

## Molecular systematics of the *Coronophorales* and new species of *Bertia*, *Lasiobertia* and *Nitschkia*

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The *Nitschkiaceae* has been placed in the *Coronophorales* or the *Sordariales* in recent years. Most recently it was accepted in the *Coronophorales* and placed in the *Hypocreomycetidae* based on sequence data from large subunit nrDNA. To confirm and corroborate the taxonomic placement and monophyly of the *Coronophorales*, additional taxa representing the diversity of the group were targeted for phylogenetic analysis using partial sequences of the large subunit nrDNA (LSU). Based on molecular data, the *Coronophorales* is found to be monophyletic and its placement in the *Hypocreomycetidae* is maintained. The order is a coherent group with morphologies that include superficial, often turbinate, often collabent ascomata that may or may not contain a quellkorper and asci that are often stipitate and at times polysporous. Three species with accepted *Nitschkia* names, together with *Fracchiaea broomeiana* and *Acanthonitschkea argentinensis*, comprise the paraphyletic nitschkiaceous complex. Two new families, *Chaetosphaerellaceae* and *Scortechiniaceae* fams nov., are described for the clades containing *Chaetosphaerella* and *Crassochaeta* and the taxa having a quellkorper (*Euacantho*, *Neofracchiaea* and *Scortechinia*) respectively. The *Bertiaceae* is accepted for the clade containing *Bertia* species. Three new species are described: *Bertia tropicalis*, *Lasiobertia portoricensis*, and *Nitschkia meniscoidea* spp. nov.

### INTRODUCTION

The family *Nitschkiaceae* (syn. *Coronophoraceae*) has been placed in different orders over the years: in the *Coronophorales* (Nannfeldt 1932, Müller & Arx 1973, Subramanian & Sekar 1990) or in the *Sordariales* (Nannfeldt 1975a, b, Barr 1990). Its inclusion in the *Sordariales* by Hawksworth *et al.* (1995) led to its study by us as part of our project to circumscribe the *Lasiosphaeriaceae* and its relationships to other families in the *Sordariales* (Huhndorf, Miller & Fernández 2004). Members of the *Nitschkiaceae* are common components of the temperate and tropical, wood-inhabiting mycobiota. As circumscribed by Nannfeldt (1975a, b) the *Nitschkiaceae* includes five genera and is characterized by taxa with mostly superficial ascomata, often becoming cupulate or collapsed. In many taxa a quellkorper is present in the centrum and often the ostiolar opening is indistinct. Munk pores are found in the ascomatal wall cells and the asci tend to be thin-walled, long-stipitate and vary in ascospore number. The ascospores range from small, allantoid, or ellipsoid

in *Nitschkia* species to large, fusoid, or cylindrical in species of *Bertia*.

Subramanian & Sekar (1990) for the most part agreed with the overall family circumscription given by Nannfeldt (1975a, b) but differed in the number of genera accepted. While Nannfeldt tended to take a very broad view of genera such as *Nitschkia*, Subramanian & Sekar (1990) chose to recognize narrower taxon limits using characters such as quellkorper and subiculum to distinguish segregates from *Nitschkia*. This view of a larger number of genera with narrower circumscriptions was also shared by Müller & Arx (1973) and Arx (1981).

Nannfeldt (1975a, b) and Subramanian & Sekar (1990) provide extensive historical reviews of the various placements of the *Nitschkiaceae* or *Coronophoraceae* and its different components. According to Nannfeldt (1975b), *Nitschkiaceae* is the earliest validly published family name, given that the Latin ending was not used by Höhnelt (1907) in his 'Familie der Coronophoreen'. Nannfeldt (1932) considered the *Coronophorales* to be ascohymenial, whereas Miller (1949) treated the group as allied with loculoascomycetous taxa. Luttrell (1951, 1955) placed the *Coronophorales* in the *Pyrenomyces* with unknown

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**Table 1.** Taxa sequenced in this study.

Taxon	Source <sup>a</sup>	Geographical locality	GenBank accession no.
<i>Acanthonitschkea argentinensis</i>	SMH1395	Puerto Rico	AY695259
<i>Bertia moriformis</i>	SMH4320	Michigan	AY695260
<i>B. moriformis</i>	SMH3344	Michigan	AY695261
<i>B. tropicalis</i>	SMH1707	Puerto Rico	AY695262
<i>B. tropicalis</i>	SMH3513	Panama	AY695263
<i>Chaetosphaerella phaeostroma</i>	SMH4257	Costa Rica	AY695264
<i>Crassochaeta nigrita</i>	SMH1667	Puerto Rico	AY695265
<i>C. nigrita</i>	SMH2931	Puerto Rico	AY695266
<i>Euacanthofoveolata</i> (syn. <i>Acanthonitschkea foveolata</i> )	SMH4408	Ecuador	AY695267
<i>Fracchiacea broomeiana</i> (syn. <i>Nitschkia broomeiana</i> )	SMH2809	Indiana	AY695268
<i>Neofracchiacea callista</i> (syn. <i>Nitschkia callista</i> )	SMH2689	Illinois	AY695269
<i>Nitschkia meniscoidea</i>	SMH1523	Puerto Rico	AY695270
<i>N. pezizoidea</i>	SMH4409	Ecuador	AY695271
<i>Scortechinia conferta</i> (syn. <i>N. confertula</i> )	SMH2648	Illinois	AY695272

<sup>a</sup> SMH, Sabine M. Huhndorf (all collections in F, The Field Museum, Chicago). DNA was extracted directly from ascospores in all collections.

affinities, while Arx & Müller (1954) placed the family in the *Plectascales*. Carroll & Munk (1964) suggested that the *Coronophoraceae* were non-ostiolate relatives of the *Lasiosphaeriaceae*, a view also shared by Nannfeldt (1975b). Using LSU sequence data, Huhndorf, Miller & Fernández (2004) found that members of the *Nitschkiaceae* formed a strongly supported clade that clustered as a sister group to the *Hypocreales*. The group was accepted as the *Coronophorales* and placed in the *Hypocreomycetidae*.

In this study additional taxa of the order have been sequenced to confirm the taxonomic placement and monophyly of the group. Representative taxa from this group and from selected orders in the *Sordariomycetes* were targeted for phylogenetic analyses using LSU sequence data. Some questions we considered were: (1) is the *Coronophorales* a natural group; (2) what are the relationships among taxa within the order; and, (3) is the placement of the *Coronophorales* as a sister group to the *Hypocreales* and a distant relative to the *Lasiosphaeriaceae* upheld?

## MATERIALS AND METHODS

### Taxon sampling

Taxa sequenced in this study are listed in Table 1 along with their source information, geographical locality, and GenBank accession numbers. Ascospores in good condition were used to extract DNA since these taxa were impossible to obtain in culture despite numerous attempts. Additional taxa obtained from GenBank are listed in Table 2. Representatives from several orders within the *Sordariomycetes* were included to determine the phylogenetic relationship of the *Nitschkiaceae*. Two loculoascomycetes, *Capnodium citri* and *Botryosphaeria ribis*, were used as outgroups. For morphological studies, ascospores were mounted first in water, then replaced with lactophenol containing azure A. Measurements were made on material in water.

**Table 2.** Sequences used in this study obtained from GenBank.

Taxon	GenBank accession no.
<i>Apiospora setosa</i>	AY346259
<i>Chaetosphaerella phaeostroma</i>	AY346274
<i>Botryosphaeria ribis</i>	AY004336
<i>Capnodium citri</i>	AY004337
<i>Chaetosphaeria innumera</i>	AY017375
<i>Daldinia concentrica</i>	U47828
<i>Diaporthe phaseolorum</i>	AY346279
<i>Diatrype disciformis</i>	U47829
<i>Eutypa</i> sp.	AY346280
<i>Hypomyces luteovirens</i>	AF160237
<i>Lasiobertaria portoricensis</i>	AY346288
<i>Lasiosphaeria ovina</i>	AF064643
<i>Microascus trigonosporus</i>	U47835
<i>Nectria cinnabarina</i>	AF193237
<i>Nectriopsis violacea</i>	AF193242
<i>Nitschkia grevillei</i>	AY346294
<i>Petriella setifera</i>	U48421
<i>Sordaria macrospora</i>	AY346301
<i>Striatosphaeria codinaeaphora</i>	AF466088
<i>Valsa ceratosperma</i>	AF408387
<i>Xylaria hypoxylon</i>	U47841

Ascospores were sectioned at 5 µm for light microscopy using the techniques of Huhndorf (1991), and structures were examined using bright field, phase contrast and differential interference microscopy. A minimum of 30 asci, paraphyses and ascospores were measured in water for each species. Images were captured and photographic plates produced following the methods of Huhndorf & Fernández (1998). Abbreviations used for collectors are: SMH, S. M. Huhndorf; and FF, F. Fernández. When no collector is listed, the collector's initials are given with the specimen number. All SMH collections are deposited in The Field Museum's Mycology Herbarium (F). Latitude and longitude are given in degrees or calculated decimal equivalents. All specimens were collected from decorticated wood unless otherwise noted, and dimensions given for the substrates are diameters.

### **DNA extraction, PCR amplification, sequencing and sequence alignment**

Detailed protocols for the extraction, amplification, and sequencing of DNA and methods for the alignment of LSU sequences are described in Huhndorf *et al.* (2004).

### **Phylogenetic analyses**

Fifteen ambiguously aligned regions were delimited and characters in these regions along with portions of the 5' and 3' ends and constant characters were excluded from all analyses. Equally-weighted (MP1) and two types of unequally-weighted (MP2, MP3) maximum parsimony analyses were performed using PAUP\* 4.0b10 (Swofford 2002): characters were equally-weighted and unordered, gaps were treated as a fifth character state, 1000 random-addition replicates were implemented with TBR branch-swapping, MULPARS option was in effect, and zero-length branches were collapsed. In the MP2 analyses, changes among transitions, transversions and gaps were subjected to a symmetric stepmatrix generated using STMatrix ver. 2.2 (François Lutzoni & Stefan Zoller, Biology Department, Duke University, Durham, NC). This program calculates the costs for changes among these character states based on the negative natural logarithm of the percentages of reciprocal changes between any two character states. The MP3 analyses were similar to the MP2 analyses except thirteen of the fifteen ambiguous regions were included as thirteen unequivocally-coded characters using INAASE (Lutzoni *et al.* 2000). The remaining two regions were excluded from all analyses because their recoded characters contained more than 32 character states, which is not allowed in PAUP\* 4.0b10. Branch support was estimated by performing 1000 bootstrap replicates (Felsenstein 1985) with a heuristic search consisting of 100 random-addition replicates for each bootstrap replicate using the above settings for each analysis.

