
Teleomorph-anamorph connections: *Chaetosphaeria raciborskii* and related species, and their *Craspedodidymum*-like anamorphs

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Several *Chaetosphaeria* species were recognised as having a distinctive ascomal wall structure and scolecosporous ascospores. Specimens fitting this description were found repeatedly in many collecting localities in temperate and tropical areas and while assignment to the overall group was easy based on the unique ascomal wall cells, determining the actual number of species was more difficult. Taxa representing the diversity of this group were targeted for phylogenetic analysis using the internal transcribed spacer region of the large subunit nrDNA (ITS). Based on the molecular data, two monophyletic clades were found which correspond with the circumscription of two existing species, *C. lapaziana* and a new combination, *C. ellisii*. Two new species are recognised, one based on distinctive teleomorph morphology and one based on culture data. *Chaetosphaeria raciborskii* is regarded as polyphyletic and the name is used for tropical specimens with small-sized, long-setose ascomata. All the species have *Craspedodidymum*-like anamorphs in culture and two species additionally have a *Chloridium*-like synanamorph. All the species are described and illustrated.

Key words: ITS, *Lasiosphaeria*, phylogeny, taxonomy.

Introduction

A recent revision of the genus *Lasiosphaeria* Ces. & De Not. based on partial sequences of the large subunit nrDNA (Miller and Huhndorf, 2004) reassigned a number of taxa unrelated to the type species, *L. ovina* (Pers.) Ces. & De Not. One of these, *Lasiosphaeria raciborskii* (Penz. & Sacc.) Carroll & Munk was found to be a member of the genus *Chaetosphaeria* Tul. & C. Tul. This species is distinguished by large, multiseptate, scolecosporous ascospores and a unique ascomal wall structure composed of enlarged, inflated, thin-walled cells. This wall structure was first noted by Carroll and Munk (1964). Specimens fitting this description have been found repeatedly in many collecting localities in temperate and tropical areas. Over 200 specimens among

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our collections fit into the broad interpretation of this species complex or group. They are found on a variety of decayed substrates especially on well-decorticated, watersoaked wood. Ascomata on the substrate are superficial, mostly gregarious ranging in size from small (Fig. 44) to very large (Fig. 26). Setae may or may not be present on the ascomata and when present may vary in length. The ascomatal wall structure is the most distinguishing feature and it serves as a major morphological character to separate this species from other *Chaetosphaeria* species.

While initial assignment to this group was easy based on the presence of the distinctive wall cells, determining the actual number of species involved proved to be more difficult. A considerable amount of variation exists within several of the morphological characters making for a very large and diverse group of specimens. In this study, specimens representing the morphological diversity were examined, characterised and described. Available cultures were targeted for phylogenetic analysis using the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA. Some questions we considered were: (1) Does variation in the ascomal or ascospore morphologies correspond to one or more species? (2) Do analyses of the ITS data provide groupings concordant with groupings having similar morphologies? (3) What information does culture and anamorph data provide?

Materials and methods

Morphological

Ascomata were mounted in water and replaced with lactophenol containing azure A. Measurements were made and images were captured of material in both mounting fluids. Ascomata were sectioned at 5 μm for light microscopy using techniques modified from Huhndorf (1991): the use of osmium tetroxide as a secondary fixative is discontinued, acetone is used for dehydration in place of ethanol and Spurr's embedding medium replaces the Low Viscosity medium no longer available. Images were captured using photomacrography, bright field (BF), phase contrast (PH) and differential interference microscopy (DIC) and photographic plates were produced following the methods of Huhndorf and Fernández (1998). Abbreviations for collectors are SMH = S.M. Huhndorf, FAF = F.A. Fernández and ANM = A.N. Miller. When no collector is listed, the collector is identified by the collection number. All SMH collections are deposited in the Field Museum Mycology Herbarium (F). Latitude and longitude are given in degrees or calculated decimal equivalents. All specimens were collected from decorticated wood unless otherwise noted.

Taxon sampling

Taxa used in this study are listed in Table 1 along with their geographical locality, collector, voucher specimen and/or isolate number, and GenBank accession number. Cultures of multispore isolates were obtained by spreading centrum material from air-dried specimens onto 1% water agar (Difco agar) in 60 mm diam. plastic Petri plates. After 24-48 hours of incubation at room temperature, germinated asci and ascospores were transferred to 60 mm diam. plastic Petri plates containing 1% corn meal agar (Difco). Cultures were maintained at 10°C on 1% potato dextrose agar (Difco) slants in screw-cap tubes (23 × 85 mm, 6 dram). All voucher specimens are deposited in the Field Museum Mycology Herbarium (F). Selected cultures are deposited at ATCC.

DNA extraction, PCR amplification, sequencing and sequence alignment

Isolates were either grown in 1.5 mL microcentrifuge tubes containing 10 mL of potato dextrose broth (Difco) or grown in 9 cm diam. Petri plates containing cornmeal agar (Difco). Mycelium was either collected from the broth or scraped from the cornmeal agar's surface in plates; washed and centrifuged twice in deionised sterile water. Total genomic DNA was extracted as described in Fernández *et al.* (1999). DNA extractions from ascomata from additional collections were also attempted but were unsuccessful.

The ITS region of the nrDNA was amplified by using reagents in a Replipack Reagent set (Boehringer Mannheim Corporation, Indianapolis, Indiana) in the following manner: 1.5 µL of 25 mM MgCl₂, 2.5 µL of 10× reaction buffer (100 mM Tris, 500 mM KCl, pH 8.3), 0.5 µL of 8 mM dNTPs, 1.25 µL each of 20 mM primers ITS5 and ITS4 (White *et al.*, 1990), 0.25 µL (1.25 units) of Taq DNA polymerase, 1 µL of the undiluted DNA extract and 16.75 µL of double distilled, sterile water for a 25 µL total reaction volume.

PCR was performed by using the following thermocycling parameters: initial denaturation temperature 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 50°C for 1 minute and extension at 72°C for 1 minute. A final extension step of 10 minutes at 72°C was added. Amplified products were separated from unincorporated nucleotides and primers by using a GeneClean III kit (Bio 101, Inc., Vista, California).

Sequencing was performed on both strands using primers ITS5/ITS1 and ITS4 (White *et al.*, 1990). Sequencing reactions were performed by using the ABI Prism Dye Terminator Cycle Sequencing kit (Perkin-Elmer Corporation). Sequenced products were cleaned by precipitation using a 70% ethanol/5 mM Magnesium chloride solution, and were run through a polyacrylamide gel using

Table 1. List of taxa used in the molecular analyses. All sequences are from the internal transcribed spacer region of the nrDNA.

Taxa	Culture designation	Geopolitical origin	GenBank Accession No.
<i>C. ellisii</i>	SMH2519	USA (Indiana)	AY906939
<i>C. ellisii</i>	SMH2758	USA (North Carolina)	AY906940
<i>C. ellisii</i>	SMH3807	USA (North Carolina)	AY906941
<i>C. ellisii</i>	SMH3809	USA (North Carolina)	AY906942
<i>C. ellisii</i>	SMH3824	USA (North Carolina)	AY906943
<i>C. ellisii</i>	SMH3860	USA (South Carolina)	AY906944
<i>C. hebetiseta</i>	SMH2729	USA (North Carolina)	AY906955
<i>C. innumera</i>	SMH2748	USA (North Carolina)	AY906956
<i>C. lapaziana</i>	SMH2182	Costa Rica	AY906945
<i>C. lapaziana</i>	SMH2900	Puerto Rico	AY906946
<i>C. lapaziana</i>	SMH3043	Puerto Rico	AY906947
<i>C. panamensis</i>	SMH3596	Panama	AY906948
<i>C. raciborskii</i>	SMH2017	Puerto Rico	AY906949
<i>C. raciborskii</i>	SMH2036	Puerto Rico	AY906950
<i>C. raciborskii</i>	SMH2132	Puerto Rico	AY906951
<i>C. raciborskii</i>	SMH3014	Puerto Rico	AY906952
<i>C. raciborskii</i>	SMH3119	Puerto Rico	AY906953
<i>C. rubicunda</i>	SMH2881	Puerto Rico	AY906954

an ABI Prism 377 DNA Sequencer (Applied Biosystems). Eighteen sequences were assembled and aligned by using Sequencher version 3.0 (Gene Codes Corporation), including *C. hebetiseta* and *C. innumera* as outgroups. Alignment was checked by eye and corrected manually when necessary. Parsimony analyses were performed using PAUP* 4.0b64a compiled for the PPC platform (Swofford, 2000). Parsimony informative characters were unordered and were unequally weighted by subjecting them to a transition/transversion-ratio step matrix. Gaps were treated as missing, 10,000 random-addition sequence replicates were implemented for the analysis, the branch-swapping algorithm was TBR, the MULPARS option was in effect, and zero-length branches were not collapsed. The support for the internodes of the most-parsimonious trees was estimated by 10,000 bootstrap replicates (Felsenstein, 1985) with a heuristic search with 10 random-addition sequences for each of 1000 bootstrap replicates.

Results

Molecular

The parsimony analysis yielded a total of 486 characters, of which 313 were constant, 57 were variable parsimony-uninformative and 99 were

parsimony-informative. The analysis generated four most parsimonious trees of 371 steps, CI = 0.68, with identical topology (Fig. 104).

Placement of *C. raciborskii* within the genus *Chaetosphaeria* is well supported according to parsimony analyses of partial sequences of the large subunit nrDNA and the β -tubulin gene (Fernández *et al.*, pers. observ.; Miller and Huhndorf, 2004). The outgroup taxa, represented by *Chaetosphaeria innumera* and *Chaetosphaeria hebetiseta*, are very unlike the ingroup taxa. Both species have small fusiform, three-septate, hyaline ascospores whereas the ingroup taxa have scolecospores, seven-septate, hyaline ascospores.

The 16 collections composing the ingroup segregated into three distinct clades that have strong bootstrap support (Fig. 104). Clade 1 (= *C. ellisii*) is composed of all six temperate collections (NC and IN) with small to medium size ascomata having short setae over the entire surface. Clades 2 and 3 contain only tropical collections (PR and CR) with clade 2 (= *C. raciborskii*; two collections) having small to medium size ascomata with long setae over the entire surface and clade 3 (= *C. lapaziana*; three collections) having large ascomata with no setae or setae only at the apex. Collection SMH3014 shows affinities with clade 3 without bootstrap support, however morphologically it more closely resembles *C. raciborskii*. The three remaining collections from Puerto Rico form a clade without bootstrap support. One collection (SMH2881) is described as a new species while the other two collections resemble *C. raciborskii*. Collection SMH3596 from Panama consistently segregates from the rest of the collections, and putatively represents an additional clade (clade 4) composed of small sized ascomata with setae. This specimen produces unique, purple aleuriospores in culture (Figs. 57, 58, 61) and is described as a new species.

