a single wall region (Fig. 3 a), which is strikingly similar to the inner region of all other species and consists of somewhat lobed cells connected by 'pseudopores' (compare to Fig. 4 e, not shown for '*N.*' *coronata*). The ostiole is surrounded by hyphal setae (not shown). Its perithecial wall anatomy is the most strongly deviant element in the *Bionectria*-clade because of its simplicity and because of the setae, which are unknown in all other groups. A hypothesis could be that these hyphae are homologous to the cells of the middle wall region of species in the *B. ochroleuca*-group. The stroma (Fig. 3 a) is similar to that produced by members of the '*N.*' *sesquicillii*-group but even less conspicuous, almost lacking. Ascospores (Fig. 5 a) are aseptate (while those of all other groups are 1-septate) and smooth. Conidiophores are typical of *Sesquicillium sensu stricto* (Fig. 5 a), more or less indistinguishable from those of the '*N.*' *sesquicillii*-group, always mononematous. The conidia are more or less cymbiform; their hilum is not laterally displaced and generally is not visible (Fig. 5 a), and conidial masses are always white to pale orange, never greenish.

'*Nectria*' *ralfsii* (Figs 1, 2) has perithecia with two wall regions, formed on an erumpent stroma (Fig. 4 g), and has rough to almost smooth ascospores (Fig. 6 e). The conidiophores are penicillate (Fig. 6 e) and are entirely sporodochial to synnematous (Fig. 7 c). Conidia are rather large (Fig. 6 e) and have strongly greenish-pigmented walls, whereas they are hyaline in all other taxa of the *Bionectria*-clade forming whitish, orange, or rarely brown conidial masses. Conidial masses in '*N.*' *ralfsii* are dark-green (myrothecium-like).

A perithecial wall with two regions characterizes the closest sister of the *Bionectria*-clade, *Stephanonectria keithii*. While the cells of the outer perithecial wall region in *Bionectria* species are angular to globose, those of *S. keithii* are rather narrow, oblong, and outwardly somewhat tooth-like. Ascospores are inconspicuously striate, 1-septate. The stroma is erumpent, as it is in members of the *B. ochroleuca*-group, or superficial when formed on *Brassica* stems. The species is particularly characterized by perithe-