Maximum-likelihood analyses were performed with the best-fit model determined by MODELTEST 3.06 (Posada & Crandall 1998) using PAUP\* 4.0b10 with 100 and 1000 random addition heuristic searches and TBR branch-swapping. Bayesian analyses employing a Markov chain Monte Carlo (MCMC) method also were performed using MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001) as an additional means of assessing branch support. Constant characters were included, the above model of evolution was implemented, and four MCMC chains were ran simultaneously for 10 000 000 generations with trees saved every 1000th generation resulting in 10 000 total trees. The MCMC chains always achieved stationarity after the first 100 000 generations (=100 trees), so the first 2000 trees, which extended well beyond the burn-in phase of each analysis, were discarded. Posterior probabilities were determined from a consensus tree generated using the remaining 8000 trees. This analysis was repeated five

times starting from different random trees to insure trees from the same tree space were being sampled during each analysis.

### **Evolutionary rate analyses**

Members of the *Coronophorales* contain a large number of indels in the LSU sequence alignment and occur on long branches suggesting they may possess an accelerated rate of evolutionary change relative to the other taxa. Therefore, relative-rate analyses were conducted using the program RRTree (Robinson-Rechavi & Huchon 2000) to determine whether the substitution rate is significantly higher within and among members of this order. RRTree compares rates among lineages containing multiple sequences relative to an outgroup and returns a probability associated with each test. Phylogenetic relationships can be integrated through topological weighting. Comparisons among lineages using the nearest outgroup were made with topological weighting only after all ambiguous sites were removed from the sequence alignment as suggested by Robinson *et al.* (1998).

## **RESULTS**

### **Sequence alignment**

The final alignment included 35 taxa and 1137 bp after the introduction of gaps. This region corresponds to bp positions 156 (5' ATATCAATAA) to 1237 (5' AAAAATGGCC) of *Saccharomyces cerevisiae* (GenBank accession no. J01355). 15 ambiguous regions representing 384 characters along with an additional 496 constant characters were excluded from all analyses. Of the remaining 257 characters, 42 were parsimony-uninformative leaving 215 parsimony-informative characters. Thirteen additional parsimony-informative characters derived from the unequivocally-coded ambiguous regions were also included in the MP3 analyses.

### **Phylogenetic analyses**

The equally-weighted MP1 analysis generated 19 equally most-parsimonious trees, which did not differ significantly in topology, all within a single island of trees (data not shown). The inclusion of the stepmatrix in the MP2 analysis produced a single most parsimonious tree (data not shown). The MP3 analysis, which included the stepmatrix along with the thirteen recoded ambiguous regions, also produced a single most parsimonious tree (Fig. 38). The topologies of the well-supported clades including the *Coronophorales* and its families were similar in all three types of analyses and primarily differed in the placement of *Nitschkea pezizoidea*.

The best-fit model determined by MODELTEST was the Tamura-Nei model (Tamura & Nei 1993) with

equal base frequencies, an assumed proportion of invariable sites of 0.52, and a gamma shape parameter of 0.67. Maximum-likelihood analyses implementing 100 or 1000 random-addition heuristic searches generated the same most-likely (ML) tree (Fig. 39), which was nearly identical in topology to the 95% majority rule consensus trees obtained from the Bayesian analyses (data not shown). The ML tree differed only slightly in topology from the MP3 tree and recovered the same well-supported families in the *Coronophorales*. The clustering of members of the *Coronophorales* could be influenced by long-branch attraction, which generally occurs when long terminal branches are separated by short internal branches (Felsenstein 1978). However, this is probably not the case since the *Coronophorales* occurs on a relatively long internal branch. In addition, members of this order are monophyletic in the maximum-likelihood analyses, a method that has been shown to be less sensitive to long-branch attraction (Huelsenbeck 1995, 1997).

#### Evolutionary rate analyses

The *Coronophorales* is characterized by a large amount of divergence, as indicated by the relatively long branches within this clade (Figs 38–39). This suggests a faster rate of evolution may have occurred within this order relative to the other orders. Therefore, relative-rate analyses were conducted between members of this order and the *Hypocreales* sister-group to determine whether their substitution rate was significantly different. Comparisons also were made among the four groups within the *Coronophorales*. As expected, the substitution rates between the *Coronophorales* and *Hypocreales*, using the *Microascales* as outgroup, were significantly different ( $P < 0.0001$ ). However, comparisons among the *Bertiaceae*, *Chaetosphaerellaceae*, *Scortechiniaceae*, and *Nitschkiaceae* (excluding *N. pezizoidea*) were not significantly different (all  $P > 0.01$ ) suggesting that while members of the *Coronophorales* are evolving at a faster rate relative to taxa in the *Hypocreales*, evolutionary rates among members within the order are similar. In addition, average sequence divergence (estimated by uncorrected p) within the other six sordariomycete orders ranged from 0.018 in the *Sordariales* to 0.034 in the *Xylariales* compared to 0.098 within the *Coronophorales* suggesting the rate of evolution may be as much as three times higher within this order.

#### TAXONOMY

The order *Coronophorales* was found to be monophyletic and its placement in the *Hypocreomycetidae* is maintained. The *Nitschkiaceae* was found to be paraphyletic and is here recognized as the 'nitschkiaceous complex'. Two new families are proposed for the clades containing *Chaetosphaerella* and *Crassochoeta*, and the taxa having a quellkorper (*Euacanthae*, *Neofracchiaea*,

and *Scortechinia*), respectively. The *Bertiaceae* is recognized for the clade containing *Bertia* species.

#### **Chaetosphaerellaceae** Huhndorf, A. N. Mill. & F. A. Fern., **fam. nov.**

Ascomata superficialia, ovoidea vel obpyriformia, ostiolata; pagina glabra vel hirsuta; subiculum praesens. Paraphyses sparsae vel copiosae, latae, inflatae. Asci clavati vel cylindracei, unitunicati, cum vel sine annulo. Ascospores ellipsoideae, oblongae vel fusiformes, septatae, fuscatae, concolorae vel versicolorae, laeves. Conidiogenesis phialidicae enteroblasticae vel treticae holoblasticae.

*Typus*: *Chaetosphaerella* E. Müll. & C. Booth 1972.

*Ascomata* superficial, ovoid to obpyriform, ostiolate, glabrous or setose; subiculum present. *Paraphyses* sparse or abundant, wide, inflated. *Asci* clavate or cylindrical, unitunicate, with or without apical ring. *Ascospores* ellipsoid, oblong or fusiform, septate, pigmented, concolorous or versicolorous, smooth. Conidiogenesis enteroblastic phialidic or holoblastic tretic.

#### **Scortechiniaceae** Huhndorf, A. N. Mill. & F. A. Fern., **fam. nov.**

Ascomata superficialia vel semi-immersa, turbinata, subglobosa vel obpyriformia, nonostiolata; pagina glabra vel hirsuta; subiculum praesens, leaves vel setosa. Paraphyses absens; quellkorper praesens. Asci clavati, unitunicati, sine annulo. Ascospores ellipsoideae, ovoideae, vel allantoidae, hyalinae vel fuscatae, laeves. Anamorpho ignota.

*Typus*: *Scortechinia* Sacc. 1891.

*Ascomata* superficial or semi-immersed, turbinate, subglobose, cupulate when dry, non-ostiolate, glabrous or setose; subiculum present, smooth or spiny. *Paraphyses* absent, quellkorper present. *Asci* clavate, unitunicate, without apical ring. *Ascospores* ellipsoid, ovoid or allantoid, hyaline or pigmented, smooth. *Anamorphs* unknown.

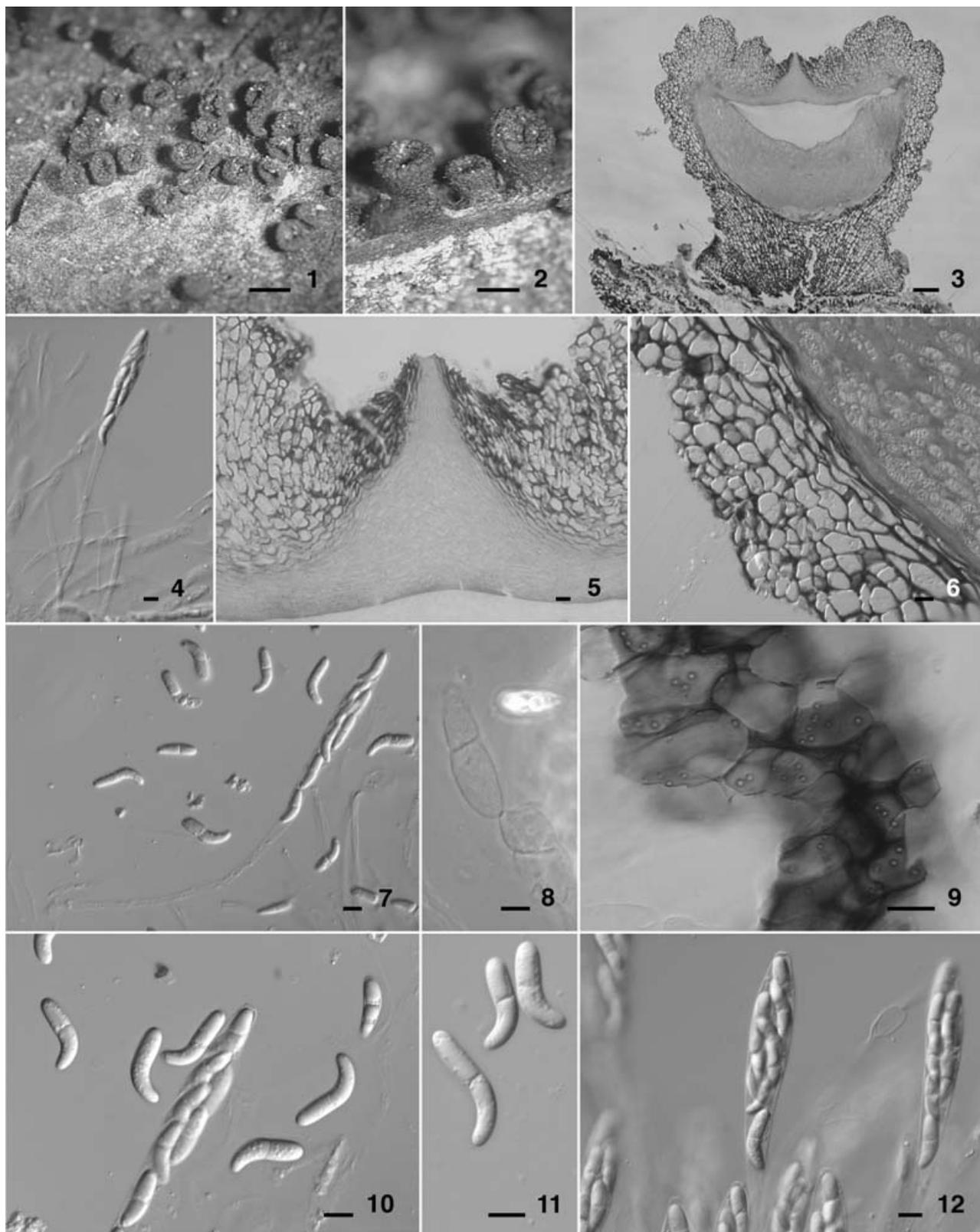
#### **Bertia multiseptata** (Sivan.) Huhndorf, A. N. Mill. & F. A. Fern., **comb. nov.**

Basionym: *Bertia moriformis* var. *multiseptata* Sivan., *Trans. Br. mycol. Soc.* **70**: 387 (1978).