Total ITS sequence variation percentages, calculated as the number of base changes divided by the total number of bases, was lower among six temperate specimens (*ca.* 4.8%) compared to that among seven specimens from Puerto Rico (*ca.* 21.4%). Based on the analysis of ITS sequence data, four separate species are recognised, three monophyletic (clades 1, 3 and 4) and one paraphyletic (clade 2 and the additional unsupported collections) (Fig. 104). Recognition of these species is supported by morphological, cultural, and geographic data. One additional species is recognised based solely on morphology of the teleomorph (SMH2881).

Taxonomy: new species and combinations

Chaetosphaeria ellisii (Barr) Huhndorf & F.A. Fernández, **comb. nov.**

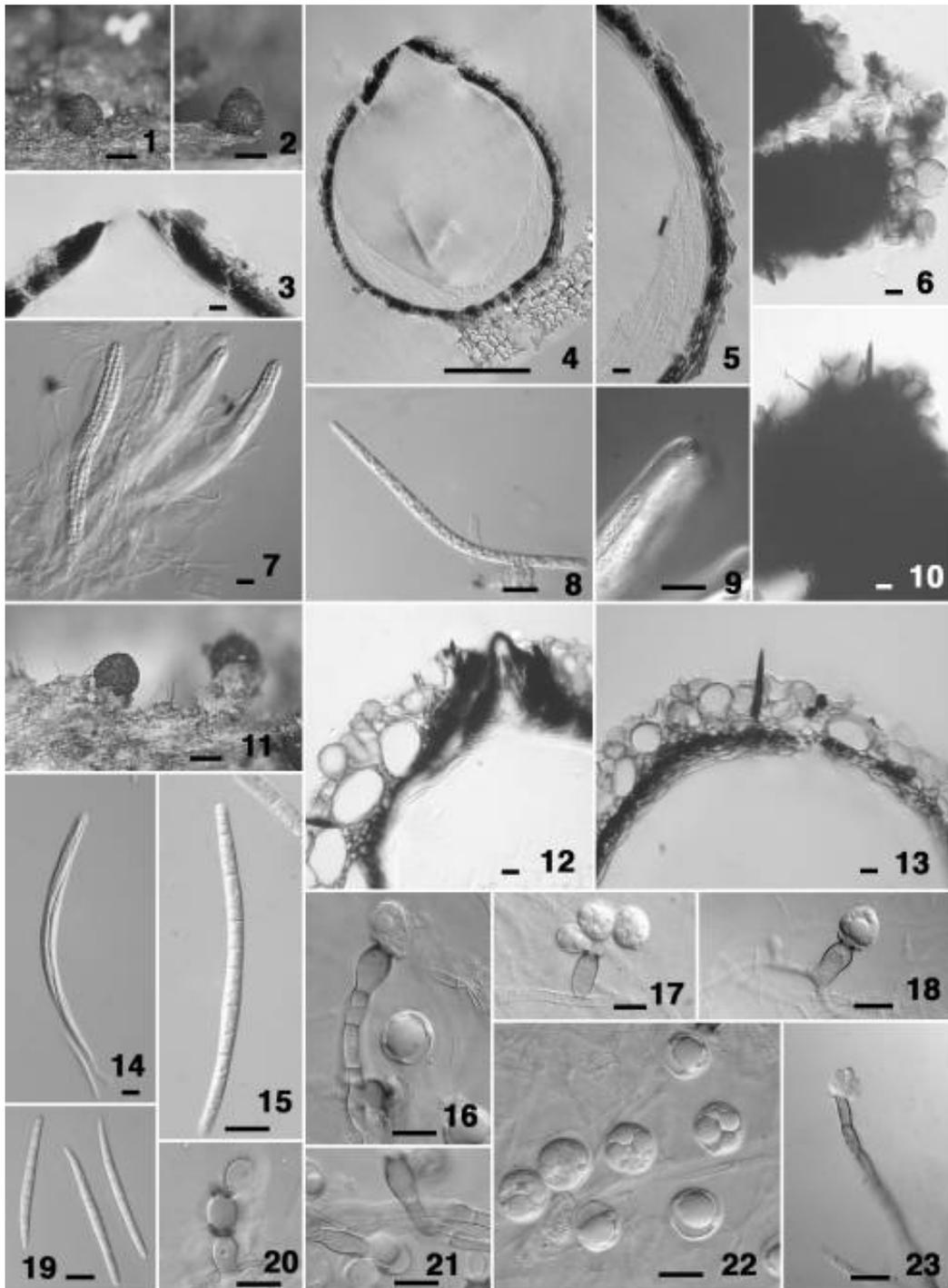
(Figs. 1-23)

≡ *Lasiosphaeria ellisii* Barr, Mycotaxon 46: 48. 1993. (Basionym)

≡ *Sphaeria longispora* Ellis, non Currey, nec Karsten (see Barr 1993 for additional synonymies).

Ascomata scattered or clustered, numerous, superficial, globose to ovoid, not collapsing, roughened, with reddish, russet or brown surface colour, (130-) 150-300(-375) μm diam., 150-325(-420) μm high, papillate. *Setae* scattered over entire ascoma, brown, stiff, pointed, arising from the inner layer of small brown cells, long, 20-45 μm , sparse or abundant. *Ascomatal wall* of *textura globosa* in surface view; in longitudinal section uniformly 36-110 μm thick; 2-layered, inner wall layer 15-19 μm thick, up to 60 μm thick at base, composed of small (4 \times 8 μm), polygonal-to-elongate, pale-to-dark brown, pseudoparenchymatic cells, 4-6 cells thick, setae arising from this wall layer; outer wall layer 34-51 μm thick, composed of large (15-30 \times 27-37 μm), isodiametric-to-polygonal, pale brown, pseudoparenchymatic cells, 5-20 cells thick, when fresh some of the cells contain pale purple pigment, disappearing when dried. *Papilla* conical, 58-60 μm high, 14-17 μm wide at the apex, 43-49 μm wide at the base; circular ostiole 15-27 μm wide, with periphyses. *Paraphyses* 3-4 μm wide, numerous, septate, tapering toward apex. *Asci* 162-180 \times 10-15 μm , numerous, arising from basal hymenium, cylindrical, rounded apex, with refractive apical ring, short-stalked, unitunicate, with 8, triseriate, ascospores. *Ascospores* (40-)50-75(-80) \times 3-4.5 μm , filiform, with apical end broadly rounded, basal end narrowly rounded, straight to slightly curved, hyaline, smooth, 7-septate, without constrictions, primary septum median, slightly bipolarly asymmetrical, without sheath or appendages. *Culture*: Colonies on WA hyaline to light brown, mostly immersed with sparse superficial hyphal growth, 12-24(-39) mm in 21 days, anamorphs present or absent. Colonies on CMA hyaline to light brown, mostly immersed with sparse superficial hyphal growth, 10-25(-40) mm in 21 days, anamorphs present or absent. Colonies on MEA light brown to dark-greenish brown, reverse dark brown, abundant immersed and superficial floccose hyphal growth, 11-20(-40) mm in 21 days, no anamorphs produced. Some isolates on WA and CMA produced two synanamorphs at 7-14 days. *Craspedodidymum*-like anamorph consists of simple phialidic conidiogenous cells on hyphae or multi-celled,

Figs. 1-23. *Chaetosphaeria ellisii*. **1, 2, 11.** Ascomata on substrate. **3, 12.** Section through ascomal neck. **4.** Longitudinal section through ascoma. **5, 13.** Section through ascomal wall. **6, 10.** Pieces of the ascomal wall showing the wall cells and setae. **7, 14.** Asci. **8, 15, 19.** Ascospores. **9.** Ascus apex. **16-18, 20, 21.** *Craspedodidymum*-like conidiophores on CMA. **22.** Conidia on CMA. **23.** *Chloridium*-like conidiophores on CMA. Figs. 1, 2, 11 by photomicrography; Figs. 3-10, 12-23 by DIC. Figs. 1-5, 7, 8 from holotype Ellis coll (NY); Figs. 6, 9, 10 from Cooke 119 (NY); Figs. 11, 12, 14, 18, 19, 23 from SMH2519; Fig. 13 from SMH3807; Fig. 15 from SMH3824; Figs. 16, 20, 22 from SMH2758; Fig. 17 from SMH3809; Fig. 21 from SMH3860. Bars: 1, 2, 11 = 200 μm ; 4 = 100 μm ; 3, 5-10, 12-23 = 10 μm .



brown conidiophores, at times more than one phialide is produced on a conidiophore or a phialide proliferates percurrently; phialides flask-shaped to obclavate, 6-8 µm diam, 7-19 µm high, brown, collarete flared or cup-shaped, 5-9 µm wide, 1.5-3(-5) µm high. Conidia globose, to subglobose, 10-13.5 µm diam, thick-walled, hyaline, one-celled; large and small guttules in the mature conidium. *Chloridium*-like anamorph consists of simple phialidic conidiogenous cells on a multi-celled, brown conidiophore, 60-125 × 2.5-3.5 µm; phialides cylindrical, mostly terminal, 2.5-4 µm diam, 8-19 µm high, sometimes with a small collarete (3-4 µm wide, 1.5-2 µm high), conidia produced percurrently, accumulating in large clusters at phialide's tip. Conidia globose to ovoid to clavate, 5-12 × 2-8.5 µm, one-celled, hyaline, sometimes with a blunt basal end.

Anamorph: Craspedodidymum-like and *Chloridium*-like.

Habitat: On decorticated wood.

Known distribution: USA.