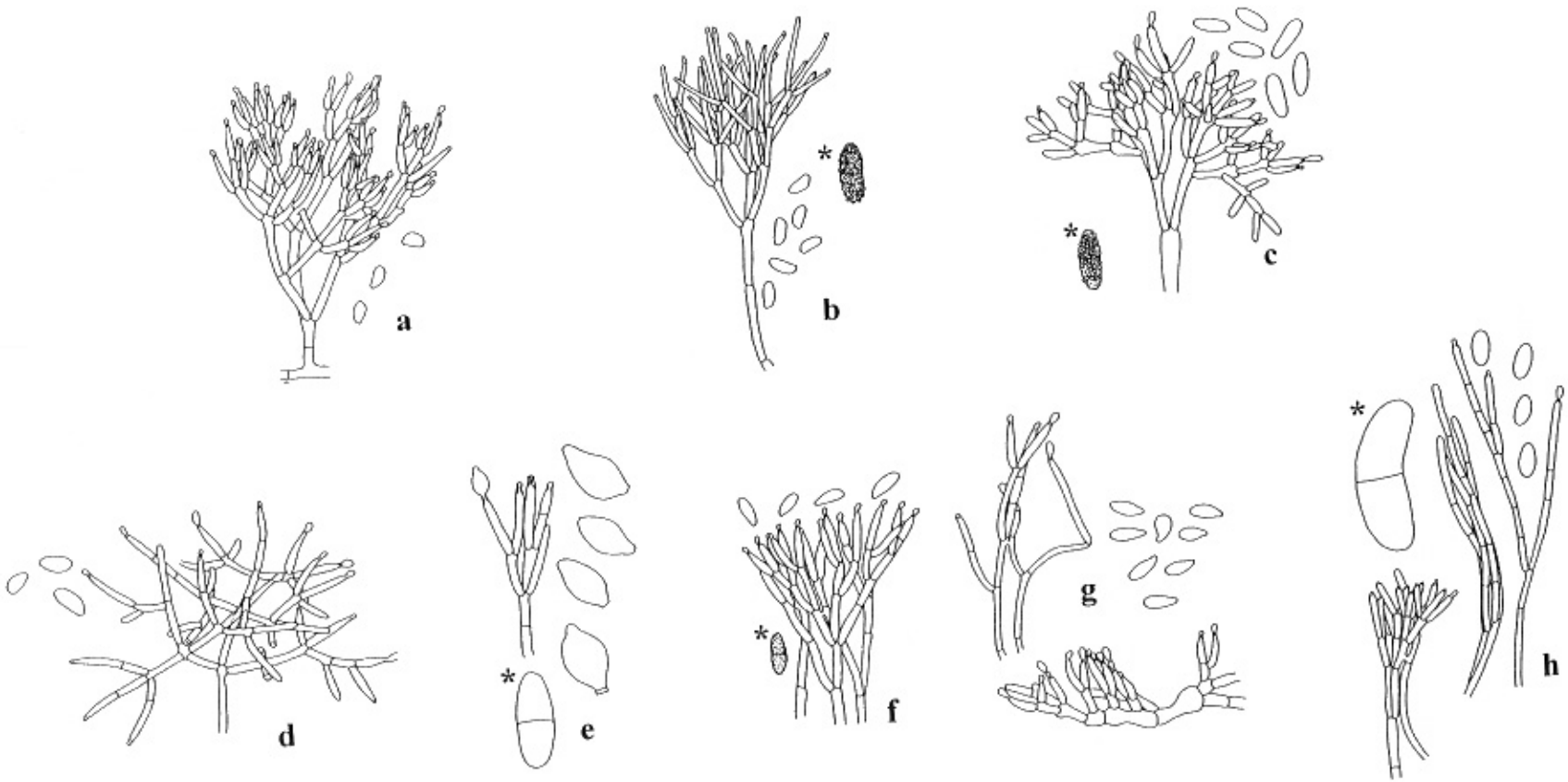

Fig. 6. Sporodochial conidiophores, conidia, and ascospores (*) of selected species; **a–c.** taxa forming white to pale orange conidial masses (dendrodochium-like); **d–h.** taxa forming green conidial masses (myrothecium-like). a. *Clonostachys* sp. 11. b. *Bionectria* sp. 10. c. *Bionectria* sp. 12. d. *Clonostachys* sp. 5. e. '*Nectria*' *ralfsii*. f, g. '*Nectria*' cf. *epichloë*-group. h. '*Nectria*' *pityrodes*. All from culture.

cial warts that are restricted to the ostiolar area containing tooth-like cells and by the brown pigmentation of the perithecia. Conidiophores are entirely sporodochial, although in culture mononematous conidiophores also occur. The morphology of the sporodochia is similar to that seen in the *Bionectria*-clade. Conidia are similar to those of '*N.*' *pityrodes*. The species is particularly characterized by brown conidial masses (Schroers *et al.*, 1999a).

Discussion

Morphology and phylogeny

Species and species-groups of the *Bionectria*-clade are monophyletic based on sequence analyses of the rDNA (Figs 1, 2; Rossman *et al.*, 2000). The main clade comprises diverse teleomorphs that partly are distributed in different subclades, rendering inference of natural relatedness based on the teleomorphs difficult. This situation contrasts with that in other genera of the *Bionectriaceae* and ascomycetes where genera are mainly defined by discontinuities in the morphology of the ascomata, stroma morphology, and ascoma–substratum interface (Rossman *et al.*, 1999).

Among the taxa of the *Bionectria*-clade, '*Nectriella*' *coronata* (F in Figs 1, 2) deviates most strongly because of its simple perithecial wall, perithecial setae, and aseptate ascospores. Mainly because of the unicellular ascospores, Juel (1925) described it originally in *Nectriella* Sacc. (homonym of the earlier and unrelated *Nectriella* Nitschke), and for the same reason Lowen (in Rossman *et al.*, 1993) transferred it to *Pseudonectria* Seaver (*Nectriaceae*). Because of its single perithecial wall region, '*N.*' *coronata* also may fit in *Nectriopsis*, where most species have thin perithecial walls with a single region. However, '*N.*' *coronata* does not fit in any of these genera, as they are understood today (Rossman *et al.*, 1999). In '*N.*' *epichloë*, as in '*N.*' *coronata*, no stroma is formed below the perithecia. Because of its fungicolous habit on ascomata of other fungi [mainly *Epichloë* (Fr.) Tul. & C. Tul. and *Balansia* Speg.], Samuels (1988b) transferred '*N.*' *epichloë* from *Nectria* to *Nectriopsis*. Species of the '*N.*' *sesquicillii*-group resemble genera such as *Hydropisphaera* and *Ochronectria* because of their superficially formed, smooth perithecia and reduced superficial stromata. Species of the *B. ochroleuca*-group, the '*N.*' *grammicospora*-group, and '*N.*' *pityrodes* differ strongly from '*N.*' *coronata*, '*N.*' *epichloë*, and the '*N.*' *sesquicillii*-group by having a well-developed, erumpent stroma and formation of perithecia in large, dense aggregates. Based on the well-developed stroma, species of the '*N.*' *ochroleuca*-group in the sense of Booth (1959) were delimited from other taxa now classified in the *Bionectriaceae* and were morphologically keyed out together with unrelated genera of the *Nectriaceae*, such as *Nectria sensu stricto* and the *Nectria coccinea* (Pers.) Fr.-group. Booth, however, did not consider the KOH reaction of the ascocarps and partly grouped together taxa of the *Nectriaceae* and *Bionectriaceae*.

Within the *Bionectria*-clade, the differences found in perithecial and stroma morphology are not or only partly corroborated by sequence data. (i) '*Nectriella*' *coronata* (F in Figs 1, 2) clusters with species of the '*N.*' *sesquicillii*-group (E). (ii) Features characterizing the '*N.*' *sesquicillii*-group (E in Figs 1, 2) and the *B. ochroleuca*-group (A in Figs 1, 2) are distributed paraphyletically because of the intercalation of *Bionectria* sp. 3 (*Sesquicillium setosum*) and taxa such as *Bionectria* sp. 13 (*Clonostachys compactiuscula*), which clusters outside the supported subclade A (Figs 1, 2). Erumpent stromata bearing large groups of perithecia occur in '*N.*' *pityrodes* in the *B. ochroleuca* group and in species of the '*N.*' *grammicospora*-group, which together form a paraphyletic group also including '*N.*' *epichloë*.