#### **Bertia tropicalis** Huhndorf, A. N. Mill. & F. A. Fern., **sp. nov.** (Figs 1–12)

*Etym.*: Named for the zone where all the collections occur.

Ascomata separata vel gregaria, superficialia, turbinatascens in statu humectato, collabens in statu sicco, ostiolata, atrobrunnea, tuberculata, 892–1135 µm alta, 785–825 µm diam. Paries ascomatis superficialis textura globosa, sectione longitudinali 95–110 µm crassus, cellulis pseudoparenchymatis, Munk pori numerosi. Paraphysoides hyalino, 13–16.5 µm crassi. Asci cylindrici-clavati, octospori, 195–246 × 14–15 µm, partibus sporiferis 86–108 µm longitudine, stipitibus 107–138 µm longitudine. Ascospores hyalinae, 1-septatae, allantoidae vel geniculatae, 22–37 × 5–8(–9.5) µm.



**Figs 1–12.** Micrographs of *Bertia tropicalis*. **Fig. 1.** Ascomata on substratum (SMH1707). Bar = 1 mm. **Fig. 2.** Ascomata on substratum (SMH1707). Bar = 0.5 mm. **Fig. 3.** Longitudinal section through ascoma (SMH1707). Bar = 100  $\mu$ m. **Fig. 4.** Long stipitate ascus (SMH1265). Bar = 10  $\mu$ m. **Fig. 5.** Section through ostiole (SMH1707). Bar = 10  $\mu$ m. **Fig. 6.** Section through ascomal wall (SMH1707). Bar = 10  $\mu$ m. **Fig. 7.** Long stipitate ascus (SMH1707). Bar = 10  $\mu$ m. **Fig. 8.** Inflated paraphysis (SMH1265). Bar = 10  $\mu$ m. **Fig. 9.** Munk pores in wall cells (SMH1773). Bar = 10  $\mu$ m. **Fig. 10.** Ascus pars sporifera (SMH1707). Bar = 10  $\mu$ m. **Fig. 11.** Ascospores (SMH1707). Bar = 10  $\mu$ m. **Fig. 12.** Ascus pars sporifera (SMH1265). Bar = 10  $\mu$ m.

*Typus*: **USA**: Puerto Rico: Caribbean National Forest, El Verde Research Area, 16-ha grid, Luquillo Mts, 370 m, grid quadrat 02.09.44, 18° 19' 31" N, 65° 49' 1" W [18.3167, -65.8167], 3 Oct. 1995, on bark of 15 cm upper branch of *Nectandra turbacensis* (*Lauraceae*; host tag no. 7553), *S. M. Huhndorf SMH1707* (F – holotypus).

*Ascomata* separate or clustered in small to large groups, superficial, turbinate when fresh, mostly collabent when dry, with a thick sterile base, apex flattened, ostiolate, dark brown, surface tuberculate, 892–1135 µm in height, 463–530 µm wide at base, 785–825 µm wide at apex. *Ascomatal wall* of *textura globosa* in surface view; in longitudinal section a single layer, 95–110 µm thick at the sides, thicker (135–200 µm) at the apex perimeter, 230–250 µm thick at the base, composed of polygonal, pseudoparenchymatic cells which become smaller in the outer surface, and flattened in the inner surface, cells at base cells radiate from the bottom, Munk pores present, numerous per cell; apex composed of a cushion of thin-walled cells. *Paraphysoids* hyaline, inflated, unbranched, 13–16.5 µm wide. *Asci* cylindrical-clavate, long-stipitate, 195–246 × 14–15 µm, part with spores 86–108 µm, pedicels 107–138 µm, with 8 biseriata ascospores. *Ascospores* cylindrical, 22–37 × 5–8(–9.5) µm, basal one third curved geniculate, hyaline, 1-septate, without sheath or appendages; spores collecting as a white droplet at the ascomal apex.

*Habitat*: On bark and decorticated wood.

*Anamorph*: Unknown.

*Distribution*: Costa Rica, French Guiana, Jamaica, Panama, USA (Puerto Rico).

*Specimens examined*: **Costa Rica**: Puntarenas, Área de Conservación Osa, Parque Nacional Corcovado, Sirena Station, Espaveles trail, 5 m, 8.4814, –83.595, 17 July 2000, on wood fragment, FF, SMH4286. – **French Guiana**: St-Laurent-du-Maroni Arrondissement, Canton de Maripasoula, Commune de Saul, Eaux Claires, up to 650 m NE, along ridge on Sentier Botanique, 200 m, 3.7, –53.2, 31 Aug. 1994, on decaying branch, SMH708; Eaux Claires, along Sentier Botanique, ca 5 km NE, 7 Sept. 1994, on decaying bark, SMH876. – **Jamaica**: Manchester Parish, Marchall's Pen, Sutton's farm, 610 m, 18.0592, –77.5314, 8 June 1999, on wood fragment, FF, SMH4046; SMH4052. – **Panama**: Barro Colorado Island National Monument, Snyder-Molino trail, 50–150 m, 9.1667, –79.8333, 19 Sept. 1997, on 5 cm branch, SMH, FF, SMH3513; Barbour-Lathrop trail, 20 Sept. 1997, on bark fragment, SMH3528. – **Puerto Rico**: Caribbean National Forest, El Verde Research Area, 16-ha grid, Luquillo Mts, 350–425 m, 18.3167, –65.8167, 1 May 1995, on 30 cm standing stump, SMH1265; along driveway, 4 May 1995, on log, DJL, SMH, SMH1323; 16-ha grid, 7 Oct. 1995, on 30 cm log, SMH1773; 16-ha grid, 18 Jan. 1996, on 12 cm log, SMH1945; 16-ha grid, 14 Jan. 1997, on wood fragment, SMH, FF, SMH2941; 16-ha grid, 25 Jan. 1997, on 20 cm log, SMH, FF, SMH3132.

**Lasiobertia portoricensis** Huhndorf, A. N. Mill. & F. A. Fern., *sp. nov.* (Figs 13–24)

*Etym.*: Refers to the collection locality.

*Ascomata* separata vel gregaria, superficialia, atrobrunnea, obpyriformia, lateralis collapsa in statu sicco. Paries ascomatis superficialis textura globosa, sectione longitudinali 30–50 µm crassus, cellulis pseudoparenchymatis, Munk pori sparsi. Paraphysibus non observatus in statu sicco. Asci cylindrici, stipitati, octospori, 140–160 × 9–10 µm, annulo apicali in liquore iodato Melzeri cyanescente, 2.5–3 × 1–1.5 µm. Ascosporeae hyalinae, fusiformes, inequilaterales, 29–38 × 6–7.5 µm.

*Typus*: **USA**: Puerto Rico: Caribbean National Forest, El Verde Research Area, 16-ha grid, Luquillo Mts, 404 m, grid quadrat 11.01.43, NW of quadrat 12.01.12, 18° 19' 26" N, 65° 48' 55" W [18.3239, –65.8153], 25 Jan. 1996, on bark of 60 cm upper trunk of *Swietenia macrophylla* (*Meliaceae*; host tag no. 334), *S. M. Huhndorf SMH2065* (F – holotypus).

*Ascomata* scattered singly or gregarious in large groups, superficial on a sparse, tomentose subiculum, obpyriform, often collapsing laterally when dry, apex papillate and ostiolate, dark brown, surface papulose to tuberculate, 192–277 µm high, 150–215 µm wide. *Ascomatal wall* of *textura globosa* in surface view; in longitudinal section 30–50 µm thick, composed of polygonal to elongate, pseudoparenchymatic cells (2.5–15 × 7.5–16 µm) which are flattened in the inner surface and become larger and slightly melanized on the outer surface, Munk pores present, few per cell; apex composed of periphyses. *Paraphyses* not observed in dry material. *Asci* cylindrical, stipitate, 8-spored, 140–160 × 9–10 µm, with a conspicuous I + apical ring, 2.5–3 × 1–1.5 µm. *Ascospores* hyaline, one celled, fusiform, inequilateral, tapering to acute ends, 29–38 × 6–7.5 µm.

*Habitat*: On bark.

*Anamorph*: Unknown.

*Distribution*: USA (Puerto Rico).