Material examined: USA, Illinois, Alexander Co., toward Bean Ridge Pond along Forest Road 262, 13-VIII-1999, ANM, *SMH4129*; Cook Co., Cook County Forest Preserve, Swallow Cliffs, 3-VII-1996, SMH, FAF, M. Huhndorf, *SMH2541*; *SMH2542*; *SMH2543*; *SMH2544*; 16-VIII-1996, *SMH2638*; 31-X-1997, *SMH3768*; Ogle Co., White Pines Forest State Park, 28-IX-1996, SMH, FAF, *SMH2690*; *SMH2699*; *SMH2703*; Vermillion Co., Kennekuk Co. Park, Lookout Point trail by Oak Bluff picnic area, W of Middle Fork Vermillion River, [40.1833, -87.7333], 29-IX-2000, ANM, *SMH4328*. Indiana, Brown Co., Yellowwood State Forest, compartment 7, tract 24, 26-VII-1996, *SMH2634*; Lake Co., Indiana Dunes National Lakeshore, Cowles Bog, 1-VII-1996, SMH, FAF, M. Huhndorf, *SMH2519*; *SMH2520*. Michigan, Berrien Co., Warren Woods, south end of trail, through picnic area, up to creek, 8-IX-1998, FAF, ANM, *SMH3884*; *SMH3889*; *SMH3893*; *SMH3898*; north end trail, 29-IX-1998, FAF, ANM, *SMH3901*; 31-VIII-1999, ANM, *SMH4134*. New Jersey, Newfield, 20-VII-1874, on branch of *Kalmia latifolia* on the ground, J.B. Ellis (NY; **holotype**). New York, Clyde, IX-1881, O.F. Cooke 119 (NY). North Carolina, Macon Co., Franklin, Standing Indian Campground, Park Creek Trail, towards gravel road, [35.0079, -83.5305], 29-VII-1998, FAF, *SMH3841*; Highlands, Blue Valley, 1000 m, [35.0192, -83.2736], 7-X-1996, SMH, FAF, Q.X. Wu, J.C. Wei, G.M. Mueller, *SMH2735*; 18-VII-1997, FAF, *SMH3267*; *SMH3272*; Highlands Biological Station, 20-VII-1997, FAF, *SMH3299*; *SMH3302*; *SMH3304*; 21-VII-1997, FAF, *SMH3312*; Horse Cove Drive & Bull Pen Road, 1000 m, [35.025, -83.1464], 5-X-1996, FAF, Q.X. Wu, J.C. Wei, *SMH2734*; 8-X-1996, SMH, FAF, Q.X. Wu, J.C. Wei, G.M. Mueller, *SMH2758*; 27-VII-1998, FAF, *SMH3816*; *SMH3824*; Otto, Cowieta Hydrological Laboratory, 27-VII-1998, FAF, *SMH3805*; *SMH3807*; *SMH3809*; 19-VII-1997, FAF, *SMH3283*; *SMH3285*; Oconee Co., Sumter National Forest Fish Hatchery, left trail through pine area to East Fork Trail, [34.9836, -83.0739], 31-VII-1998, FAF, *SMH3860*. Wisconsin, Fond du Lac Co., Kettle Moraine State Forest, Mantle Lake Rec. Area, wet area scenic trail, 24-IX-1999, SMH, *SMH4190*.

Chaetosphaeria lapaziana (Carroll & Munk) F.A. Fernández & Huhndorf, *Fungal Diversity* 18: 48 (2005) (Figs. 24-43)

Ascomata clustered, numerous, superficial, globose to ovoid, not collapsing, roughened, with reddish, russet or brown surface colour, (400-)500-

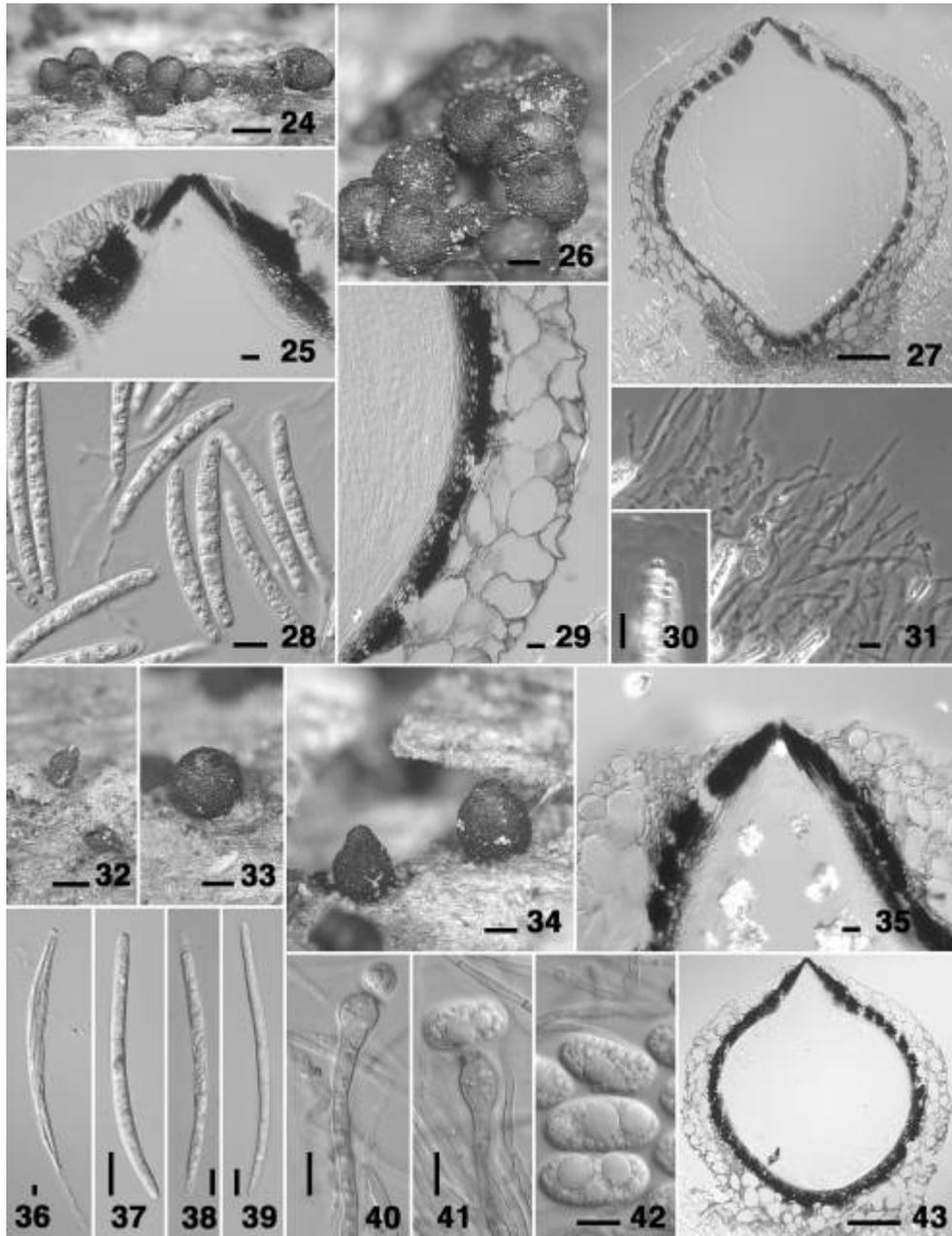
950 μm diam., 525-825(-1025) μm high, papillate. *Setae* absent or only surrounding the ostiole, brown, stiff, pointed, short, 25-35 μm , sparse. *Ascomatal wall* of *textura globosa* in surface view; in longitudinal section uniformly 50-90(-145) μm thick; 2-layered, inner wall layer 10-30(-45) μm thick, composed of small, polygonal-to-elongate, pale-to-dark brown, pseudoparenchymatic cells, 4-6 cells thick, setae arising from this wall layer; outer wall layer 20-65(-115) μm thick, composed of variable-sized (largest range from 30-39 \times 46-56 μm), isodiametric-to-polygonal, pale brown, pseudoparenchymatic cells, 5-20 cells thick, when fresh some of the cells contain pale purple pigment, disappearing when dried. *Papilla* conical, at times composed of apical setae 25-35 μm long; (50-)85-125 μm high, 25-45 μm wide at the apex, 65-150 μm wide at the base; circular ostiole 35-85 μm wide, with periphyses. *Paraphyses* 3-4 μm wide, numerous, septate, tapering towards apex. *Asci* 170-290 \times 11-25 μm , numerous, arising from basal hymenium, cylindrical, rounded apex, with refractive apical ring, short-stalked, unitunicate, with 8, triseriate, ascospores. *Ascospores* (45-)50-100(-120) \times (3-)4.5-6(-7) μm , filiform to cylindrical, with apical end broadly rounded, basal end narrowly rounded, straight to slightly curved, hyaline, smooth, 7-septate, without constrictions, primary septum median, slightly bipolarly asymmetrical, without sheath or appendages. *Culture*: Colonies on WA hyaline to light brown, mostly immersed with sparse superficial hyphal growth, 17 mm in 21 days, anamorph present over entire colony in 2 months. Colonies on CMA hyaline to light brown, mostly immersed with sparse superficial hyphal growth, 21 mm in 21 days, anamorph present over entire colony in 2 months. Colonies on MEA light brown to dark-greenish brown, reverse dark brown, abundant immersed and superficial floccose hyphal growth, 24 mm in 21 days, no anamorphs produced. *Craspedodidymum*-like anamorph consists of simple phialidic conidiogenous cells on a multi-celled, brown conidiophore; phialides clavate, 8-10 μm diam, 13-20 μm high, brown, collarete flared, rarely cup-shaped, 5-6.5 μm wide, 1-2 μm high. Conidia oblate to horizontally oblong, 10.5-12.5 \times 20-28 μm , hyaline, one-celled, with short abscission scar or frill located medially on one long side; large and small guttules in the mature conidium; more than one conidium produced per phialide.

Anamorph: *Craspedodidymum*-like.

Habitat: On decorticated wood

Known distribution: Costa Rica, French Guiana, Jamaica, Puerto Rico.

Material examined: COSTA RICA, Alajuela, La Fortuna de San Carlos, Parque Nacional Volcan Arenal, Pilón trail, [10.4419, -84.7167], 15-VII-2001, SMH, FAF, ANM, M.P. DaRin, SMH4532; SMH4551; La Paz, by waterfall, 1600 m, 13-VI-1962, G. Carroll, GC87 (NY; **holotype**; IMI100173, **isotype**); Guanacaste, Parque Nacional Guanacaste, Santa Cecilia, Sector Pitilla, 700 m, [10.9889, -85.4261], 23-VI-1997, SMH3211; Puntarenas, San Vito, Las Cruces Biological Station, Rio Jaba trail, 1050 m, [8.7858, -82.9586], 5-V-1996, SMH, FAF,



Figs. 24-43. *Chaetosphaeria lapaziana*. **24, 26, 32-34.** Ascomata on substrate. **25, 35.** Section through ascomal neck. **27, 43.** Longitudinal section through ascoma. **28, 37-39.** Ascospores. **29.** Section through ascomal wall. **30.** Ascus apex. **31.** Paraphyses. **36.** Ascus. **40, 41.** *Craspedodidymum*-like conidiophores on CMA. **42.** Conidia on CMA. Figs. 24, 26, 32-34 by photomacrography; Figs. 25, 27-30, 35-43 by DIC; Fig. 31 by PH. Figs. 24-31 from holotype GC87 (NY); Figs. 32, 35, 38 from SMH3043; Figs. 34, 39 from SMH2900; Figs. 33, 36, 37, 40-43 from SMH2182. Bars: 24, 32 = 500 μ m; 26, 33, 34 = 200 μ m; 27, 43 = 100 μ m; 25, 28-31, 35-42 = 10 μ m.