Characters of the anamorphs apparently better illustrate the relatedness of the taxa in the *Bionectria*-clade. All species or species-groups form penicillate conidiophores and most of them form imbricately aggregated conidia held in chains or columns, sometimes collapsing into slimy masses. No such character combinations occur in closely related genera of the *Bionectriaceae*, such as *Hydropisphaera*, *Ochronectria*, *Kallichroma* Kohlm. & Volkm.-Kohlm., *Nectriella*, and *Nectriopsis* (Rossman, this volume). Conidiophores of these taxa frequently are simple, acremonium-like or otherwise penicillate (Schroers *et al.*, 1999b). The presence of the anamorphic character combination in the *Bionectria*-clade, and the absence of these combined characters in closely related genera, demonstrate their value in recognizing species of the *Bionectria*-clade as naturally related. Arguments for including '*N.*' *coronata* in this clade, therefore, are mainly taken from the characters of the conidiophores.

Penicillate conidiophores, however, are found in unrelated fungal orders and families e.g. in *Penicillium* Link and *Paecilomyces* Bainier (*Eurotiales*), *Sphacelia* Lév. (with teleomorphs in *Claviceps* Tul., *Clavicipitaceae*), and *Stachybotrys* Corda (with teleomorphs in *Melanopsamma* Niessl, *Niessliaceae*, Samuels & Barr, 1997). Within the remaining families of the *Hypocreales* (*Hypocreaceae*, *Nectriaceae*, and *Bionectriaceae*), penicillate conidiophores forming slimy heads of conidia have mostly been placed in

Gliocladium Corda. They can be segregated in more narrowly defined units when more subtle features of conidia and/or conidiophores, biology, DNA sequence characters, and teleomorphs are taken into account (Rehner & Samuels, 1994; reviewed in Schroers *et al.*, 1999b). Taxa with gliocladium-like anamorphs include (i) *Gliocladium* Corda *sensu stricto* (Samuels, 1976; Seifert, 1985; Schroers *et al.*, 1999b), with species that form long-stalked, appressed conidiophores and yellowish watery heads of conidia. The type species of *Gliocladium*, *G. penicillioides* Corda, is related to several species forming synnematous conidiomata, with teleomorphs classified in *Sphaerostilbella* or *Hypocrea* series *Pallidae* (Doi & Yamatoya, 1989), which grow on basidiocarps of aphyllophoralean fungi or on rotten wood, (ii) *Gliocephalotrichum* J.J. Ellis & Hesselt. characterized by setose conidiophores and a teleomorph classified in *Leuconectria* Rossman, Samuels & Lowen (*Nectriaceae*; Rossman *et al.*, 1993), (iii) *Roumegueriella rufula* (Berk. & Broome) Malloch & Cain (*Bionectriaceae*), with conidiophores that are almost indistinguishable from those of species of *Gliocladium sensu stricto*, but normally have short stipes and off-white conidial masses, with a cleistothecial ascoma, (iv) the myxomyceticolous *Rhopalocladium myxophilum* Schroers, Samuels & W. Gams (Samuels, 1973; Schroers *et al.*, 1999b), which forms penicilli consisting of clavate cells and a teleomorph classified in *Nectriopsis* (*Bionectriaceae*), (v) *Didymostilbe* Henn., *Albosynnema* E.F. Morris (both *Bionectriaceae*), and the presumably related *Septomyrothecium* Matsush., with penicillate conidiophores forming synnemata, and dark-green conidial masses (yellow, orange or red in *Didymostilbe* species). In contrast to these taxa, the secondary conidiophores of the *Bionectria*-clade have loose to appressed penicilli, generally on rather short stipes, imbricate conidial columns, and verticillium-like or penicillate synanamorphs. Although the gross penicillate branching system is found in unrelated orders and genera of the ascomycetes, it can be concluded that the combination of anamorphic characters of species in the *Bionectria*-clade allows their recognition as a group of naturally related species.

Certain anamorphic characters found in the *Bionectria*-clade also occur in unrelated hyphomycetes. These include intercalary phialides, imbricately arranged conidia, sporodochia, and dimorphic conidiophores. Intercalary phialides or acropleurogenously arising phialidic necks have been described in diverse and unrelated fungal taxa, e.g. in *Tolypocladium microsporum* (Jaap) Bissett (Bissett, 1983), *Nectria cinnabarina* (Seifert, 1985), *Trichoderma* Fr. (anamorphs of the *H. schweinitzii* complex, sect. *Longibrachiatum*, Samuels *et al.*, 1998), in the lasiosphaeriaceous genus *Cladorrhinum* Sacc. & Marchal (Mouchacca & Gams, 1993), in the clavicipitalean *Drechmeria* W. Gams & H.-B. Jansson (Gams & Jansson, 1985), and the genus *Zakatoshia* B. Sutton (Sutton, 1973; Gams, 1986). In *Trichoderma* anamorphs of the *H. schweinitzii* complex, intercalary phialides are irregularly scattered in the complex branching systems of the conidiophores, while in *N. cinnabarina* they are found in long hyphal chains, particularly of the sporodochia. In contrast, in the *Bionectria*-clade, intercalary phialides are exclusively formed in well-defined penicilli of the conidiophores and should not be compared with the intercalary phialides in other genera. Similar distributions of intercalary phialides, however, are found in *T. microsporum*. This species, although considered congeneric with *Sesquicillium buxi* (Schmidt) W. Gams (1968) by some authors, differs strongly from the anamorphs of species in *Bionectria* by the smaller dimensions of phialides and conidia (Samuels, 1989, Fig. 7–9), the phialides that frequently have a swollen base and a rather long narrow neck, and the conidial masses held in heads instead of columns. It is not considered closely related to species of the *Bionectria*-clade.