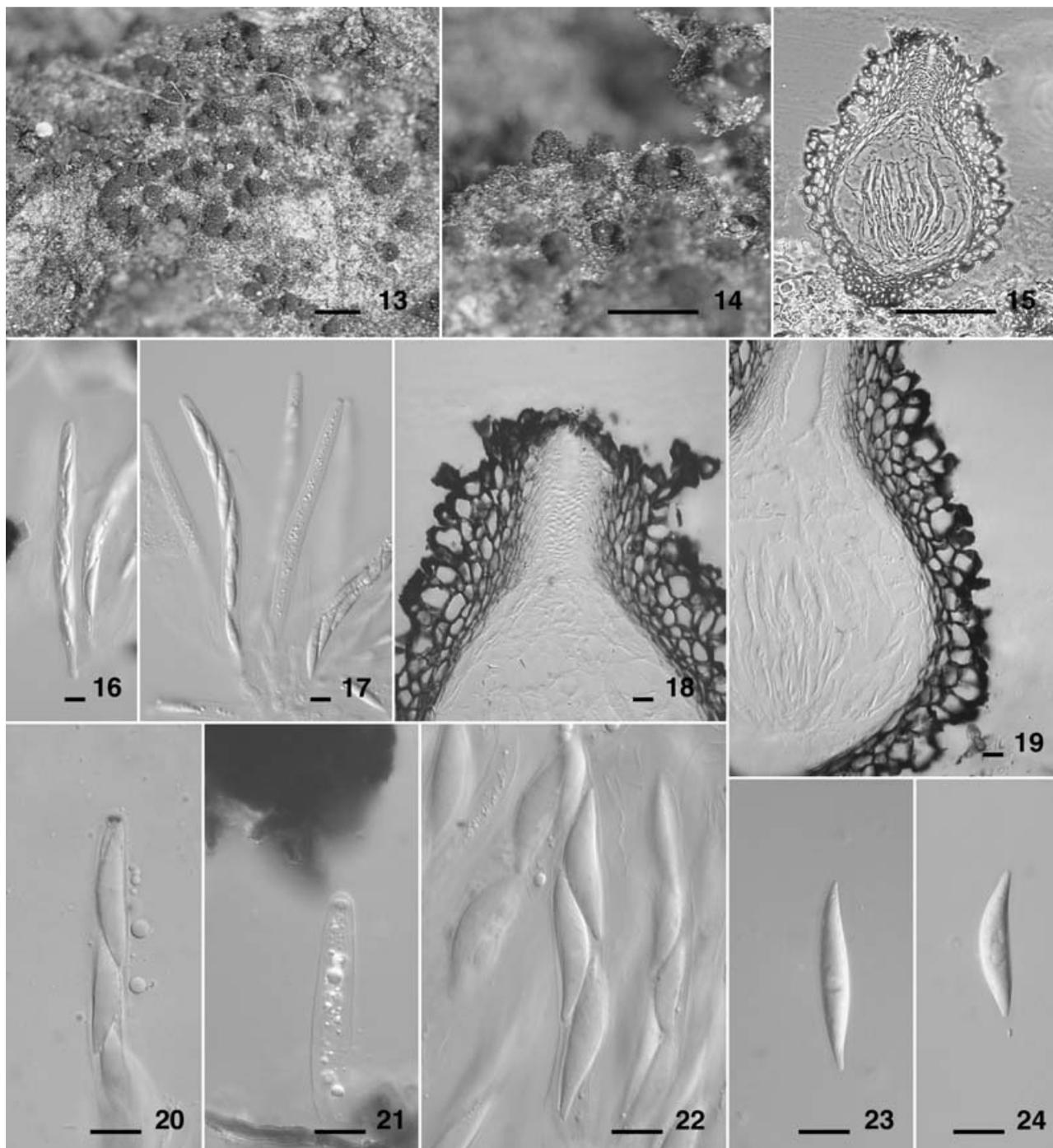
*Additional specimen examined*: **USA**: Puerto Rico: Caribbean National Forest, El Verde Research Area, 16-ha grid, Luquillo Mts, 350 to 425 m, 18.3167, –65.8167, 25 Jan. 1996, on bark of 60 cm log, SMH2045.

**Nitschkia meniscoidea** Huhndorf, A. N. Mill. & F. A. Fern., *sp. nov.* (Figs 25–37)

*Etym.*: Refers to the dry ascomal shape, thin, concavo-convex and hemisphaerical, resembling a watch glass.

*Ascomata* separata, nonnihil gregaria, superficialia, discoidea in statu sicco, tuberculata, atrobrunnea, 680–850 µm diametro, 170–290 µm alta in statu sicco, setae sparsis. Paries ascomatis superficialis textura globosa, sectione longitudinali 70–90 µm crassus, cellulis pseudoparenchymatis, Munk pori numerosi; sine quellkörper. Paraphyses nulla. Asci clavati, octospori, longissime stipitati, 52–56 × 6.5–8.5 µm, partibus sporiferis 33.5–36.5 µm longitudine, stipitibus 18.5–19.5 µm longitudine. Ascosporeae hyalinae, cylindrici vel ellipsoidea, uniseptatae, 6.5–9 × 2.5–3.5 µm.

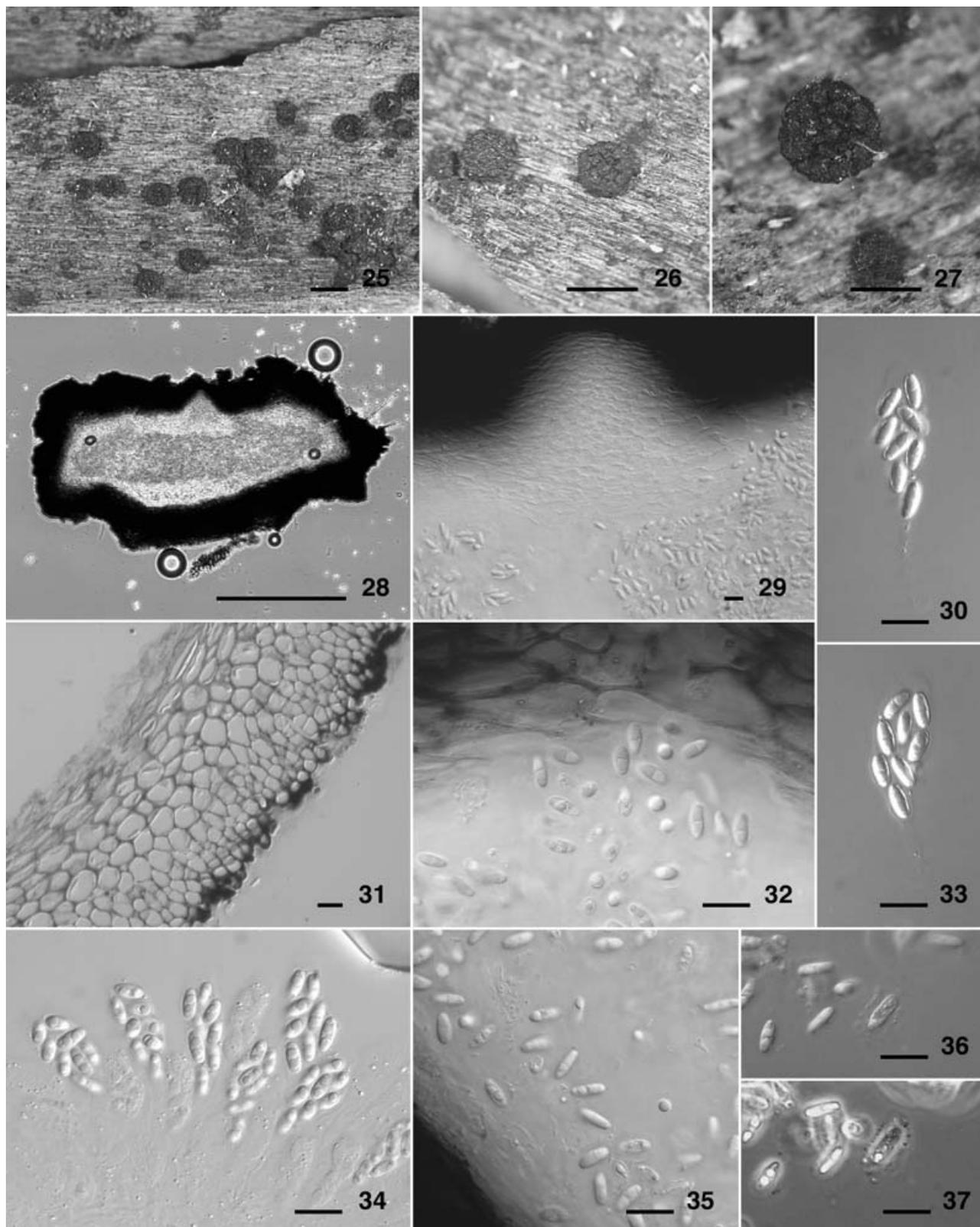
*Typus*: **USA**: Puerto Rico: Caribbean National Forest, El Verde Research Area, 16-ha grid, Luquillo Mts, 380 m, W of grid quadrat 05.04.42, just W of tree no. 23063, 18° 19' 28" N, 65° 49' 0" W [18.3167, –65.8167], 18 June 1995, on 25 cm log, *S. M. Huhndorf SMH1523* (F – holotypus).



**Figs 13–24.** Micrographs of *Lasiobertia portoricensis* (SMH2065). **Fig. 13.** Ascomata on substratum. Bar = 0.5 mm. **Fig. 14.** Ascomata on substratum. Bar = 0.5 mm. **Fig. 15.** Longitudinal section through ascoma. Bar = 100 µm. **Figs 16–17.** Asci. Bar = 10 µm. **Fig. 18.** Section through ascomal neck. Bar = 10 µm. **Fig. 19.** Section through ascomal wall. Bar = 10 µm. **Fig. 20.** Ascus apex with amyloid ring. Bar = 10 µm. **Fig. 21.** Ascus apex with ring. Bar = 10 µm. **Figs 22–24.** Ascospores. Bar = 10 µm.

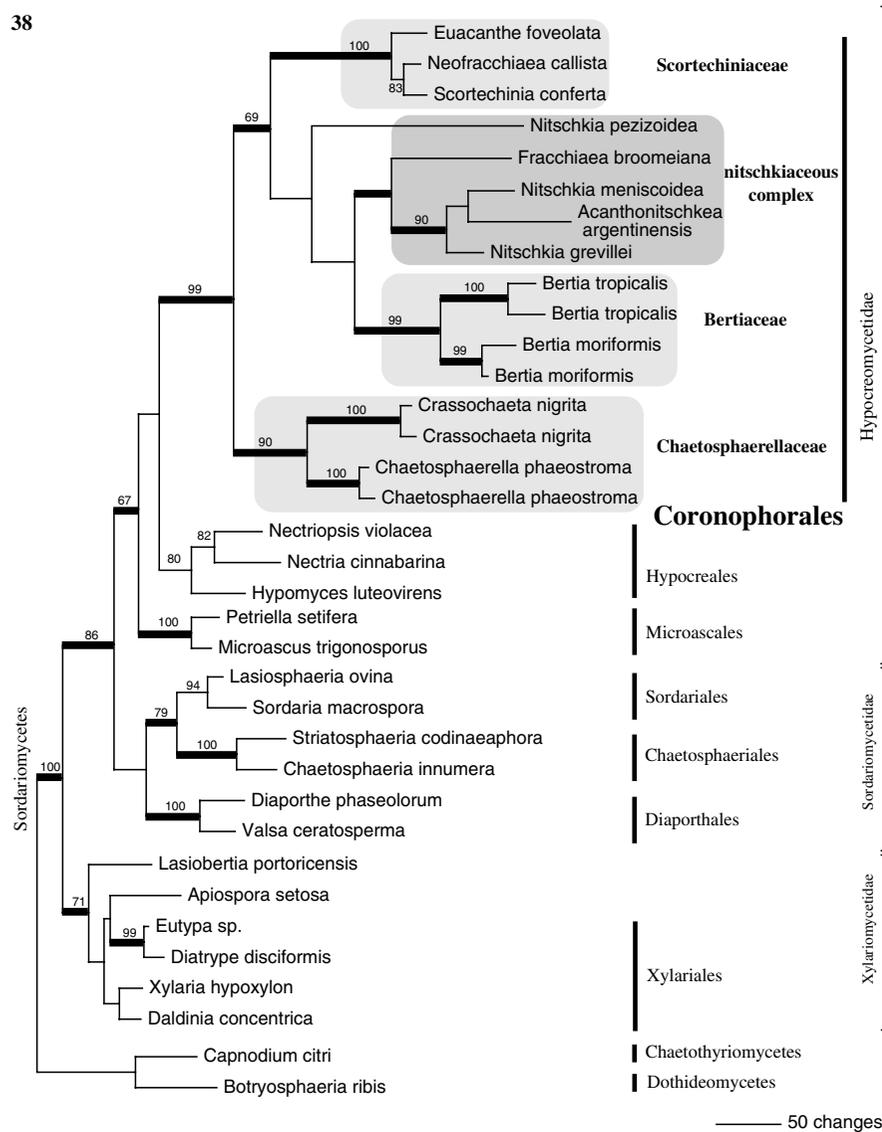
*Ascomata* separate to somewhat clustered in small groups, superficial, discoid when dry, dark brown, surface tuberculate, 680–850 µm diameter, 170–290 µm high when dry, with sparse setae. *Ascomatal wall* of *textura globosa* in surface view; in longitudinal section 70–90 µm thick, composed of polygonal to elongate, pseudoparenchymatic cells (5.5–12.5 × 11.5–22.5 µm)