SMH2182; *SMH2188*; *SMH2193*; Puntarenas, Parque Internacional La Amistad Pacifico, Los Alturas Biological Station, trail to Cerro Echandi, 1st 500 m, 1580 m, [8.95, -82.8333], 6-V-1996, SMH, FAF, *SMH2244*; *SMH2251*; *SMH2253*; San Jose, La Chonta, Km 55 on Interamerican Hwy, 2400 m, [9.6994, -83.9419], 9-V-1996, SMH, FAF, *SMH2304*; San Gerardo de Dota, Albergue de Montana, Savegre, Sendero la Quebrada, 2300 m, [9.55, -83.8], 12-V-1996, SMH, FAF, *SMH2408*; 14-V-1996, SMH, FAF, *SMH2458*; *SMH2464*; *SMH2466*; Bosque los Ninos, 18-V-1996, SMH, FAF, *SMH2502*; *SMH2505*; *SMH2509*. FRENCH GUIANA, St-Laurent-du-Maroni Arrondissement, Canton de Maripasoula, Commune de Saul, Eaux Claires, 3rd stream crossing *ca.* 1 hr walk N along Route de Belizon, 200 m, [3.7, -53.2], 4-IX-1994, *SMH797*. JAMAICA, St. Andrew Parish, Black Mts. Nat. Park, Fairy Glade trail, [18.0433, -76.3108], 17-VI-1999, FAF, *SMH4103*. PUERTO RICO, El Verde Research Area, Luquillo Mts., 16-ha Grid, 350 to 425 m, [18.3167, -65.8167], 12-I-1997, SMH, FAF, *SMH2900*; 16-I-1997, SMH, FAF, *SMH3004*; 18-I-1997, SMH, FAF, *SMH3043*.

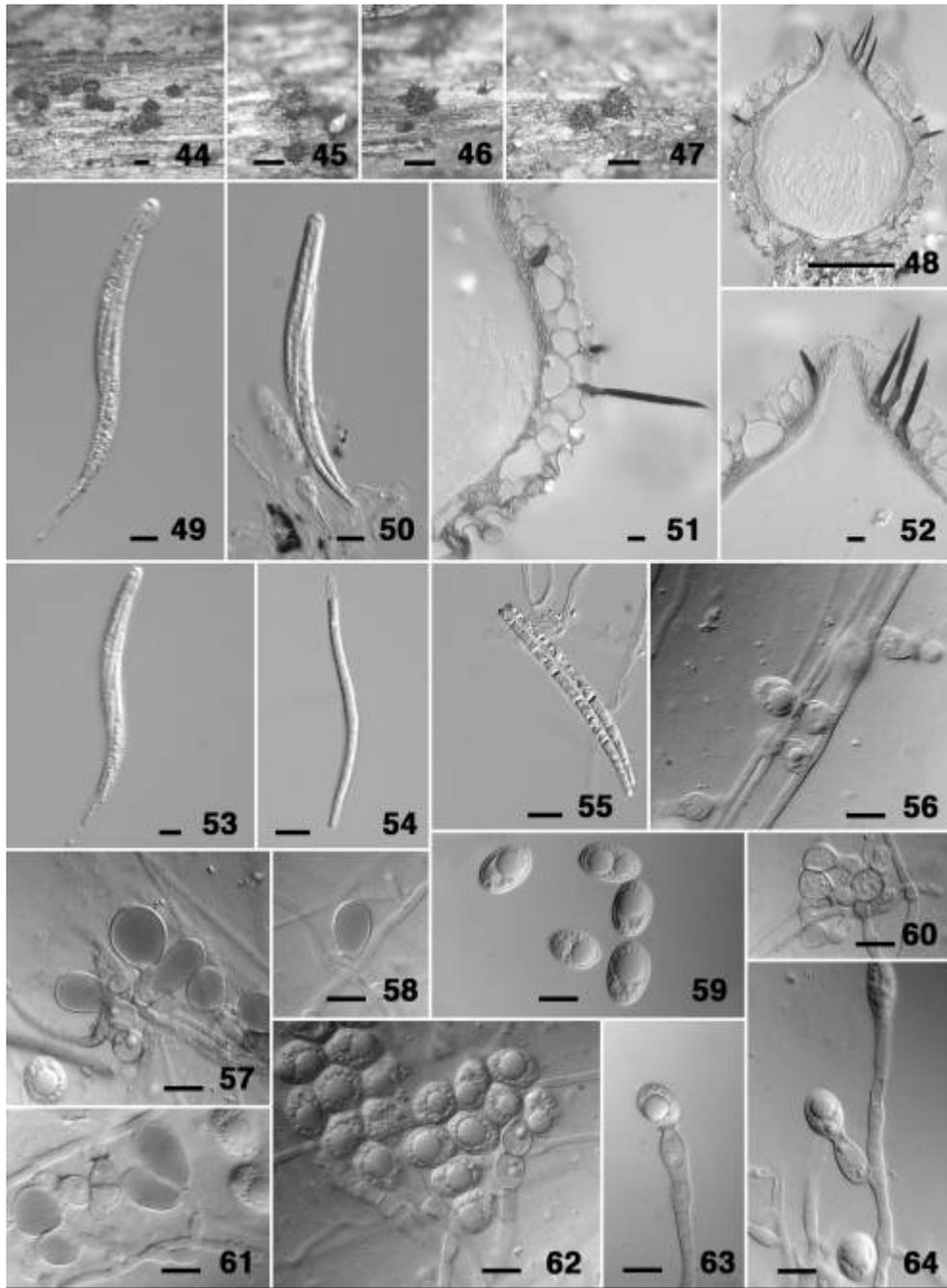
***Chaetosphaeria panamensis* Huhndorf & F.A. Fernández, sp. nov.**

(Figs. 44-64)

Etymology: Refers to the collection locality.

Ascomata dispersa, superficialia, globosa, 185-235 μm diametro, 190-270 μm alta, papillata, setae sparsae vel abundantes, brunneae, 60-75 μm altae. *Parietes* superficie e textura globosa compositus, 30-40 μm crassus, pseudoparenchymaticus, e cellulis multangularibus vel elongatis compositus. *Paraphyses* simplices, septatae, hyalinae. *Asci* cylindrici, 123-140 \times 10-11 μm , octospori. *Ascospores* filiformes, 65-75 \times 3-4 μm , hyalinae, 7-septatae.

Ascomata scattered, sparse, superficial, globose, not collapsing, roughened, with reddish, russet or brown surface colour, setose, 185-235 μm diam., 190-270 μm high, papillate. *Setae* scattered over entire ascoma, brown, stiff, pointed, arising from the inner layer of small brown cells, 60-75 μm long, sparse or abundant. *Ascomatal wall* of *textura globosa* in surface view; in longitudinal section uniformly 30-40 μm thick; 2-layered, inner wall layer 5-10 μm thick, composed of small, polygonal-to-elongate, pale-to-dark brown, pseudoparenchymatic cells, 4-6 cells thick, setae arising from this wall layer; outer wall layer 25-30 μm thick, composed of large (15-20 \times 20-30 μm), isodiametric-to-polygonal, pale brown, pseudoparenchymatic cells, 5-20 cells thick, pale purple pigment not seen. *Papilla* conical, *ca.* 60 μm high, *ca.* 20 μm wide at the apex, *ca.* 45 μm wide at the base; circular ostiole *ca.* 12 μm wide, with periphyses. *Paraphyses* 4-5 μm wide, numerous, septate, tapering toward apex. *Asci* 123-140 \times 10-11 μm , numerous, arising from basal hymenium, cylindrical, rounded apex, with refractive apical ring, short-stalked, unitunicate, with 8, triseriate ascospores. *Ascospores* 65-75 \times 3-4 μm , filiform, with apical end broadly rounded, basal end narrowly rounded, straight to slightly curved, hyaline, smooth, 7-septate, without constrictions, primary septum median, slightly bipolarly asymmetrical, without sheath or appendages. *Culture*: Colonies on WA hyaline to light brown, mostly immersed with sparse superficial hyphal growth, 15 mm in 21 days, anamorph present. Colonies on CMA hyaline to light brown, mostly immersed with sparse superficial hyphal



Figs. 44-64. *Chaetosphaeria panamensis*. 44-47. Ascomata on substrate. 48. Longitudinal section through ascoma. 49, 50, 53. Asci. 51. Section through ascomal wall. 52. Section through ascomal neck. 54, 55. Ascospores. 56, 60, 63, 64. *Craspedodidymum*-like conidiophores on CMA. 57, 58, 61. Aleuriospore-like cells on CMA. 59, 62. Conidia on CMA. Figs. 44-47 by photomacrography; Figs. 48-64 by DIC. Figs. 44-64 from SMH3596. Bars: 44-47 = 200 μ m; 48 = 100 μ m; 49-64 = 10 μ m.

growth, 20 mm in 21 days, anamorph present. Colonies on MEA light brown to dark-greenish brown, reverse dark brown, abundant immersed and superficial floccose hyphal growth, 26 mm in 21 days, anamorphs absent. *Craspedodidymum*-like anamorph consists of simple phialidic conidiogenous cells on hyphae or multi-celled, brown conidiophores, at times more than one phialide is produced on a conidiophore; phialides flask-shaped to clavate, 6.5-10 μm diam, 10-16 μm high, hyaline to pale brown, collarette flared, rarely cup-shaped, 4.5-5.5 μm wide, 1.5-2 μm high. Conidia ellipsoid to subglobose, 13-17 \times 9.5-12 μm , hyaline, one-celled; large and small guttules in the mature conidium. Hyphae on WA and CMA produce aleuriospore-like cells on short stalks, cells ellipsoid to clavate, 14.5-17 \times 10-15 μm , filled with purple pigment.

Anamorph: *Craspedodidymum*-like.

Habitat: On decorticated wood.

Known distribution: Panama.

Material examined: PANAMA, Barro Colorado Island National Monument, Shannon trail, 50 to 150 m, [9.1667, -79.8333], 23-VIII-1997, SMH, FAF, SMH3596 (F; **holotype designated here**).

Chaetosphaeria raciborskii (Penz. & Sacc.) F.A. Fernández & Huhndorf, in Miller and Huhndorf, Mycol. Res. 108: 29 (2004) (Figs. 65-86)

≡ *Ophiochaeta raciborskii* Penz. & Sacc., Malpighia 11: 406 (1897). (Basionym)

≡ *Lasiosphaeria raciborskii* (Penz. & Sacc.) Carroll & Munk, Mycologia 56: 91 (1964).