Within the *Bionectria*-clade, however, intercalary phialides are formed in several species-groups. They frequently can be seen below solitary terminal phialides (as in '*N.*' *coronata*, the '*N.*' *sesquicillii*-group, and several species of the '*N.*' *grammicospora*-group, Fig. 5 a–d, e), rarely and mostly below whorls of terminal phialides (as in the *B. ochroleuca*-group), or they are completely lacking (as e.g. in '*N.*' *pityrodes*, '*N.*' *epichloë*-group, and several species of the *B. ochroleuca*-group).

Conidiomata are common in unrelated taxa, mostly either synnematous or sporodochial. Characters of synnemata that sometimes delimit naturally related species are the sterile hyphal elements arising at the margin of sporodochia or the synnema stipe, such as in *Gracilistilbella* Seifert species (Seifert & Samuels, this volume; Seifert, 1985), or combinations of characters such as morphology of sterile hyphal elements of the synnema stipe and conidial size and septation (such as in *Didymostilbe* species), or the *textura* of hyphae forming the synnema stipe (Seifert & Okada, 1991). Characters for the delimitation of sporodochial genera have included presence or absence of sterile elements such as setae or hyphae, the morphology and ornamentation of these sterile elements, the pigmentation of the conidial masses, the texture of the subhymenial cells or hyphae, and sometimes teleomorph–anamorph connections. The genus *Myrothe-*

cium Tode is well-defined based on its type species, *M. inundatum*, with suspected affinities to the *Bionectriaceae* (Rossman *et al.*, 1999). Its main characters are flat sporodochia, green conidial masses, and thick, straight setae or at least 'marginal hairs' arising from the sporodochial margin. Numerous other similar fungi were placed in *Myrothecium* by Tulloch (1972), but their phylogenetic relatedness to *M. inundatum* has not yet been ascertained. Similar setae, but more stipitate sporodochia and pale, light, non-green conidial masses, are found in species of *Volutella* (with teleomorphs in *Cosmospora* Rabenh. and *Pseudonectria* Seaver, *Nectriaceae*). In species of *Sarcopodium* Ehrenb. (Sutton, 1981), setae extending beyond the hymenium are partly undulate, warted, and pigmented (with teleomorph in *Nectriella*, *Bionectriaceae*). Sporodochial taxa lacking distinct sterile elements such as setae have been classified in genera such as *Tubercularia* Tode (teleomorphs classified in *Nectria sensu stricto*) or *Dendrodochium* Bonorden (1851). At least the type species of *Tubercularia*, *T. vulgaris* Tode, is well-defined as the anamorph of *N. cinnabarina* and relatively easily recognizable by the positive KOH reaction of the rather well-developed pseudoparenchymatous stroma (Seifert, 1985) and intercalary phialides formed in long chains in the sporodochial margin and hymenium.

Setae are not found in sporodochia of *Bionectria*, neither on the natural substratum nor in culture, while in several *Myrothecium* species setae predominantly occur, at least in nature. In the *Bionectria*-clade, conidiomata are generally sporodochial (Fig. 7 a–d), or exceptionally somewhat funnel-shaped, as in '*N.*' *ralfsii* (Fig. 7 c). Setae, however, can occur in species of the *N. sesquicillii*-group formed from mononematous conidiophores (Fig. 5b) or the aerial mycelium.

Sporodochia were never observed in '*N.*' *coronata*, or in species of the '*N.*' *sesquicillii*- and '*N.*' *grammicospora*-groups, but they are commonly formed on the natural substratum in '*N.*' *pityrodes*, species of the '*N.*' *epichloë*-group, '*N.*' *ralfsii*, and most species of the *B. ochroleuca*-group.

Conidiophores of sporodochia and solitary secondary conidiophores are considered homologous. Both possess similar branching patterns and conidial chains at least when young. On the natural substratum, sporodochia are generally formed on stromata erumpent through the bark (Fig. 7 a). Remnants of old sporodochia sometimes were found covering perithecial stromata, indicating that development of perithecia followed that of sporodochia. More typically, however, perithecial and sporodochial stromata occur independently on the same specimen. The pigmentation of conidial masses in taxa of the *Bionectria*-clade is diverse; white, pale yellowish, pale to light orange, or pale to dark green. Taxa forming green-coloured conidial masses do not form a natural group. They are found in the somewhat isolated '*N.*' *pityrodes* (D in Figs 1, 2), the '*N.*' *epichloë*-group (C in Figs 1, 2), and species that are linked to the *B. ochroleuca*-group such as '*Gliocladium*' *catenulatum* Gilman & Abbott (*Clonostachys*), '*N.*' *ralfsii* and *Clonostachys* sp. 5. The '*N.*' *grammicospora*- and '*N.*' *sesquicillii*-groups and '*N.*' *coronata*, on the other hand, always lack green hues of conidial masses, and in these groups sporodochia were not observed at all. Within the *Bionectria*-clade, sporodochia of '*N.*' *pityrodes* deviate most strongly because of a palisade of marginal, sterile or conidiogenous hyphae (Fig. 6 h, right) that extend slightly beyond the hymenium and give the sporodochia a somewhat cup-shaped appearance. Sporodochia formed by taxa of the *Bionectria*-clade all have in common conidial chains and columns that may collapse into slimy masses, and a lack of setae and ornamented hyphae. The terms dendrodochium-like, for sporodochia with white to pale-orange conidial masses, and myrothecium-like, for those with greenish hues, may be used to acknowledge these phenotypically striking characters.