which become smaller in the outer surface (4–6 µm diam), and flattened in the inner surface, with a thin, external melanized crust, Munk pores present, few per cell; apex composed of a cushion of thin-walled cells, quellkorper absent. *Paraphyses* absent. *Asci* clavate, octosporous, long stipitate, 52–56 × 6.5–8.5 µm, part with spores 33.5–36.5 µm, pedicels

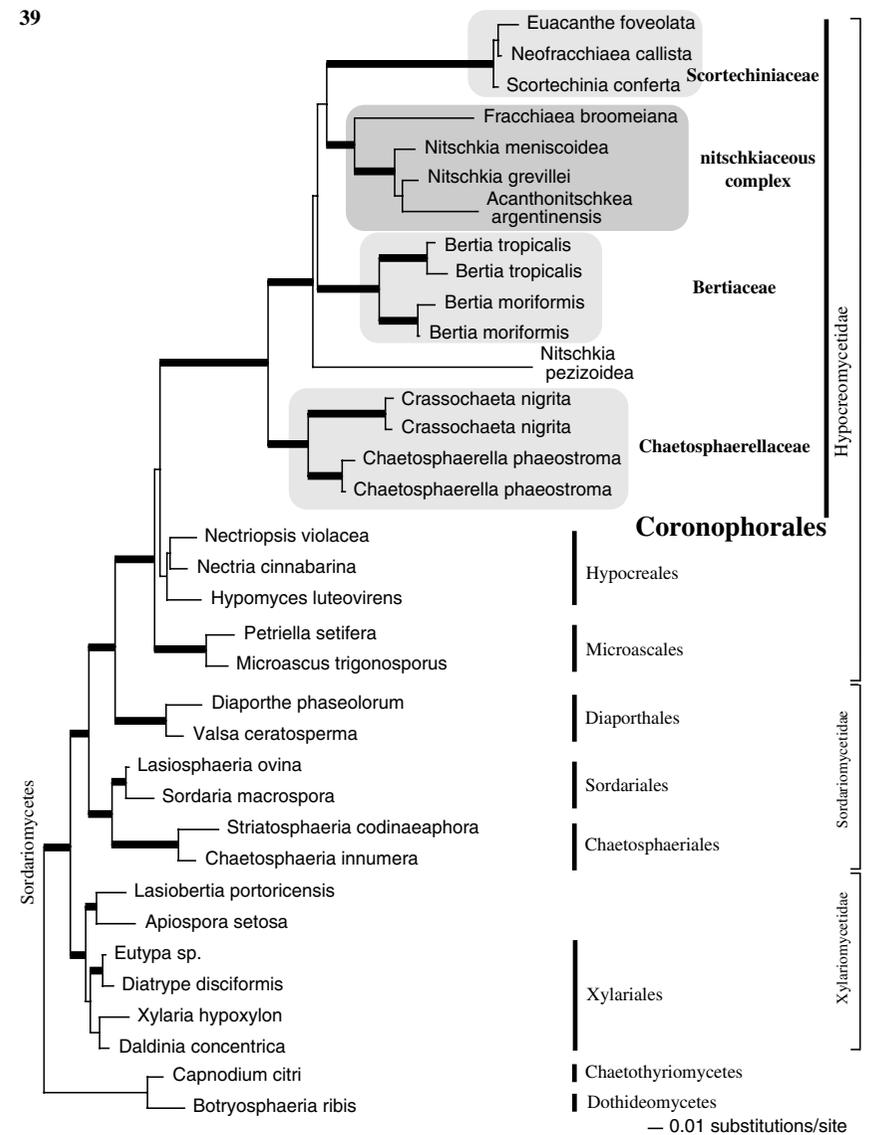


**Figs 25–37.** Micrographs of *Nitschka meniscoidea*. **Figs 25–26.** Ascomata on substratum (SMH1523). Bar = 1 mm. **Fig. 27.** Ascomata on substratum (SMH1523). Bar = 0.5 mm. **Fig. 28.** Longitudinal section through ascoma (SMH1657). Bar = 0.5 mm. **Fig. 29.** Centrum showing cushion of apical cells (SMH1657). Bar = 10  $\mu$ m. **Fig. 30.** Ascus (SMH1665). Bar = 10  $\mu$ m. **Fig. 31.** Section through ascomal wall (SMH1523). Bar = 10  $\mu$ m. **Fig. 32.** Munk pores in wall cells (SMH1657). Bar = 10  $\mu$ m. **Figs 33–34.** Asci (SMH1665). Bar = 10  $\mu$ m. **Fig. 35.** Ascospores (SMH1657). Bar = 10  $\mu$ m. **Figs 36–37.** Ascospores with a gelatinous sheath (SMH1657). Bar = 10  $\mu$ m.

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**Fig. 38.** Phylogram of the single most parsimonious tree generated from the unequally-weighted MP3 analysis of 1137 bp of the 5' end of nuclear LSU rDNA for 35 ascomycete sequences; length = 2380.98 steps, CI = 0.628, RI = 0.734, RC = 0.461. Bootstrap values  $\geq 60\%$  are shown above or below branches. Thickened branches indicate Bayesian posterior probabilities  $\geq 95\%$ . The three families in the *Coronophorales* are in lightly shaded boxes while the *Nitschkiaceae* complex is in a heavily shaded box. Subclass and order designations following Huhndorf *et al.* (2004) are given along the right side. **Fig. 39.** Phylogram of the single most likely tree ( $-\ln L = 4836.69$ ) generated from the maximum-likelihood analysis of 1137 bp of the 5' end of nuclear LSU rDNA for 35 ascomycete sequences. Bayesian support, shading, and taxonomy as in Fig. 38.

18.5–19.5  $\mu\text{m}$ . *Ascospores* small, cylindrical to ellipsoid, hyaline, one celled or 1-septate, 6.5–9  $\times$  2.5–3.5  $\mu\text{m}$ .

*Habitat*: On decorticated wood.

*Anamorph*: Unknown.

*Distribution*: Costa Rica, Panama, USA (Puerto Rico).

*Additional specimens examined*: **Costa Rica**: Limón, Área de Conservación La Amistad Caribe, Parque Nacional Cahuita, Sector Humedal, 0–100 m, 9.7133, –82.8183, 17 Jan. 2000, on wood, *M. Umaña* MU727. – **Panama**: Barro Colorado Island National Monument: Donato trail, 50–150 m, 9.1667, –79.8333, 16 Sept. 1997, on 2 cm liana, SMH, FF, SMH3440; Thomas Barbour trail, 18 Sept. 1997, on 25 cm log, SMH, FF, SMH3503. – **USA**: **Puerto Rico**: Caribbean National Forest, El Verde Research Area, 16-ha grid, Luquillo Mts, 350–425 m, 18.3167, –65.8167, 25 Sept. 1995, on 4 cm branch, SMH1572; 30 Sept. 1995, on 10 cm log, SMH1657.1; 30 Sept. 1995, on 30 cm log, SMH1665; 4 Jan 1997, on 30 cm log, SMH, FF, SMH2952; 20 Jan 1997, on log, SMH, FF, SMH3058.

## DISCUSSION

### *Placement of the Coronophorales*

Based on LSU data, Huhndorf *et al.* (2004) accepted the *Nitschkiaceae* in the *Coronophorales* and placed the order in the *Hypocreomycetidae* where it occurred as an unsupported sister group to the *Hypocreales*. Subsequent analyses using additional genes ( $\beta$ -tubulin, RPB2) and similar taxon sampling give further support to this placement (Miller & Huhndorf, unpubl.). The analyses presented here corroborate previous findings (Huhndorf *et al.* 2004) in that the *Coronophorales* maintains its position in the *Hypocreomycetidae* and occurs as a distantly related group to the *Sordariales*, which includes the *Lasiosphaeriaceae*. In this dataset the *Sordariales* is represented by two taxa, *Lasiosphaeria ovina* and *Sordaria macrospora*, but our overall concept of the group is based on additional morphological and molecular work (Huhndorf *et al.* 2004, Miller & Huhndorf 2004, unpubl.) and includes *Lasiosphaeria*, *Cercophora*, *Bombardia*, *Podospora* and other elements consistent with those found in Lundqvist's (1972) concept of the *Lasiosphaeriaceae*. A large number of taxa included in the *Lasiosphaeriaceae s. lat.* (Eriksson & Hawksworth 1998, Eriksson *et al.* 2001, 2003) do not belong in the group and have been removed (Huhndorf *et al.* 2004). The removed taxa are ones that do not share a common pattern of ascospore morphology that ranges from a one-celled hyaline, cylindrical ascospore in *Lasiosphaeria* to a one-celled, brown, ellipsoid ascospore in *Sordaria*. Intermixed between these two extremes are many genera which possess two-celled ascospores with cylindrical to ellipsoidal, brown cells and different degrees of cylindrical to triangular (often basal), hyaline cells (Huhndorf *et al.* 2004). The putative relationship between the *Coronophorales* and the *Lasiosphaeriaceae* was tested by conducting a maximum-likelihood analysis in which

the *Coronophorales* and *Sordariales* were constrained to be sister taxa. This analysis generated a ML tree which was significantly less likely ( $-\ln L=4911.76$ ) than the unconstrained ML tree ( $-\ln L=4836.69$ ) as determined by both the Kishino-Hasegawa (KH) test ( $P=0.00005$ ) and the Shimodaira-Hasegawa (SH) test ( $P=0.00004$ ). Thus, the hypothesis that the *Coronophorales* and the *Lasiosphaeriaceae* are closely related can be rejected.