Ascomata scattered or clustered, sparse or numerous, superficial, globose, to obpyriform or ovoid, not collapsing, roughened, with reddish, russet or brown surface colour, setose, (150-)200-450 μm diam., (160-)200-450 μm high, papillate. *Setae* scattered over entire ascoma, brown, stiff, pointed, arising from the inner layer of small brown cells, short or long, 40-120 μm , sparse or abundant. *Ascomatal wall* of *textura globosa* in surface view; in longitudinal section uniformly 45-57(-77) μm thick; 2-layered, inner wall layer 6-17 μm thick, composed of small, polygonal-to-elongate, pale-to-dark brown, pseudoparenchymatic cells, 4-6 cells thick, setae arising from this wall layer; outer wall layer 30-50(-60) μm thick, composed of variable-sized (largest range from 15-30 \times 20-40 μm), isodiametric-to-polygonal, pale brown, pseudoparenchymatic cells, 5-20 cells thick, when fresh some of the cells contain pale purple pigment, disappearing when dried. *Papilla* conical, 50-100 μm high, 25-40 μm wide at the apex, 35-80 μm wide at the base; circular ostiole (12-)16-30(-45) μm wide, with periphyses. *Paraphyses* 2.5-5(-7) μm wide, numerous, septate, tapering toward apex. *Asci* (150-)180-250(-350) \times 10-20(-27) μm , numerous, arising from basal hymenium, cylindrical, rounded apex, with refractive apical ring, short-stalked, unitunicate, with 8, triseriate, ascospores. *Ascospores* (50-)60-100(-150) \times 3-3.75(-4.5) μm , filiform, with

apical end broadly rounded, basal end narrowly rounded, straight to slightly curved, hyaline, smooth, 7-septate, without constrictions, primary septum median, slightly bipolarly asymmetrical, without sheath or appendages.

Culture: Colonies on WA hyaline to light brown, mostly immersed with sparse superficial hyphal growth, 17-20 mm in 21 days, anamorphs present, scanty or abundant conidiophores, conidia and setae (85-90 μm long, 4-5.5 μm wide) on the surface. Colonies on CMA hyaline to light brown, in concentric rings, mostly immersed with sparse superficial hyphal growth, 17-20 mm in 21 days, anamorphs present over entire colony. Colonies on MEA light brown to dark-greenish brown, reverse dark brown, abundant immersed and superficial floccose hyphal growth, 11-20 mm in 21 days, no anamorphs produced. Some isolates on WA and CMA produced two synanamorphs at 7-14 days. *Craspedodidymum*-like anamorph consists of simple phialidic conidiogenous cells on hyphae or multi-celled, brown conidiophores; phialides flask-shaped to obclavate, 6.5-10 μm diam., 8.5-19 μm high, brown, collarete flared or cup-shaped, 7-13 μm wide, 2-7(-10) μm high. Conidia globose, to subglobose, 9.5-15.5 μm diam, thick-walled, subhyaline, one-celled; large and small guttules in the mature conidium; one isolate (*SMH 3119*) has conidia triangular in shape, with 3 or more elongate, lash-like setulae or appendages (10-12 μm long, 1 μm wide). *Chloridium*-like anamorph consists of simple phialidic conidiogenous cells on a multi-celled, brown conidiophore, 50-70 \times 3-4.5 μm ; phialides cylindrical, mostly terminal, at times proliferating percurrently, 3-5 μm diam, 18-26 μm high, sometimes with a small collarete (3-4 μm wide, 1.5-4 μm high), conidia produced percurrently, accumulating in large clusters at phialide's tip. Conidia globose to ovoid to clavate, 3.5-6.5 \times 2.5-3.5 μm , one-celled, hyaline, sometimes with a blunt basal end.

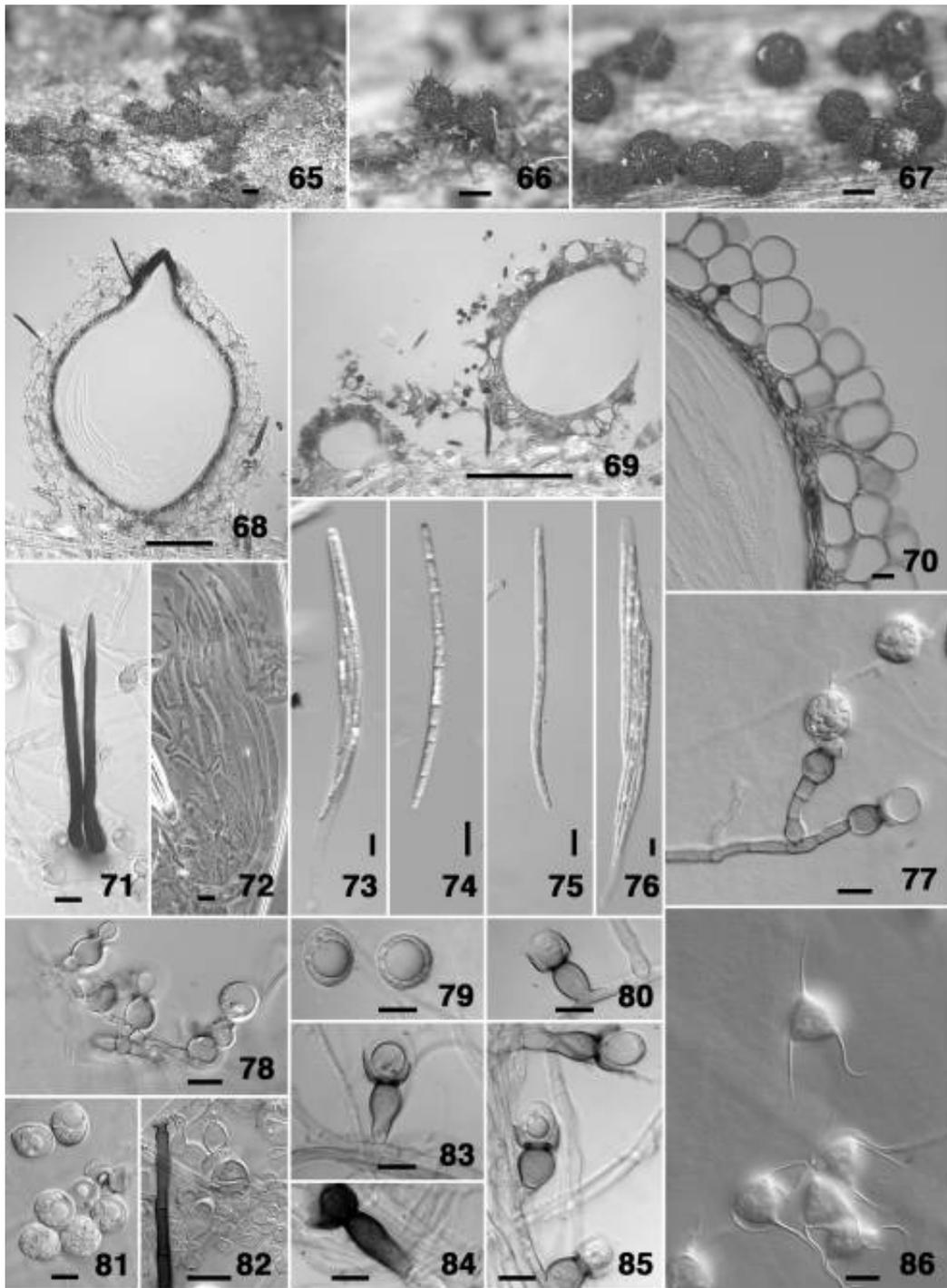
Anamorph: *Craspedodidymum*-like and *Chloridium*-like.

Habitat: On decorticated wood, rotting stems, palms, bamboo.

Known distribution: Costa Rica, Cuba, Ecuador, French Guiana, Indonesia (Java), Jamaica, New Zealand, Panama, Puerto Rico, Venezuela; probably tropical worldwide.

Material examined: COSTA RICA, Alajuela, Cantón Upala, District Bijagua, Heliconias Station, Heliconias trail, 1190 m, [10.7081, -85.0453], 12-VII-2001, SMH, FAF, ANM, M.P. DaRin, *SMH4457*; *SMH4463*; *SMH4473*; *SMH4475*; *SMH4480*; *SMH4481*; La Fortuna de San Carlos, Parque Nacional Volcan Arenal, Pílon trail, [10.4419, -84.7167], 15-VII-2001, SMH,

Figs. 65-86. *Chaetosphaeria raciborskii*. **65-67.** Ascomata on substrate. **68, 69.** Longitudinal section through ascoma. **70.** Section through ascomal wall. **71.** Setae on CMA. **72.** Paraphyses. **73, 76.** Asci. **74, 75.** Ascospores. **77.** *Craspedodidymum*-like conidiophores on WA. **78, 80, 83-85.** *Craspedodidymum*-like conidiophores on CMA. **79, 81.** Conidia on CMA. **82.** *Chloridium*-like conidiophores on CMA. **86.** Conidia on WA. Figs. 65-67 by photomacrography; Figs. 68-71, 73-86 by DIC; Fig. 72 by PH. Fig. 65 from SMH2132; Figs. 66, 68, 71-74, 78, 81, 82 from SMH2036; Figs. 67, 70, 75-77, 86 from SMH3119; Fig. 69 from holotype (PAD); Figs. 79, 80, 83-85 from SMH2017. Bars: 65-67 = 200 μm ; 68, 69 = 100 μm ; 70-86 = 10 μm .