Primary conidiophores that form watery heads of conidia are considered synanamorphs and considered as not strictly homologous to the secondary conidiophores. A distinct conidiophore dimorphism is particularly found in species of the *B. ochroleuca*-group (A in Figs 1, 2; Fig. 5 i, j) but also in *Bionectria* sp. 13 and *Clonostachys* sp. 14 that fall outside of the supported clade A, and in most species of the '*N.*' *grammicospora*-group (B in Figs 1, 2; Fig. 5 g, h). In culture, both kinds of conidiophores generally are present, but in certain species or strains one type is rare or even absent. For most holomorphic species possessing dimorphic conidiophores in culture, both types of conidiophores were also sometimes found on the natural substratum, accompanying the perithecia. The primary conidiophores generally arise from scanty aerial mycelium or directly from perithecia or the plant substratum. The secondary conidiophores are either formed solitarily, as are the primary conidiophores, or aggregated in well-developed sporodochia (Fig. 7 a).

Conidiophore dimorphism is also known in other hypocrealean fungi. In *Sphaerostilbella lutea* (Henn.) Sacc. (*Hypocreaceae*), verticillium-like conidiophores occur alongside synnemata (Seifert, 1985); in *Mariannaea elegans* (Corda) G. Arnaud ex Samson (1974)

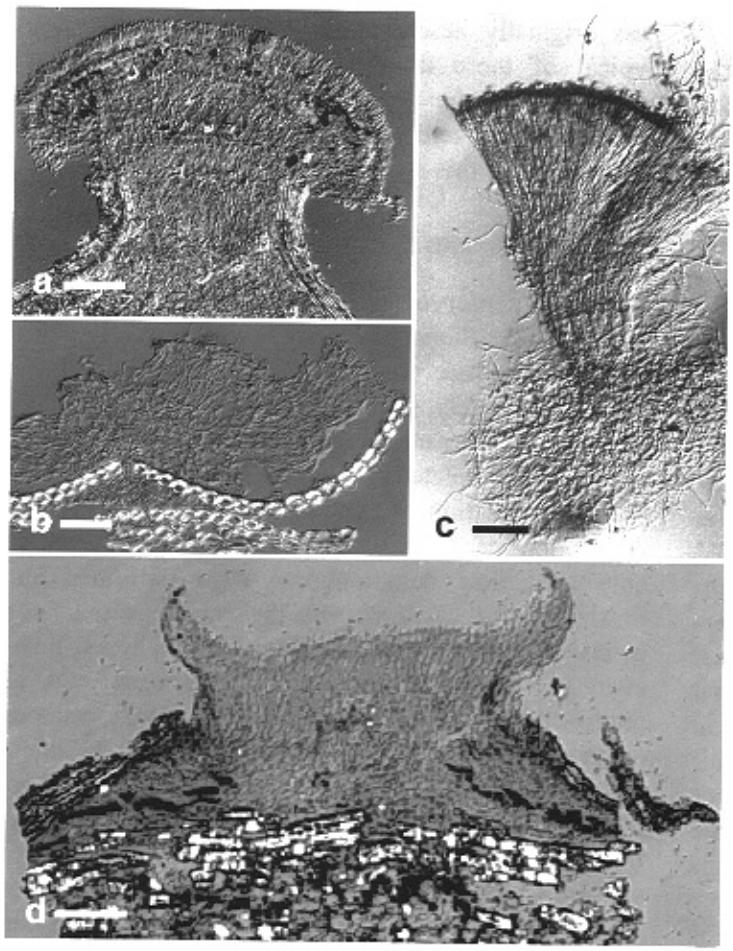

Fig. 7. Sections through sporodochia of selected species. **a.** *Clonostachys* sp. 11. **b.** '*Nectria*' cf. *epichloë*/myrothecium-like. **c.** '*Nectria*' *ralfsii*/myrothecium-like. **d.** '*Nectria*' *pityrodes*/myrothecium-like. a, b, d from nature; c from culture. Scale bars: a, c, d: 100 μm; b: 50 μm. a: Herb. CBS 04367; b: PDD 46484; c: CBS 703.97; d: Ve 3222.

more penicillate and short-stiped conidiophores sometimes are found next to verticillium-like conidiophores; in *Stilbocrea macrostoma* (Berk. & M.A. Curtis) Höhn. two types of synnemata are formed (Seifert, 1985; Seifert & Samuels, this volume); in *Albonectria rigidiuscula* (Berk. & Broome) Rossman & Samuels (anamorph = *F. decemcellulare* Brick) and species of the *F. solani* (Mart.) Sacc. complex (Gerlach & Nirenberg, 1982), microconidia are frequently formed on unbranched to sparsely branched conidiophores often in the aerial mycelium and with rather long phialides, while macroconidia are generally formed from sporodochial conidiophores and relatively short phialides.

Conidiophore dimorphism either developed independently in unrelated genera (a case of analogy) or might be traced to a common ancestor (case of homology). However, no data are available to support the idea that the sparsely branched aerial conidiophores of e.g. *Fusarium* species (*Nectriaceae*) are homologous to the primary conidiophores in *Bionectria*/*Clonostachys* species (*Bionectriaceae*) or that the macroconidium-forming sporodochia of *Fusarium* are homologous to the penicillate secondary conidiophores in *Clonostachys* species. If conidiophore dimorphism has evolved independently in unrelated taxa, different ecological roles for the conidiophores can be assumed. In cases of homology such dimorphisms may have been of ecological significance in ancestral species but not necessarily in the species living today.