### *Morphological concepts within the Coronophorales*

The concept of the *Coronophorales* accepted here includes the genera *Acanthonitschkea*, *Bertia*, *Chaetosphaerella*, *Crassochaeta*, *Euacantho*, *Fracchiacea*, *Neofracchiacea*, *Nitschkia*, and *Scortechinia*. Among our collections were several genera that were not included in our analyses either because the collections were too scanty or insufficient amounts of DNA were obtained from the samples, but morphology suggests that they belong in the group. *Gaillardella*, *Spinulosphaeria*, and *Thaxteria* show characteristics consistent with inclusion in the order and are accepted. Some of the genera have been at one time placed in the *Lasiosphaeriaceae*, but they do not fit our concept of that family (Huhndorf *et al.* 2004).

The *Coronophorales* contains taxa that have the characteristics of erumpent to superficial ascomata sometimes with an extensive hyphal subiculum or well developed basal stroma. The basic shape is often turbinate becoming collabent on drying and the surface is often tuberculate, at times setose. The ascomatal wall is composed of coriaceous, not carbonized, large-celled pseudoparenchyma. Munk pores (a term coined by Nannfeldt 1975a) are small circular pores, ca. 1  $\mu\text{m}$  diam., found in the ascomatal cell walls in many but not all taxa in the order (see Fig. 9). Munk pores are also found in a few taxa outside the order, e.g. *Lasiobertia*.

Taxa with or without a quellkorper in the centrum occur in the *Coronophorales* but this structure is not found in taxa outside the order. Our definition of a quellkorper follows that given by Nannfeldt (1975b): a structure consisting of 'concentrically and transversely oriented, firmly conglutinated cells with very thick, hyaline, strongly refractive walls and narrow lumina.' Additionally the quellkorper is described as being ' $\pm$ prolonged, subcylindrical to inverted-conical... , which may even reach almost down to the bottom of the locule.' The low, perforated cushion (also described as an incipient or reduced quellkorper) (Nannfeldt 1975b) is not considered by us to be homologous to the quellkorper. In our analyses, taxa that possess a quellkorper include *E. foveolata*, *N. callista*, and *S. conferta*; taxa that possess a cushion include *F. broomeiana*, *N. grevillei*, *N. meniscoidea*, *N. pezizoidea*, and *A. argentinensis*.

In taxa with a quellkorper, the ascoma is considered non-ostiolate and spore discharge apparently occurs

through a rupture in the apical wall caused by swelling and pressure from the quellkorper (Nannfeldt 1975b). In the taxa without a quellkorper, the nature of the ostiole has been debated, whether it is 'true' (perforated) or not, and whether the ostiole is filled with periphyses or indistinct hyaline tissue that may or may not be periphyses-like. Taxa such as *Bertia tropicalis* have been observed in nature with droplets of ascospores collected at their ascomatal apices, indicating they emerged from an opening at the top. There is a preformed area of hyaline tissue which eventually opens and through which the spores are released. The order as circumscribed here accommodates taxa with a variety of ascomatal apices that range from a true, perforated, periphysate ostiole (as in *Chaetosphaerella*) to indistinct hyaline tissue that may or may not be periphyses-like (as in *Bertia*) to a non-ostiolate perithecium in taxa with a quellkorper (as in *Scortechinia*).

Generally the asci are thin-walled, clavate, and stipitate. They occur basally or laterally in fascicles within the centrum. The apex can be rounded, truncate, invaginated or slightly thickened, and lacks a ring in most taxa; there are exceptions, as in *Crassochaeta*, with a distinct ring and some *Bertia* species with small rings. Asci can have eight ascospores, or be polysporous with normally 32 ascospores, although *F. broomeiana* offers an extreme case with >200 (Nannfeldt 1975b). Filiform paraphyses are lacking in the group, but widely inflated paraphyses are found in *Chaetosphaerella* and *Spinulosphaeria* and shriveled sterile threads are present in *Bertia*. Ascospores can be small or large, slightly or strongly allantoid, ellipsoid, ovoid or cylindrical, one or several septate and hyaline or pale brown, but in many species the ascospores are generally hyaline, relatively small, and suballantoid.

### **Relationships within the Coronophorales**

Within the *Coronophorales*, the analyses show several strongly supported subclades which we propose as the families *Bertiaceae*, *Chaetosphaerellaceae* and *Scortechiniaceae* and a single unsupported, paraphyletic *Nitschkiaceae*, here denoted as the nitschkiaceous complex.

#### **Bertiaceae**

*Bertia* has generally been considered to take an isolated position within the *Nitschkiaceae* (Corlett & Krug 1984) or has been placed into its own family (Smyk 1981, Eriksson 1984, Subramanian & Sekar 1990). Our data places it within the *Coronophorales* on a well-supported branch that we accept as the *Bertiaceae*. The genus contains 40 names, most of which have not been looked at since their description. Five species and two varieties have been added in recent years (Sivanesan 1978, Corlett & Krug 1984, Krug & Corlett 1988, Subramanian & Sekar 1990, Hsieh, Chen & Sivanesan 1995, Hyde 1995, Yuan & Mohammed 1997) and

together with the type species, these eight taxa make up the current concept of the genus. *Bertia* species are found in the tropical and temperate zones.

Our two collections of *B. moriformis* agree well with those examined by Corlett & Krug (1984) and are characterized by large, tuberculate ascomata and hyaline, fusiform, one-septate ascospores. *B. tropicalis* differs from *B. moriformis* by geniculate ascospores and ascomata that become collabent. *B. tropicalis* resembles *B. convolutispora* in the shape and size of the ascospores but differs in its terrestrial habit and in ascomata that are strongly clustered or gregarious. *B. convolutispora* was described from submerged wood and the ascomata are solitary or very rarely clustered (Hyde 1995). *B. tropicalis* also resembles *B. latispora* in the geniculate morphology of the ascospores but differs in ascospore and ascomatal size, a tropical versus temperate distribution, and the apparent limitation of *B. latispora* to coniferous hosts (Corlett & Krug 1984). *B. tropicalis* was commonly found in Puerto Rico and Panama and was also occasionally encountered in French Guiana, Costa Rica, and Thailand. It was never found in our temperate collecting sites.

*B. multiseptata* was encountered a few times in Puerto Rico and its long fusiform, multiseptate ascospores that become brown provide sufficient difference for its elevation to species rank from variety status under *B. moriformis*.

#### **Chaetosphaerellaceae**

The strongly supported basal branch within the *Coronophorales* contains two taxa previously thought to be unrelated to the others in the order and to each other. The genus *Chaetosphaerella* was described for two species in *Chaetosphaeria* that had versicolorous ascospores and ascomata with a setose basal subiculum (Müller & Booth 1972). Sivanesan (1976) added a third species. No comments were made on its affinities at that time but its placement eventually settled in the *Lasio-sphaeriaceae* along with *Chaetosphaeria* and other genera. Réblová (1999a, b, c, d) in an attempt to clarify some species of *Chaetosphaeria* and allied genera, rearranged the versicolorous taxa into several groups. *Chaetosphaerella* was accepted for two species (*C. phaeostroma* and *C. fusca*) and placed in the *Helminthosphaeriaceae* (Réblová 1999a). New genera were introduced to accommodate additional versicolorous taxa placed in the *Helminthosphaeriaceae* (*Tengiomyces indicus*) and *Trichosphaeriaceae* (*Crassochaeta nigrita* and *C. fusispora*) (Réblová 1999a, d). Characters emphasized for the placement into the different families include the kind of ascomal wall, character of the setae, ascal anatomy and conidogenesis. Réblová (1999a, d) segregated *Chaetosphaerella* from *Crassochaeta* based on differences in associated anamorphs, and perithecial and ascal anatomy. *Chaetosphaerella* has associated *Oedemium* and *Veramycina* synanamorphs, obpyriform perithecia with a stout

sterile base formed of divergent rows of vertically arranged cells, dark subiculum with thick-walled setae only forming around the base of the perithecium, inflated, broadly cellular paraphyses, and asci with an indistinct apical ring. *Crassochaeta* has an unknown anamorph with *Arthrimum*-like conidia, globose to ovoid perithecia, a leathery, two-layered peridium with angular thick-walled cells, thick-walled perithecial setae and a dense subiculum of multi-branched setae, persistent cylindrical paraphyses, and asci with a distinct apical ring. Both taxa possess Munk pores in the perithecial wall cells and three-septate versicolorous ascospores.

Réblová (1999a) acknowledged that *Chaetosphaerella* bears resemblance to the *Nitschkiaceae* based on perithecial morphology (both species have Munk pores in the perithecial wall cells, and both have a sterile perithecial base formed of divergent rows of vertically arranged cells) but believed that the differences (i.e. presence of a definite ostiole, lack of quellkorper, and the ascus and ascospore morphology) were more indicative of relationships and placed it in the *Helminthosphaeriaceae*. Huhndorf *et al.* (2004) have shown that *Chaetosphaerella* is unrelated to the *Helminthosphaeriaceae*.

We sampled two collections of *C. phaeostroma* and the European specimen (SMH4585) displays the morphological characteristics typical for the species as given by Réblová (1999a) (viz. ascomata growing in glistening, black, velvety colonies and ascospores generally longer than 30 µm). However, the Costa Rican specimen (SMH4257) has ascospores of the size given for *C. fusca* (less than 30 µm long) but ascomata identical to those of *C. phaeostroma*. Based on molecular data we consider both to be *C. phaeostroma*. The two collections of *Crassochaeta nigrita* were identical. Sequences of the other species in both *Chaetosphaerella* and *Crassochaeta* as well as *Tengiomyces indicus* are needed to further clarify the classification. It remains to be seen whether these taxa are species in a single genus or can be maintained as separate genera as Réblová outlined (1999a, d). Our data show that both *Chaetosphaerella* and *Crassochaeta* have their relationships in the *Coronophorales* but there are distinctive morphological differences that warrant the creation of a new family.