FAF, ANM, M.P. DaRin, *SMH4529*; *SMH4529*; *SMH4536*; *SMH4540*; Heredia, Puerto Vieho, Finca la Selva, 300 m, 12-VI-1962, G. Carroll, *GC88* (NY); Guanacaste, Liberia ACG, Sector Santa Maria, trail to Bosque Encantado at Estacion Biologica, 750 m, [10.7647, -85.3033], 26-VI-1997, *SMH3241*; *SMH3242*; *SMH3249*; *SMH3252*; Parque Nacional Guanacaste (ACG), Sector Cacao, trail to Estacion Biologica Cacao, 1100 m, [10.9264, -85.4686], 24-VI-1997, *SMH3222*; *SMH3228*; Santa Cecilia, Sector Pitilla, 700 m, [10.9889, -85.4261], 23-VI-1997, *SMH3202*; *SMH3205*; *SMH3208*; Pasmompa, S of St. Cecilia, 4 km N of La Pitilla, 700 m, [11.0333, -85.4333], 23-VI-1997, *SMH3217*; Limon, Estacion RB Hitoy Cerare, La Catarata, 120 m, [9.6717, -83.0289], 20-I-1999, FAF, *SMH4040*; Puntarenas, Area de Conservacion Osa, Parque Nacional Corcovado, Sirena Station, Las Ollas trail, 10 m, [8.4806, -83.5917], 16-VII-2000, FAF, G.M. Mueller, B. Strack, J.P. Schmit, L. Umaña, *SMH4271*; 18-VII-2000, FAF, *SMH4296*; *SMH4299*; Monteverde, Santa Elena Cloud Forest Reserve, 1190 m, [10.7081, -85.0453], 13-VII-2001, SMH, FAF, ANM, M.P. DaRin, *SMH4508*; Parque Internacional La Amistad Pacifico, Las Tablas, Coto Brus, 1700 m, [8.9492, -82.7772], 14-I-1999, FAF, *SMH4020*; La Montura trail, 1900 m, [8.9542, -82.8361], 13-I-1999, FAF, *SMH4009*; Los Alturas Biological Station, trail to Cerro Echandi, 1st 500 m, 1580 m, [8.95, -82.8333], 6-V-1996, SMH, FAF, *SMH2225*; *SMH2237*; San Vito, Las Cruces Biological Station, Rio Jaba trail, 1050 m, [8.7858, -82.9586], 5-V-1996, SMH, FAF, *SMH2192*; San Jose, Bosque los Ninos, 18-V-1996, SMH, FAF, *SMH2499*; *SMH2517*; Canton Guareo, La Esperanza, 2-XI-1999, FAF, *SMH4204*; *SMH4206*; Dist. Acosita, ca. de Palmichal, Rio San Pablo, 1500 m, [9.8397, -84.1761], 17-V-1996, SMH, FAF, *SMH2490*; La Chonta, Km 55 on Interamerican Hwy, 2400 m, [9.6994, -83.9419], 9-V-1996, SMH, FAF, *SMH2298*; Perez Zeledon, Villa Mills, Catie Experimental Forest, 2850 m, [9.55, -83.6833], 15-V-1996, SMH, FAF, *SMH2482*; San Gerardo de Dota, Albergue de Montana, Savegre, 2350 m, [9.55, -83.8], on *Chusquea* bamboo, 8-V-1996, SMH, FAF, *SMH2289*; on *Chusquea* bamboo, *SMH2290*; 10-V-1996, *SMH2318*; on bamboo, *SMH2340*; trail to waterfall along Rio Savegre, 2150 m, [9.5439, -83.8142], 11-V-1996, SMH, FAF, *SMH2375*; *SMH2382*; 13-V-1996, *SMH2439*; Sendero la Quebrada, 2300 m, 12-V-1996, *SMH2396*; *SMH2397*; *SMH2399*; *SMH2412*; *SMH2413*. CUBA, Pinar del Rio, ca. 3 km E of El Cuzco, Sierra del Rosario, Loma El Salon, 450 m, 6-VII-1993, SMH, *SMH590*. ECUADOR, Orellana Prov., Yasuni National Park, Tinamou trail, [-0.6713, -77.4005], 5-III-2001, FAF, ANM, R. Briones, *SMH4343*; Garza trail, *SMH4352*; Laguna trail, 7-III-2001, *SMH4372*; along road near Ceiba tree, 8-III-2001, *SMH4393*; Chorongu trail, 9-III-2001, *SMH4399*; on palm trunk, *SMH4405*; Mirador trail, 10-III-2001, *SMH4420*; Peru trail, *SMH4430*. FRENCH GUIANA, Cayenne Arrondissement, Canton de Approuague-Kaw, Commune de Regina, 34.9 km SE of Roura, immed NW of logging camp on N side of road, Piste au Grotto, 270 m, [4.55, -52.1333], 17-IX-1994, SMH, *SMH1055*; Canton de Matoury, Commune de Matoury, end of Route de la Desiree, W of N2 between Matoury and road to Aeroport de Rochambeau (N4), [4.8333, -52.35], 3-XI-1997, SMH, *SMH3660*; St-Laurent-du-Maroni Arrondissement, Canton de Maripasoula, Commune de Saul, up to 1 km north of Eaux Claires, along Route de Belizon, 200 m, [3.7, -53.2], 30-VIII-1994, *SMH676*; *SMH689*; *SMH691*; *SMH697*; Eaux Claires, 8-IX-1994, *SMH885*; north of Eaux Claires, trail to army camp along Route de Belizon, Crique de l'Est, 30-VIII-1994, SMH699; 6-XI-1997, *SMH3666*; *SMH3669*; *SMH3674*; 3rd stream crossing ca. 1 hr walk N along Route de Belizon, on decaying *Cecropia* petiole, 4-IX-1994, *SMH795*; *SMH799*; *SMH800*; at Crique Tortue ca 1 km E on Sentier Botanique, 12-IX-1994, *SMH971*; *SMH973*; on *Cecropia* petiole, 7-XI-1997, *SMH3693*; *SMH3694*; 5 km NE along the Sentier Botanique, on old palm fruit, 1-IX-1994, *SMH735*; S along Route de Belizon, 11-IX-1994, *SMH964*; SMH966; 14-IX-1994, *SMH1034*; Mont Galbao base, along upper part of La Mana Fleuve, 380 m, [3.6167, -53.2833], 12-XI-1997, *SMH3723*; *SMH3724*; Mont Galbao, source of La Mana Fleuve, 500 m, [3.6, -53.2667],

14-XI-1997, *SMH3738*; *SMH3739*. INDONESIA, Java, Kota Batoe, on rotting wood, 5-I-1897, M. Raciborski (PAD; **holotype**). JAMAICA, Clarendon Parish, Ritchies village, Quaco Rock, 762 m, 9-VI-1999, FAF, *SMH4054*; Trelawny Parish, 1.5 miles beyond the village of Crown Lands, 610 m, [18.2608, -77.6517], 10-VI-1999, FAF, *SMH4057*; Winsor Trail, 115 m, [18.3556, -77.6472], 13-VI-1999, FAF, *SMH4085*. PANAMA, Panama, Barro Colorado Island National Monument, Fausto trail, 50 to 150 m, [9.1667, -79.8333], 15-IX-1997, SMH, FAF, *SMH3395*; *SMH3409*; *SMH3415*; Donato trail, 16-IX-1997, *SMH3425*; *SMH3442*; *SMH3443*; *SMH3447*; *SMH3450*; Donato and Thomas Barbour trails, 17-IX-1997, *SMH3459*; *SMH3461*; *SMH3462*; Thomas Barbour trail, 18-IX-1997, *SMH3481*; on palm petiole, *SMH3488*; *SMH3499*; Snyder-Molino trail, 19-IX-1997, *SMH3508*; *SMH3510*; 24-VIII-1997, *SMH3634*; Barbour-Lathrop trail, 20-IX-1997, *SMH3533*; Wheeler-Schneirla trail, 21-IX-1997, *SMH3541*; *SMH3545*; *SMH3556*; *SMH3557*; *SMH3558*; Shannon trail, 22-IX-1997, *SMH3579*; 23-VIII-1997, *SMH3602*; 24-VIII-1997, *SMH3623*; Armour trail, 22-IX-1997, *SMH3583*. PUERTO RICO, Bosque Estatal de Guajataca, [18.4833, -66.9500], 22-I-1996, *SMH2017*; Luquillo Mts., Bisley Watershed 3, [18.3167, -65], 27-I-1997, FAF, *SMH3140*; *SMH3143*; *SMH3150*; *SMH3153*; 28-I-1997, FAF, SMH3161; El Verde Research Area, 16-ha Grid, 350 to 425 m, [18.3167, -65.8167], 25-IV-1995, SMH, D.J. Lodge, *SMH1150*; 27-IV-1995, *SMH1170*; 6-V-1995, *SMH1375*; 10-V-1995, *SMH1443*; 12-VI-1995, *SMH1460*; 13-VI-1995, *SMH1474*; 26-IX-1995, *SMH1594*; 27-IX-1995, *SMH1597*; *SMH1608*; *SMH1619*; 29-IX-1995, *SMH1634*; 5-X-1995, *SMH1758*; 8-X-1995, *SMH1800*; 9-X-1995, *SMH1817*; *SMH1843*; 10-X-1995, *SMH1852*; 18-I-1996, *SMH1949*; SMH1951; 25-I-1996, *SMH2036*; 26-I-1996, *SMH2078*; *SMH2083*; 30-I-1996, *SMH2116*; *SMH2118*; *SMH2132*; *SMH2144*; *SMH2163*; *SMH2164*; 11-I-1997, SMH, FAF, *SMH2869*; *SMH2887*; 12-I-1997, *SMH2890*; *SMH2895*; *SMH2904*; *SMH2906*; 14-I-1997, *SMH2937*; *SMH2944*; *SMH2946*; 15-I-1997, *SMH2972*; *SMH2974*; *SMH2978*; *SMH2983*; 16-I-1997, *SMH2991*; *SMH2994*; *SMH3008*; *SMH3014*; 18-I-1997, *SMH3029*; *SMH3032*; *SMH3033*; *SMH3035*; *SMH3039*; *SMH3045*; 20-I-1997, *SMH3059*; *SMH3062*; *SMH3067*; 25-I-1997, *SMH3119*; *SMH3137*; 9-VI-1998, FAF, ANM, *SMH3788*; Guzman Abajo, off Camino los Tapias, 23-I-1996, *SMH2025*; near Rio Sabana, NW of junction of Rte 983 & 991, 70 m, [18.35, -65.725], 22-I-1997, SMH, FAF, *SMH3091*. THAILAND, Khao Sok National Park, 19-XI-1996, *SMH2812*; *SMH2828*; *SMH2830*; *SMH2832*; 20-XI-1996, *SMH2837*; *SMH2841*; *SMH2842*; 21-XI-1996, *SMH2854*; *SMH2858*. VENEZUELA, Edo. Aragua, Parque Nacional Rancho Grande, Periquito Peak, 1100 m, [10.3489, -67.6856], 30-VIII-1999, FAF, *SMH4155*; *SMH4157*.

***Chaetosphaeria rubicunda* Huhndorf & F.A. Fernández, sp. nov.**

(Figs. 87-103)

Etymology: Refers to the red outer wall structure.

Ascomata dispersa vel congregata, superficialia, globosa vel obpyriformia, tunica crystallorum rubrorum, 275-390 μm diametro, 385-450 μm alta, papillata, pagina ad apicem setosa. *Paries* superficie e textura globosa compositus, 60-70 μm crassus, pseudoparenchymaticus, e cellulis multangularibus vel elongatis compositus. *Paraphyses* simplices, septatae, hyalinae. *Asci* cylindrici, 180-210 \times 9-15 μm , octospori. *Ascospores* filiformes, 80-100 \times 3.5-4.2 μm , hyalinae, 7-septatae.