The six teleomorph groups distinguished (A–F, Figs 1, 2) also differ in certain character patterns of the anamorphs: (i) The lack of an erumpent stroma in '*N.*' *coronata* and the '*N.*' *sesquicillii*-group (Fig. 3 a–c) is accompanied by the formation of intercalary phialides in conidiophores that are similar in different species (Fig. 5 a–d). (ii) The prosenchymatous stroma (Fig. 3 e, f), the absence of hyphal layer in the perithecial wall (Fig. 3 d), and striate ascospores (Fig. 5 g, f) of the '*N.*' *grammicospora*-group are generally accompanied by almost straight conidia (Fig. 5 e–h); (iii) The pseudoparenchymatous stromata (Fig. 4 a, b), median hyphal layer in the perithecial wall (Fig. 4 d), and warted ascospores in the *B. ochroleuca*-group (Fig. 5 i, 6 b, c) are mostly accompanied by dimorphic conidiophores (Fig. 5 i, j) and asymmetric conidia.

Intermediate character combinations in strains or species within the groups A–F, however, weaken the taxonomic significance of the above characters: (i) Although the conidiophores of '*Nectriella*' *coronata* and the '*N.*' *sesquicillii*-group are generally monomorphic, at least one species, '*N.*' *sesquicillii*, frequently develops additional verticillium-like conidiophores (Fig. 5 c; Samuels, 1989: Fig. 12). Such a conidiophore dimorphism is more typical of the *B. ochroleuca*-group and the '*N.*' *grammicospora*-group (arrow 1 in Fig. 5). (ii) In one of the six species of the '*N.*' *sesquicillii*-group, an erumpent stroma was found that is typical of species of the *B. ochroleuca*-group. (iii) Although intercalary phialides are characteristic of the '*N.*' *sesquicillii*-group and for '*N.*' *coronata*, they also occur in some species of the '*N.*' *grammicospora*-group (Fig. 5 e, arrow 2 in Fig. 5). (v) In two species of the *B. ochroleuca*-group (not shown here), the hyphal region of the perithecial wall could not be detected, although all other characteristics of the teleomorph and the anamorph clearly place them in the *B. ochroleuca*-group.

INFERENCES FROM ECOLOGY

The nature of the perithecial stroma and the substratum–ascocarp interface is possibly influenced by the life-style of the fungus, its ecological strategies, or the substratum. The occupation of different ecological niches is an important factor in speciation processes. Different ecological strategies or life-styles, therefore, may help to explain morphological differences in species or species-groups originating from the same ancestor. However, it should be emphasized that different ecological strategies can be realized in closely related species and apparently similar strategies can occur in unrelated taxa. Three main ecological patterns have been found in the *Bionectria-clade*: (i) Subcortical growth in recently dead trees (seen in '*N.*' *pityrodes*, species of the *B. ochroleuca*- and '*N.*' *grammicospora*-groups); stromata may help the fungus to break through the outer bark cells. (ii) Superficial formation of perithecia and possibly superficial growth on lichens, decaying leaves, immersed fungal ascomata, or bark [seen in the '*N.*' *sesquicillii*-group (Samuels, 1989) including '*N.*' *coronata*], or (iii) superficial growth on rachides of ferns and grasses or on fungal hosts inhabiting such substrata (seen in the '*N.*' *epichloë*-group). Species of the latter two groups possibly lack erumpent stromata because they grow superficially, because the cortex of the plant surface is easy to penetrate, or because of the more ephemeral nature of the substratum that does not allow stroma formation.

While these observations exclusively refer to the habitat of the perithecia, several species of the *Bionectria*-clade have been isolated from soil or decaying plant material in absence of perithecia or ascospores (reviewed in Domsch *et al.*, 1980). As saprophytes, they grow in soils and on recently dead trees or decaying leaves. Several records (listed in Domsch *et al.*, 1980; Schroers *et al.*, 1999b) consider certain species of the *Bionectria*-clade also as destructive and non-specific mycoparasites, mainly on other ascomycetes or hyphomycetes. The observation of stromata or perithecia that frequently are seated close to or on fruiting structures of other ascomycetes, in some cases even embedded within them (Schroers *et al.*, 1999b: Fig. 5), also hints at the role of these species as mycoparasites. The observations suggest that mycoparasitism by species of the *Bionectria*-clade may play a main role, but certain species within this clade may also be specialized on particular plant substrata or fungal hosts living in such plant substrata. The behaviour of some strains or species deviating from these concepts could possibly be explained by activity as mycoparasites. For example, *B. tonduzii*, which is regarded as a member of the *B. ochroleuca*-group, was originally described from a leaf, which is not typical of these taxa. However, Samuels (1988b) observed that the perithecia inhabit the stroma of another ascomycete and this possibly better reflects the biology of this species.