#### *Nitschkiaceous complex*

The genus *Nitschkia* has been narrowly (Fitzpatrick 1923a, Subramanian & Sekar 1990; *s. str.*) or widely (Nannfeldt 1975a, b; *s. lat.*) circumscribed over the years. Characters such as the presence or absence of a quellkorper and a subiculum, eight vs multi-spored asci, and ascospore morphology have been used to segregate taxa at the genus and species levels. Nannfeldt (1975b) included 22 species in the genus, whereas Subramanian & Sekar (1990) treated ten species.

Six species that fit *Nitschkia s. lat.* and represent some of the morphological variability present under Nannfeldt's wide concept were included in our analyses: *Fracchiacea broomeiana*, *N. callista*, *N. grevillei*, *N. meniscoidea*, *N. pezizoidea*, and *Scortechinia conferta*. These six species segregate into two separate well-supported clades and one lone branch (Figs 1–2). The ML tree generated from a maximum-likelihood analysis, which constrained these six taxa to be monophyletic, was significantly less likely than the unconstrained ML tree (Fig. 2) as determined by a KH test ( $P < 0.0001$ ) and a SH test ( $P < 0.0001$ ). Therefore, *Nitschkia s. lat.* cannot be maintained. Some of these included species represent genera that are accepted as being separate from *Nitschkia* (see below). We were unable to find fresh material of the type of the genus, *N. parasitans* to include in our molecular analyses. Two recent collections of a new variety of this species (Vujanovic 2002) were determined to be too scanty to be used for ascomatal DNA extraction. This is unfortunate because only the type species will indicate where the genus *Nitschkia s. str.* belongs in the tree. *N. parasitans* is described as having small, gregarious ascomata that occur on stromata of *Nectria cinnabarina*. Asci are 8-spored and the ascospores are hyaline, suballantoid, and eventually develop a faint septum (Nannfeldt 1975b). Nannfeldt (1975b) illustrated the centrum of *N. parasitans* and in median sections showed that no quellkorper is present. He describes the 'cushions' as composed of 'hyphae that are unusually periphysis-like and leave a relatively large, upwards tapering, empty canal.' Vujanovic (2002) describes a quellkorper as present in *N. parasitans* var. *mijuskovicii* and illustrates liberated cells enlarged in water. The cells do not appear convincingly like the enlarged quellkorper present in the three species we sampled (see below) nor like those of *N. acanthostroma* or *N. chaetomioides* illustrated by Nannfeldt (1975b). We interpret them as belonging to a cushion-like structure in the centrum apex rather than a quellkorper.

Two species maintain their *Nitschkia* names and one additional species, *N. meniscoidea* is described as new. The ascospores of *N. meniscoidea* are very small, similar in size to those of *N. grevillei* and *N. calyculus*, but the ascomata are quite different. *N. grevillei* and *N. calyculus* both have clustered, gregarious ascomata with large, basal stromatic tissues. *N. meniscoidea* has ascomata that are sessile on the substrate, mostly found separately but sometimes in small groups. The tuberculate ascomata are disk- or saucer-shaped, lenticular in side view, that become collabent (flattened, actually) or shaped like a watch glass. The shape of fresh ascomata (before becoming flattened) is reminiscent of ascomata of *Fracchiacea*. *N. collapsa* also has ascomata that are sessile and disk-shaped but it differs from *N. meniscoidea* in having larger ascospores and a subiculum surrounding the ascomata. *N. meniscoidea* occurs in a well-supported clade with *N. grevillei* and *Acanthonitschkea argentinensis*. *N. grevillei* is the type

species of the segregate genus *Calyculosphaeria*, distinguished according to Fitzpatrick (1923b) by having hyaline, 1-septate ascospores. We refrain from accepting *Calyculosphaeria* at this time because *N. parasitans*, the type species for *Nitschkia*, might reside in this clade. The name *N. pezizoidea* is maintained because we also cannot rule out its possible affinity to *N. parasitans*. *N. pezizoidea* occupies a lone position on a long branch not showing clear affinities to any of the sampled taxa. Fitzpatrick (1923b) considered this a species of *Calyculosphaeria* but if that genus were recognized for *N. grevillei*, *N. pezizoidea* would not be a member based on the molecular data.

Accepting these three *Nitschkia* names leaves us with a paraphyletic genus at this time. Our concept of *Nitschkia* includes taxa having ascomata separate or gregarious, mostly collabent, quellkorper absent, eight-spored asci, ascospores fusiform, ovoid or allantoid, hyaline, one celled or one septate. However there are many more *Nitschkia* species that must be considered and the entire group would benefit from a re-evaluation and molecular analyses that take into account as many of the morphological variations as possible.

The genus *Acanthonitschkea* has been accepted for two (Fitzpatrick 1923a), four (Nannfeldt 1975b) or only a single species (Subramanian & Sekar 1990). Both Nannfeldt (1975b) and Fitzpatrick (1923a) included species with or without a quellkorper. The type species, *A. argentinensis* has superficial, setose, collabent ascomata without a quellkorper, thin-walled clavate asci and strongly curved, allantoid ascospores. *A. argentinensis* occurs in the well-supported clade that contains *N. grevillei* and *N. meniscoidea*. Another species that Nannfeldt considered an *Acanthonitschkea* was also included in this study but it does not group with *A. argentinensis* and is here treated under *Euacanthie*.

*Fracchiaea broomeiana* is the type of the genus and occurs on a single branch with Bayesian support as a sister group to the highly-supported clade of *N. grevillei*, *N. meniscoidea* and *A. argentinensis*. Fitzpatrick (1924) synonymized 16 species and varieties under *F. broomeiana* and considered the genus to be monotypic. Among the various species, he found differences in asci and ascomatal structure to be inconsistent or lacking and therefore not useful for the separation of these species. *Fracchiaea broomeiana* is characterized by clustered, subglobose, spinulose ascomata only rarely collapsing to cupulate. The centrum contains a low flat cushion at the apex and no quellkorper is present. Asci are clavate, long stipitate, and polysporous with numerous allantoid ascospores arranged in obliquely overlapping parallel series. We accept *Fracchiaea* as a genus separate from *Nitschkia*.

#### Scortechiniaceae

In these analyses, three taxa with a quellkorper occur in one well-supported clade, while taxa that possess a low perforated cushion occur outside this clade. The

presence or absence of a quellkorper was not taken by Nannfeldt (1975a, b) to be a genus-level character and he circumscribed genera regardless of this feature. The three species we sampled were considered by Nannfeldt to belong either to his broadly circumscribed *Acanthonitschkea* or *Nitschkia*. Our analyses indicate that species with a quellkorper are more closely related to each other than they are to other non-quellkorper species in these broad genera. A new family is described for taxa with a quellkorper.

*Euacanthie* is monotypic for *E. foveolata* and its synonyms. Nannfeldt (1975b) considered it a species of *Acanthonitschkea* and Arx & Müller (1954) treated it as a species under *Scortechinia* (as *S. usambarensis*). Because it does not group with *A. argentinensis* it does not belong in *Acanthonitschkea*. Recognizing it as a *Scortechinia* would make that genus paraphyletic, so the genus *Euacanthie* is accepted. Morphologically *E. foveolata* resembles *A. argentinensis* in its setose ascomata and 8-spored asci.

*Neofracchiaea* is monotypic for *N. callista* (Teng 1938). It was placed at one time in *Fracchiaea* (Saccardo 1882) and later Fitzpatrick (1924) referred it to *Cryptosphaerella* but never made the combination. Nannfeldt (1975b) treated it as a species of *Nitschkia*. *Cryptosphaerella*, a genus conceived for taxa with a quellkorper and multi-spored asci, could also be a possible disposition for this species. However, we have not seen type or other material of the genus. We utilize an existing name and accept the taxon as *Neofracchiaea callista*. It is easily distinguished by its dark brown, felty subiculum and polysporous asci.

*Scortechinia* was described by Saccardo (*in* Saccardo & Berlese 1885) for a species having a dense subiculum, collabent ascomata with a quellkorper and 8-spored asci. Fitzpatrick (1923b) treated *Scortechinia* species under the genus *Tympanopsis*. Arx & Müller (1954) recognized six species in *Scortechinia* but Nannfeldt (1975b) treated it as a synonym of *Nitschkia*. Subramanian & Sekar (1990) again recognized the genus and made the combination of *S. conferta*, the taxon sampled here. *S. conferta* has a basal, hyphal subiculum, collabent ascomata with a quellkorper, and 8-spored asci. It differs from the type species, *S. acanthostroma* by its non-spiny hyphal subiculum.

#### Other taxa

Three genera known from our collections show morphological affinities to the other taxa in the *Coronophorales*. *Gaillardella* has collabent ascomata with a circular thickening around the edge of the 'cup', ascomatal cells with Munk pores, long-stipitate, eight-spored asci, and ellipsoid, one-septate, brown ascospores. *Spinulosphaeria* is very similar to *Chaetosphaerella* in having obpyriform to clavate ascomata with a stout sterile base, seated on a dense hyphal subiculum. Similar also are the broadly cellular, inflated paraphyses and asci without any distinct apical

apparatus. *Spinulosphaeria* differs in its tooth-like spines on the ascumata and its ellipsoid, one-septate, brown ascospores. *Thaxteria* shows similarities to *Bertia* in its long-stipitate asci and its curved-cylindrical to allantoid ascospores. Nannfeldt (1975b) accepted *Gaillardia* in the *Nitschkiaceae* but included *Spinulosphaeria* and *Thaxteria* in the *Lasiosphaeriaceae*, whereas Subramanian & Sekar (1990) accepted *Gaillardia* in the *Nitschkiaceae*, *Spinulosphaeria* in the *Bertiaceae* and placed *Thaxteria* into *Nitschkiaceae*. We recently accepted all three genera in the *Coronophorales* (Huhndorf *et al.* 2004) and presently place *Spinulosphaeria* in the *Chaetosphaerellaceae* and *Gaillardia* and *Thaxteria* in the *Bertiaceae* based on morphological characters.

There are additional genera in Subramanian & Sekar (1990) that can be accepted in the order, but we have not encountered them among our collections, viz. *Biciliospora*, *Biciliosporina*, *Coronophora*, *Cryptosphaerella*, *Janannfeldtia*, *Neotrotteria*, and *Schizocapnodium*. A few of these genera have been taken out of synonymy from *Nitschkiaceae* and accepted. *Lasiosphaeriopsis* and *Rhagadostoma* are two lichenicolous genera that possibly are related to *Bertia* but no molecular data are available. There are numerous additional genera still in synonymy as well as other poorly known taxa that need to be re-examined.