Ascomata scattered or clustered, sparse, superficial, obpyriform to ovoid, not collapsing, roughened, with red surface crystals not dissolving in water, 3% KOH or lactophenol, (230-)275-390 μm diam., (340-)385-450 μm high, papillate. *Setae* only surrounding the ostiole, brown, stiff, pointed, short, 40-50

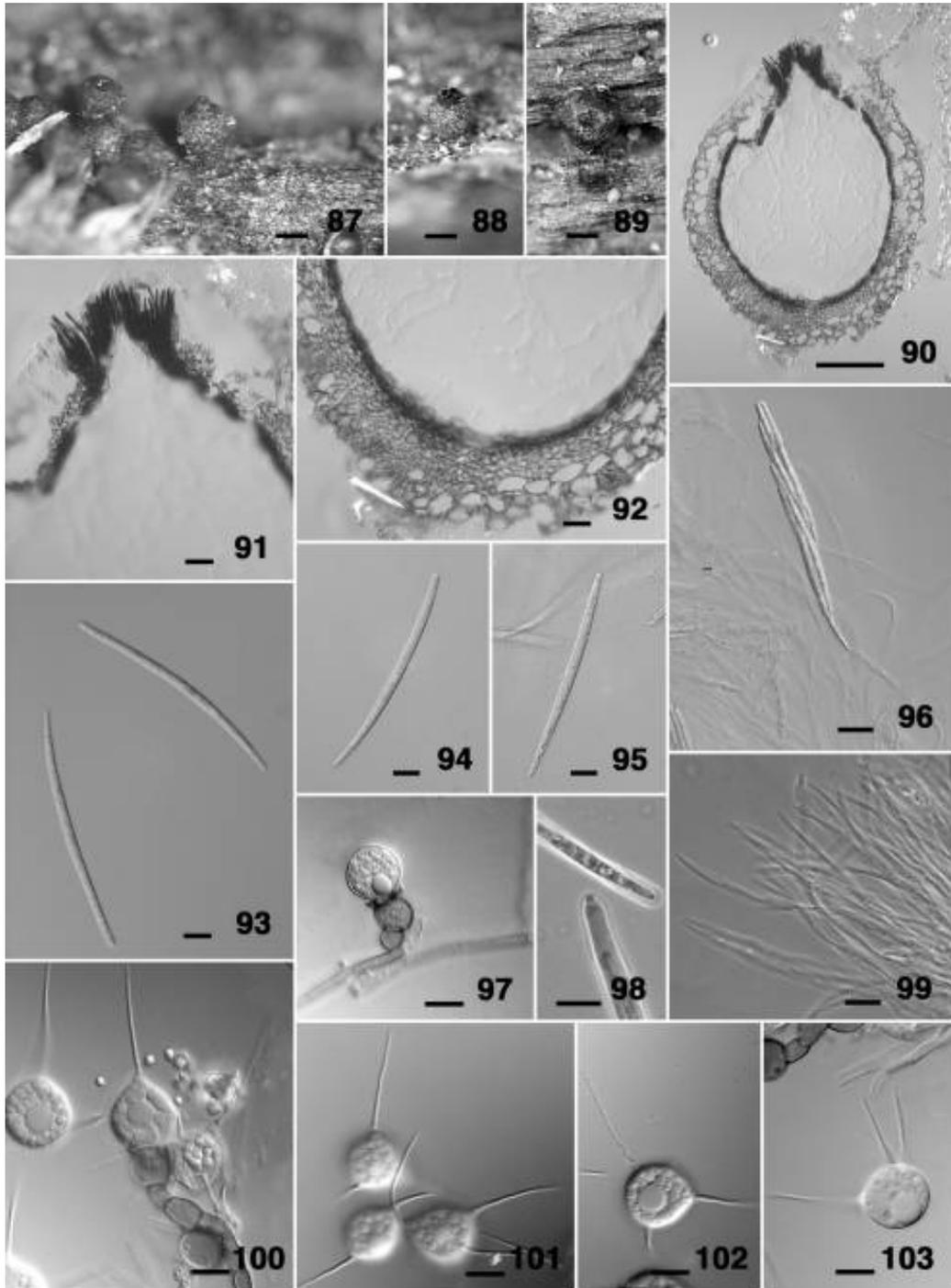
μm , sparse. *Ascomatal wall* of *textura globosa* in surface view; in longitudinal section uniformly 60-70 μm thick; 2-layered, inner wall layer 8-12 μm thick, composed of small, polygonal-to-elongate, pale-to-dark brown, pseudoparenchymatic cells, 4-6 cells thick, setae arising from this wall layer; outer wall layer 50-60 μm thick, composed of variable-sized (largest range from 15-22 \times 9-13 μm), isodiametric-to-polygonal, pale brown, pseudoparenchymatic cells, at times intermixed with smaller, pale brown, pseudoparenchymatic cells, 10-20 cells thick; purple pigment not seen. *Papilla* conical to cylindrical, composed of apical setae 40-50 μm long; 50-60 μm high, *ca.* 50 μm wide at the apex, *ca.* 70 μm wide at the base; circular ostiole 20-25 μm wide, periphyses not seen. *Paraphyses* 1.8-2 μm wide, numerous, septate, tapering toward apex. *Asci* 180-210 \times 9-15 μm , numerous, arising from basal hymenium, cylindrical, rounded apex, with refractive apical ring, short-stalked, unitunicate, with 8, triseriate, ascospores. *Ascospores* 80-100 \times 3.5-4.2 μm , filiform, ends rounded, with apical end slightly wider, basal end slightly narrower, straight to slightly curved, hyaline, smooth, 7-septate, without constrictions, primary septum median, slightly bipolarly asymmetrical, without sheath or appendages. *Culture*: Colonies on WA hyaline, mostly immersed with sparse superficial hyphal growth, 18-21 mm in 21 days, anamorphs present. Colonies on CMA hyaline to light brown, mostly immersed with sparse superficial hyphal growth, 25-27 mm in 21 days, anamorphs present over entire colony. Colonies on MEA light brown to dark-greenish brown, reverse dark brown, abundant immersed and superficial floccose-aggregated hyphal growth, 29-30 mm in 21 days, no anamorphs produced. *Craspedodidymum*-like anamorph consists of simple phialidic conidiogenous cells on a 1-4 celled, brown conidiophore; phialides subglobose, 10-11 μm diam, brown, collarete cup-shaped, 10-11 μm wide, 2-3 μm high. Conidia globose, subglobose to subangular (15-19 μm diam), thick-walled, hyaline, one-celled, 3 setulae or appendages present (20-25 μm long, 1-1.2 μm wide), with flattened areas between setulae; large and small guttules in the mature conidium; more than one conidium produced per phialide.

Anamorph: *Craspedodidymum*-like.

Habitat: On decorticated wood.

Known distribution: Costa Rica, Puerto Rico

Figs. 87-103. *Chaetosphaeria rubicunda*. **87-89.** Ascomata on substrate. **90.** Longitudinal section through ascoma. **91.** Section through ascomal neck. **92.** Section through ascomal wall. **93-95.** Ascospores. **96.** Ascus. **97, 100.** *Craspedodidymum*-like conidiophores on CMA. **98.** Ascus apex. **99.** Paraphyses. **101-103.** Conidia on CMA. Figs. 87-89 by photomacrography; Figs. 90-97, 100-103 by DIC; Figs. 98, 99 by PH. Fig. 87 from SMH3221; Figs. 88-92, 97, 100-103 from SMH2881; Figs. 94-96, 99 from SMH4920; Fig. 93 from SMH4970. Bars: 87-89 = 200 μm ; 90 = 100 μm ; 91, 92, 96, 99 = 20 μm ; 93-95, 97, 98, 100-103 = 10 μm .



Material examined: COSTA RICA, Guanacaste, Liberia ACG, Sector Santa Maria, trail to Bosque Encantado at Estacion Biologica, 750 m, [10.7647, -85.3033], 26-VI-1997, *SMH3245*; Parque Nacional Guanacaste (ACG), Sector Cacao, trail to Estacion Biologica Cacao, 1100 m, [10.9264, -85.4686], 24-VI-1997, *SMH3221* (F; **holotype designated here**); Pasmompa, S of St. Cecilia, 4 km N of La Pitilla, 700 m, [11.0333, -85.4333], 23-VI-1997, *SMH3214*; Prov. Puntarenas, Reserva Biologica Bosque Nuboso Monteverde, Centro Cientifico Tropical, Sendero Rio, 1536 m, [10.3058, -84.7933], 3-XI-2003, SMH, FAF, *SMH4907*; Sendero Roble, 4-XI-2003, SMH, FAF, *SMH4920*; Sendero El Camino, 5-XI-2003, SMH, FAF, *SMH4970*; 6-XI-2003, SMH, FAF, *SMH4990*. PUERTO RICO, El Verde Research Area, Luquillo Mts., 16-ha Grid, 350 to 425 m, [18.3167, -65.8167], 11-I-1997, SMH, FAF, *SMH2881* (F; **paratype designated here**); 14-I-1997, SMH, FAF, *SMH2930*; 20-I-1997, SMH, FAF, *SMH3055*.