CONCLUSIONS AND TAXONOMIC INFERENCES

The most conserved morphological characteristic of species in the *Bionectria*-clade is the relatively short-stalked penicillate conidiophore; at least initially, it forms imbricately arranged conidia. The secondary conidiophore apparently separates *Bionectria*/*Clonostachys* from related genera of the *Bionectriaceae*. This interpretation is supported by Corda's original description of *Clonostachys* (Corda, 1839), where only penicillate conidiophores were described and illustrated (for *C. araucaria*, the type species of the genus). The phenotype of these conidiophores, however, is variable in some other characters such as pigmentation of conidial masses, their mononematous or sporodochial nature, frequent occurrence of intercalary phialides, and presence of synanamorphs. Although proven only for a few species, the destructive and unspecific nature of their (myco-)parasitism possibly is of taxonomic significance. Characters of the ascomata and stromata are diverse and these can be regarded as adaptations to the diverse nature of the plant substrata on which perithecia are formed.

Sequence data of the rDNA, the overall occurrence of penicillate conidiophores, and suspected similarities in life-style, have led to the conclusion that *Bionectria* is best circumscribed in a broad sense comprising all species-groups discussed here. A broad generic description of the teleomorphs, however, might overlap with the delimitation of other genera in the *Bionectriaceae*. To account for the heterogeneous patterns of the teleomorphs, the delimitation of infrageneric taxa seems to describe the taxonomic situation of the holomorphs best. Contrary to the situation of the teleomorphs, informative names of the anamorphs would be lost if the genera *Sesquicillium* and *Dendrodochium* were synonymized under *Clonostachys*. Rather than describing infrageneric taxa for anamorphic groups, the different patterns found in the anamorphs can best be referred to as clonostachys-, sesquicillium-, dendrodochium-, or myrothecium-like, while *Clonostachys* is to be retained as the formal anamorph genus.

This system has several advantages: **(i)** *Bionectria* is a monophyletic genus in a circumscription that is concordant with a phylogeny inferred from rDNA sequences. **(ii)** The genus *Bionectria* is correlated with only one anamorphic genus, *Clonostachys*. The ana-

morphic and the holomorphic generic names can be considered taxonomic synonyms because they comprise the same species, for some of which no teleomorph is known. **(iii)** Taking into account the variation of the teleomorphs, infrageneric taxa to be described within *Bionectria* increase the number of taxonomic units that can be applied to the more complex branching patterns and nested clades observed in the phylogenetic trees (Figs 1, 2). The infrageneric taxa seem to reflect differences in life-styles or plant/substratum preferences, at least to a certain degree. Infrageneric categories also serve to delimit *Bionectria* from other taxa in the *Bionectriaceae*. **(iv)** Considering the additional infrageneric categories in *Bionectria*, problems of paraphyletic distribution of some teleomorph characters are partly solved; if *Sesquicillium* and *Dendrodochium* are recognized as synonyms of *Clonostachys*, the problem of paraphyletic distribution of anamorphic characters is formally solved. **(v)** In describing the anamorphs as clonostachys-, sesquicillium-, dendrodochium-, myrothecium-, and verticillium-like (the latter to be used for the synanamorphs in species of the *B. ochroleuca*-group), the differences observed in the anamorphs are sufficiently taken into account.

The acceptance of diverse teleomorph characters in *Bionectria*, on the one hand, and the significance of anamorphs for generic circumscription on the other, leads to the inclusion of *Stephanonectria keithii* in the *Bionectria*-clade. *Stephanonectria keithii* was mainly described on the basis of discontinuities of the teleomorphs. Its conidiophores are entirely sporodochial, showing a similar branching pattern to those of '*N.*' *pityrodes*, but the sporodochia lack a distinct margin and the conidial masses are brown, which is relatively rare in hypocrealean fungi. However, perithecia of *S. keithii* are generally seated on well-developed stromata as they are in '*N.*' *pityrodes*, the *B. ochroleuca*-group, and the '*N.*' *grammicospora*-group. The molecular data neither support nor reject its inclusion in *Bionectria* because the clade including *S. keithii* is highly supported relative to *N. sporangiicola*, which is excluded from the *Bionectria*-clade with high support.

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Table 1. Strains included in the trees of Figs 1, 2 with references to the morphological groups indicated in Figs 1, 2 and described in the text. For abbreviations of culture collections, see CBS (1996); C.T.R = C.T. Rogerson, New York Botanical Garden, New York, USA; G.J.S. = G.J. Samuels, Beltsville, USA.