#### Taxa removed from the Nitschkiaceae

Based on molecular data, *Lasiobertia* was placed in the *Xylariomycetidae* (Huhndorf *et al.* 2004). This monotypic genus was described by Sivanesan (1978) as differing only from the *Coronophorales* and *Lasiosphaeriaceae* in the presence of an amyloid ring. It does show some of the morphological characteristics of the *Nitschkiaceae*, such as superficial, tuberculate ascumata with a basal stalk of vertical cells, an indistinct apical ostiolar region, and ascumatal wall cells with Munk pores. Our new species *Lasiobertia portoricensis* differs from the type *L. africana* in having shorter, wider ascospores ( $29\text{--}38 \times 6\text{--}7.5$  vs  $55\text{--}74 \times 4.5\text{--}6$   $\mu\text{m}$ ) and in ascumata that are less coarsely tuberculate and have a smaller sterile base. The hyphomycete similar to *Melanographium* found associated with *L. africana* was not found associated with *L. portoricensis*. The long-fusiform, apiculate ascospores of *L. portoricensis* resemble *Oxydothis*, a generic relationship first suggested for *Lasiobertia* by Hyde (1993).

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#### REFERENCES

- Arx, J. A. von (1981) On *Monilia sitophila* and some families of ascomycetes. *Sydowia* **34**: 13-29.
- Arx, J. A. von & Müller, E. (1954) Die Gattungen der amerosporen Pyrenomyceten. *Beiträge zur Kryptogamenflora der Schweiz* **11**(1): 1-434.
- Barr, M. E. (1990) Prodrum to nonlichenized, pyrenomycetous members of class *Hymenoascomycetes*. *Mycotaxon* **39**: 43-184.
- Carroll, G. & Munk, A. (1964) Studies on lignicolous *Sordariaceae*. *Mycologia* **56**: 77-98.
- Corlett, M. & Krug, J. C. (1984) *Bertia moriformis* and its varieties. *Canadian Journal of Botany* **62**: 2561-2569.
- Eriksson, O. E. (1984) Outline of the ascomycetes - 1984. *Systema Ascomycetum* **3**: 1-72.
- Eriksson, O. E. & Hawksworth, D. L. (1998) Outline of the ascomycetes - 1998. *Systema Ascomycetum* **16**: 83-296.
- Eriksson, O. E., Baral, H.-O., Currah, R. S., Hansen, K., Kurtzman, C. P., Rambold, G. & Læssøe, T. (Eds). (2001) Outline of *Ascomycota* - 2001. *Myconet* **7**: 1-88.
- Eriksson, O. E., Baral, H.-O., Currah, R. S., Hansen, K., Kurtzman, C. P., Rambold, G. & Læssøe, T. (Eds). (2003) Outline of *Ascomycota* - 2003. *Myconet* **9**: 1-89.
- Felsenstein J. (1978) Cases in which parsimony or compatibility methods will be positively misleading. *Systematic Zoology* **27**: 401-410.
- Felsenstein, J. (1985) Confidence intervals on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783-791.
- Fitzpatrick, H. M. (1923a) Monograph of the *Nitschkiaceae*. *Mycologia* **15**: 1-44.
- Fitzpatrick, H. M. (1923b) Monograph of the *Nitschkiaceae*. *Mycologia* **15**: 45-67.
- Fitzpatrick, H. M. (1924) The genus *Fracchiacea*. *Mycologia* **16**: 101-114.
- Hawksworth, D. L., Kirk, P. M., Sutton, B. C. & Pegler, D. N. (1995) *Ainsworth & Bisby's Dictionary of the Fungi*. 8th edn. CAB International, Wallingford.
- Höhnelt, F. von (1907) Fragmente zur Mykologie (4 Mitteilung, nr 156-168. *Akademie der Wissenschaften in Wien. Sitzungsberichte, Mathematisch-naturwissenschaftliche Klasse, Abteilung 1*, **116**: 615-647.
- Hsieh, W. H., Chen, C. Y. & Sivanesan, A. (1995) Taiwan fungi: new species and new records of ascomycetes. *Mycological Research* **99**: 917-931.
- Huelsbeck, J. P. (1995) Performance of phylogenetic methods in simulation. *Systematic Biology* **44**: 17-48.
- Huelsbeck, J. P. (1997) Is the Felsenstein zone a fly trap? *Systematic Biology* **46**: 69-74.
- Huelsbeck, J. P. & Ronquist, F. R. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Biometrics* **17**: 754-755.
- Huhndorf, S. M. (1991) A method of sectioning ascomycete herbarium specimens for light microscopy. *Mycologia* **83**: 520-524.
- Huhndorf, S. M. & Fernández, F. A. (1998) Neotropical *ascomycetes* 7. *Caudatipora biapiculatis* sp. nov. from Puerto Rico. *Sydowia* **50**: 200-204.
- Huhndorf, S. M., Miller, A. N. & Fernández, F. A. (2004) Molecular systematics of the *Sordariales*: the order and the family *Lasiosphaeriaceae* redefined. *Mycologia* **96**: 368-387.

- Hyde, K. D. (1993) Fungi from palms. VI. Reflections on *Oxydothis* and related genera. *Sydowia* **45**: 204–225.
- Hyde, K. D. (1995) Tropical Australian freshwater fungi. VIII. *Bertia convolutispora* sp. nov. *Nova Hedwigia* **61**: 141–146.
- Krug, J. C. & Corlett, M. (1988) A new species of *Bertia* from China. *Canadian Journal of Botany* **66**: 1256–1258.
- Lundqvist, N. (1972) Nordic *Sordariaceae* s. lat. *Symbolae Botanicae Upsalienses* **20** (1): 1–374.
- Luttrell, E. S. (1951) Taxonomy of the *pyrenomycetes*. *University of Missouri Studies* **24**: 1–120.
- Luttrell, E. S. (1955) The ascostromatic *ascomycetes*. *Mycologia* **47**: 511–532.
- Lutzoni, F., Wagner, P., Reeb, V. & Zoller, S. (2000) Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. *Systematic Biology* **49**: 628–651.
- Miller, A. N. & Huhndorf, S. M. (2004) A natural classification of *Lasiosphaeria* based on nuclear LSU rDNA sequences. *Mycological Research* **108**: 26–34.
- Miller, J. H. (1949) A revision of the classification of the ascomycetes with special emphasis on the *pyrenomycetes*. *Mycologia* **41**: 99–127.
- Müller, E. & Arx, J. A. von (1973) *Pyrenomycetes: Meliolales, Coronophorales, Sphaeriales*. In *The Fungi: an advanced treatise* (G. C. Ainsworth, F. K. Sparrow & A. S. Sussman, eds) **4A**: 87–132. Academic Press, New York.
- Müller, E. & Booth, C. (1972) Generic position of *Sphaeria phaeostroma*. *Transactions of the British Mycological Society* **58**: 73–77.
- Nannfeldt, J. A. (1932) Studien über die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten. *Nova Acta Regiae Societatis Scientiarum Upsaliensis, ser. 4*, **8**(2): 1–368.
- Nannfeldt, J. A. (1975a) Stray studies in the *Coronophorales* (*Pyrenomycetes*) 1–3. *Svensk Botanisk Tidskrift* **69**: 49–66.
- Nannfeldt, J. A. (1975b) Stray studies in the *Coronophorales* (*Pyrenomycetes*) 4–8. *Svensk Botanisk Tidskrift* **69**: 289–335.
- Posada, D. & Crandall, K. A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* **49**: 817–818.
- Réblová, M. (1999a) Studies in *Chaetosphaeria sensu lato* I. The genera *Chaetosphaerella* and *Tengiomyces* gen. nov. of the *Helminthosphaeriaceae*. *Mycotaxon* **70**: 387–420.
- Réblová, M. (1999b) Studies in *Chaetosphaeria sensu lato* II. *Coniobrevicolla* gen. & sp. nov. *Mycotaxon* **70**: 421–429.
- Réblová, M. (1999c) Studies in *Chaetosphaeria sensu lato* III. *Umbrinosphaeria* gen. nov. and *Miyoshiella* with *Sporodesmium* anamorphs. *Mycotaxon* **71**: 13–43.
- Réblová, M. (1999d) Studies in *Chaetosphaeria sensu lato* IV. *Crassochoeta* gen. nov., a new lignicolous genus of the *Trichosphaeriaceae*. *Mycotaxon* **71**: 45–67.
- Robinson, M., Gouy, M., Gautier, C. & Mouchiroud, D. (1998) Sensitivity of the relative-rate test to taxonomic sampling. *Molecular Biology & Evolution* **15**: 1091–1098.
- Robinson-Rechavi, M. & Huchon, D. (2000) RRTree: Relative-Rate Tests between groups of sequences on a phylogenetic tree. *Bioinformatics* **16**: 296–297.
- Saccardo, P. A. (1882) *Sylloge Fungorum omnium hucusque cognitorum*. Vol. 1. P. A. Saccardo, Padova.
- Saccardo, P. A. & Berlese, A. N. (1885) *Miscellanea mycologica ser II. Atti del Reale Istituto Veneto di Scienze, Lettere ed Arti, serie 3* **43**: 711–742.
- Sivanesan, A. (1976) New British species of *Rhamphoria*, *Trematosphaeria* and *Chaetosphaerella*. *Transactions of the British Mycological Society* **67**: 469–475.
- Sivanesan, A. (1978) *Lasiobertia africana* gen. et sp. nov. and a new variety of *Bertia moriformis*. *Transactions of the British Mycological Society* **70**: 383–387.
- Smyk, L. V. (1981) Fungi of the order *Coronophorales* in the Ukraine mycoflora. *Ukrainskii Botanichnii Zhurnal* **38**: 46–49.
- Subramanian, C. V. & Sekar, G. (1990) *Coronophorales* from India – a monograph. *Kavaka* **18**: 19–90.
- Swofford, D. L. (2002) *PAUP\*: phylogenetic analysis using parsimony (\*and other methods)*. Version 4. Sinauer Associates, Sunderland, MA.
- Tamura, K. & Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**: 512–526.
- Teng, S. C. (1938) Additional fungi from China VIII. *Sinensia* **9**: 219–258.
- Vujanovic, V. (2002) A new variety of *Nitschkia parasitans* hyperparasitic on *Nectria cinnabarina* from American beech. *Mycotaxon* **82**: 121–126.
- Yuan, Z. Q. & Mohammed, C. (1997) New species and new records of ascomycetes on stems of eucalypts from Tasmania, Australia. *Mycotaxon* **63**: 9–23.

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