Taxon name	Morphological groups (A–F) and anamorphs	Strain number(s), collector, and isolator	Origin and substratum	Sequence accession number
Nectria cinnabarina	–, sporodochial (*Tuberculariavulgaris*) (outgroup)	NRRL 20484, MUCL 19 (Hennebert)	Belgium; on bark	L36625*** (O'Donnell, 1993); L36626** (O'Donnell & Gray, 1995)
Nectriopsis sporangiicola	–, gliocladium-like (*Rhopalocladium myxophilum*) (outgroup)	CBS 166.74, ATCC 26542, C.T.R. 67-136 (Rogerson)	USA, New Jersey; *Physarum polycephalum*; ex-type of *Nectriopsis sporangiicola*	AF210661** AF210662***
Sesquicillium sp. 1	E, sesquicillium-like	CBS 685.96, INIFAT C96/37 (Castañeda)	Cuba: 19 Mar. 1996; R. F. Castañeda	AF210663**
Sesquicillium sp. 1	E, sesquicillium-like	CBS 921.97 (Schroers)	France; fallen leaf of *Viscum album*	AF210664*
'*Nectria*' *sesquicillii*	E, sesquicillium-like	CBS 180.88, G.J.S. 87-23 (Samuels)	Guyana; twigs and lichen; ex-type culture	AF210666*
'*Nectria*' sp. 2	B, sesquicillium-like	CBS 211.93, G.J.S. 86-246	French Guiana; on twigs of recently dead tree	AF210665*
'*Nectriella*' *coronata*	E, sesquicillium-like (*Sesquicillium buxi*)	CBS 696.93, G.J.S. 93-53 (Candoussau, Samuels)	France; leaf of *Buxus sempervirens*	AF210667*
Sesquicillium candelabrum	E, sesquicillium-like	CBS 504.67 (von Emden)	Netherlands; wheat-field soil	AF210668**
Bionectria sp. 3	E, sesquicillium-like (*Sesquicillium setosum*)	CBS 917.97 (Schroers)	U.S.A., Puerto Rico; wet, decayed twig on wet ground	AF210669**
Sesquicillium setosum	E, sesquicillium-like	CBS 834.91, INIFAT C91/96 (Castañeda)	Cuba; *Trophis racemosa*	AF210670*
'*Nectria*' *pityrodes*	D, myrothecium-like	CBS 102033, IMI 3663387, G.J.S. 95-26	Mauritius; bark	AF210672*
'*Nectria*' *pityrodes*	D, myrothecium-like	CBS 246.78 (Samuels)	Brazil; bark	AF210673**
Clonostachys sp. 4	C, myrothecium-like	CBS 997.69 (Veenbaas-Rijks)	Netherlands; agricultural soil	AF210674*
'*Nectria*' cf. *epichloë*	C, myrothecium-like	CBS 101037, PDD 46484, G.J.S. 83-301 (Korf, Samuels)	Japan; *Sasa* sp.	AF210675*
'*Nectria*' *ralfsii*	A, myrothecium-like	CBS 129.87, G.J.S. 86-548 (Doi, Samuels)	New Zealand; bark	AF210676*
Clonostachys sp. 5	A, myrothecium-like	CBS 967.73b (Gams)	Germany; soil from wheat-field	AF210677*
'*Nectria*' *grammicospora*	B, clonostachys-like	CBS 209.93, G.J.S. 86-123 (Samuels)	French Guiana; trunk of standing dead tree	AF210678*

'Nectria' lucifer	B, gliocladium-like	CBS 100008, G.J.S. 96-52 (Samuels *et al.*)	U.S.A., Puerto Rico; bark of recently dead *Casicena arborea*	AF210683**
'Nectria' grammicosporopsis	B, gliocladium-, clonostachys-like	CBS 115.87; PDD 50054, G.J.S 85-44 (Samuels & Kohn)	New Zealand; *Metrosideros* sp.	AF210679*
'Nectria' sp. 6	B, clonostachys-like	CBS 948.97, G.J.S. 93-4 (Candoussau, Samuels)	France; branch of ?dead *Buxus sempervirens*	AF210680*
Sesquicillium sp. 7	B, sesquicillium-like	CBS 287.90 (Pfenning)	Brazil; soil under *Theobroma cacao*	AF210681**
Clonostachys sp. 8	B, clonostachys-like	CBS 508.82 (Nylander)	Netherlands; agricultural soil	AF210682**
Bionectria sp. 9	A, clonostachys-like	CBS 232.80, PDD 32501, G.J.S. 73-283 (Dingley *et al.*)	New Zealand; bark of *Coprosma australis*	AF210684*
Bionectria sp. 10	A, dendrodochium-like	CBS 101921 (Schroers *et al.*)	U.S.A., Puerto Rico; bark of recently dead branches	AF210685**
Bionectria ochroleuca	A, clonostachys-like (*Clonostachys rosea*)	CBS 193.94, G.J.S. 90-167 (Samuels *et al.*)	Venezuela; bark	U00750*** (Rehner & Samuels, 1994) AF210686**
Clonostachys sp. 11	A, clonostachys-like	CBS 702.97 (Schroers)	France; rotten fruit of *Aesculus hippocastanum*	AF210687**
Bionectria apocyni	A, clonostachys-like (*Dendrodochium macrosporum*)	CBS 130.87, G.J.S. 86-552 (Samuels & Rogerson) ; conidial isolate	U.S.A., New York; dead stem of *Apocynum cannabinum*	AF210688**
Bionectria sp. 12	A, dendrodochium-like	CBS 700.97 (Samuels *et al.*)	U.S.A., Puerto Rico; bark of recently dead tree	AF210689**
Bionectria sp. 13	A, clonostachys-like (*Clonostachys compactiuscula*)	CBS 919.97, G.J.S. 91-120 (Samuels *et al.*)	U.S.A., Virginia; twigs of *Acer* sp.	AF210690*
Clonostachys sp. 14	A, clonostachys-like	CBS 582.89 (Pfenning)	Brazil; rain forest soil	AF210691**
Stephanonectria keithii	–, myrothecium-like (closest sistergroup)	CBS 100005, G.J.S. 92-133 (Candoussau, Samuels)	France; bark of *Elaeagnus* sp.	AF210671*

A: *B. ochroleuca*-group
B: '*N.*' *grammicospora*-group
C: '*N.*' *epichloë*-group
D: '*N.*' *pityrodes*
E: '*N.*' *sesquicillii*-group
F: '*Nectriella*' *coronata*

* ITS 1–5.8S–ITS 2–LSU (*ca* 500 bp)
** ITS 1–5.8S–ITS 2
*** LSU (*ca* 500 bp)
Sequence accession numbers beginning with 'AF2106' represent newly generated sequences.