Key to species treated

1. Ascomatal setae present only at the apex or absent 2
1. Ascomatal setae present scattered on entire surface 3
2. Red coloration on the outer wall cells, ascomata 385-450 μm , ascospores 75-120 μm long *C. rubicunda*
2. Wall not red, ascomata 400-900 μm , ascospores 50-100 μm long and up to 6 μm wide *C. lapaziana*
3. *Craspedodidymum*-like anamorph with globose conidia, at times with appendages; *Chloridium*-like synanamorph present; aleuriospore-like cells absent 4
3. *Craspedodidymum*-like anamorph with ovoid conidia, no appendages; purple-pigmented aleuriospore-like cells present in culture *C. panamensis*
4. Ascomatal setae up to 120 μm long; ascospores 60-100 μm long; tropical, subtropical distribution *C. raciborskii*
4. Ascomatal setae shorter (20-45 μm); ascospores 50-75 μm long; north temperate distribution *C. ellisii*

Discussion

Relationships within the genus Chaetosphaeria

Phylogenetic analyses of nuclear ribosomal and β -tubulin sequences indicate that *C. raciborskii* belongs to a well-supported monophyletic group which includes the type species of *Chaetosphaeria*, *C. innumera* (Miller and Huhndorf, 2004; Fernández *et al.*, pers. observ.). *Chaetosphaeria raciborskii* forms a highly supported monophyletic group with *Chaetosphaeria conirostris* F.A. Fernández & Huhndorf and *Chaetosphaeria spinosa* F.A. Fernández & Huhndorf, two recently described species (Fernández and Huhndorf, 2005). Morphological similarities among the three species are not ample although they all produce setose ascomata. The setae in *C. spinosa* are stout, opaque, and

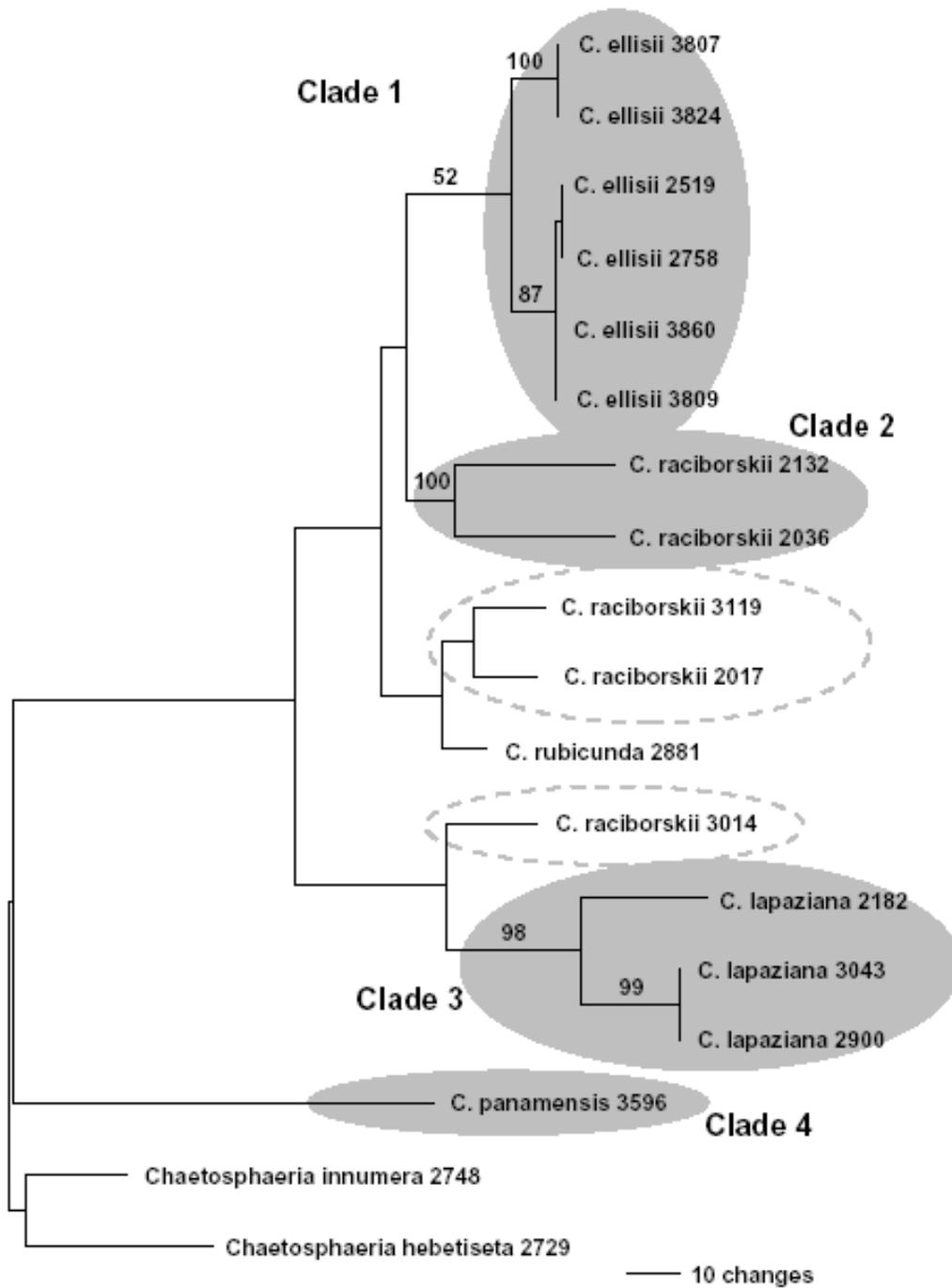


Fig. 104. One of two most parsimonious trees based on 110 parsimony informative characters from an ITS dataset. Numbers above the branches indicate bootstrap support based on 1000 replicates.

apically tapering as in *C. raciborskii* whereas in *C. conirostris* they are capitate with apical yellow-coloured exudate droplets that persist after drying. As in *C. raciborskii* the ascospores of *C. spinosa* are filiform, but they differ in being nonseptate and *C. conirostris* differs with cylindrical-fusiform, inequilateral, one-septate ascospores (Fernandez and Huhndorf, 2005). *Chaetosphaeria spinosa* has a simple phialidic anamorph that resembles the *Chloridium*-like synanamorph of *C. raciborskii* and *C. ellisii*. Neither *C. spinosa* nor *C. conirostris* have ascomal walls that resemble *C. raciborskii*.

Relationships among the scolecosporous species

In this group of five *Chaetosphaeria* species, the ascomal wall structure is the most distinguishing feature and it serves as a major morphological character to separate these species from other *Chaetosphaeria* species. The unique wall composed of an outer layer of large, thin-walled, predominantly globose cells is the repository of pale purple pigments, mainly present when the collections are fresh. When specimens are squash-mounted the pigments appear as if emanating from the centrum, a characteristic noted by Carroll and Munk (1964) for *C. lapaziana* and *C. raciborskii*. The type specimens of *C. ellisii* and *C. raciborskii* were not in good condition but in both collections the unique wall cells were present. All five species share the characteristic wall cells. While the thickness of this layer of cells can vary considerably between the species, the wall layer also shows a similar variation in thickness among collections of the same species. We were not able to use wall thickness as a characteristic to distinguish species. *Chaetosphaeria rubicunda* is distinguished from the others by the bright red coloration of crystals on the external ascomal surface. Although they are not dissolved in water the red crystals are easily dislodged or washed away so if they are not present this species can be confused with the others. The crystals do not appear on the surface of the ascoma in sectioned material.

The unique wall is further distinguished by stout, pointed setae that originate from the inner wall layer, growing through the outer layer of globose cells. In *C. rubicunda* and in some specimens of *C. lapaziana* the setae are concentrated only at the ascomal apex, either surrounding the ostiole forming the apex, or surrounding the apical wall cells. Some specimens of *C. lapaziana* may have no setae at all. In *C. ellisii*, *C. raciborskii* and *C. panamensis* the setae tend to be scattered over the entire surface of the ascomata, however some specimens of *C. ellisii* many have setae concentrated only at the apex. In *C. ellisii* the setae tend to be at the shorter end of the range (20-45 μm) whereas *C. raciborskii* can have setae up to 120 μm .

The size of the ascomata is one character used to distinguish *C. lapaziana* from the other species. Among the five species there is some overlap in the size of ascomata, with a tendency toward larger ascomata occurring in *C. lapaziana* (400-950 μm diam.) and some of the smallest ascomata found in *C. raciborskii* (150 μm diam. at lower end of range) and *C. panamensis* (185 μm diam. at lower end of range).

Among the five species there is considerable overlap in ascospore sizes, with a tendency toward shorter spores occurring in *C. ellisii* (50-75 μm long) and a tendency toward longer spores in *C. rubicunda* (75-120 μm long). In published descriptions of *C. raciborskii* and *C. lapaziana* spore lengths do not exceed 70 μm , yet among our collections of these species spores routinely are greater in length, reaching up to 150 μm in one collection of *C. raciborskii*. *Chaetosphaeria lapaziana* tends to have the widest spores reaching up to 6 μm in the Costa Rican type specimen. Ascospore septation is uniform for the five species. Among all of our collections, no ascospores greater than 7-septate were seen. In the original publication of *C. raciborskii* the ascospores are described as being 13-17 septate. This feature could not be verified however, as no asci or ascospores were seen in the type specimen.

The anamorphs of *C. ellisii*, *C. raciborskii*, and *C. rubicunda* form inflated phialides with shallow to deep collarettes, as wide or wider than the venter. The phialides are brown, pale brown or in one collection (SMH2017) dark purplish brown. Successive hyaline conidia are produced endogenously. In *C. rubicunda* and one collection of *C. raciborskii* (SMH3119) the conidia form distinctive long, slender setulae or appendages (Figs. 86, 100-103). When appendages are present the shape of the conidia can become almost triangular (Fig. 86); without appendages the conidia are globose (Figs. 79, 81). The anamorph of *C. panamensis* differs from the others in having hyaline-to-pale brown, ampulliform conidiogenous cells with shallow collarettes and hyaline, ovoid conidia (Figs. 59, 64). It is further distinguished by forming large thin-walled aleuriospore-like cells filled with purple pigment (Figs. 57, 58, 61). The anamorph of *C. lapaziana* differs in having clavate conidiogenous cells with shallow collarettes and hyaline, oblate to horizontally oblong conidia with a short abscission scar or frill located medially on one long side (Figs. 40-42). Several collections of *C. ellisii* and *C. raciborskii* additionally formed a *Chloridium*-like synanamorph with simple brown conidiophores, a terminal phialide with inconspicuous collarette and hyaline, ovoid conidia (Figs. 23, 82). The synanamorph and conidial appendages were also noted by Samuels (pers. observ.) from several New Zealand collections of *C. raciborskii*.

Morphologically the anamorphs of all five *Chaetosphaeria* species most closely resemble *Craspedodidymum* Hol.-Jech. In *Craspedodidymum*, ellipsoid

or angular brown conidia are produced from conidiogenous cells with funnel-shaped collarettes atop dichotomously branched or erect, elongate conidiophores (Holubová-Jechová, 1972; Rao and de Hoog, 1986; Bhat and Kendrick, 1993; Yanna *et al.*, 2000). Multiple collarettes may be formed by percurrent conidial production at one level in the conidiogenous cell as in *Craspedodidymum elatum* Hol.-Jech. or at multiple levels as in *C. proliferans* V. Rao & de Hoog. The anamorphs of all five species have distinctive collarettes on the conidiogenous cells resembling the flared collarettes on the conidiogenous cells in *Craspedodidymum cubense* J. Mena & Mercado and *C. proliferans*. In *C. ellisii* phialides occasionally proliferate percurrently (Fig. 20) as seen also in *Craspedodidymum abigianense* Lunghini & Onofri (1980). The very conspicuous collarettes of *C. raciborskii* are the most extreme in the group, resembling those found in *Conioscypha* Höhn. *Conioscypha* also has multilayered collarettes formed by repeated conidium production. In this genus, the conidia are pigmented and conidiophores are lacking. The resemblance in the conidiogenous cells of *C. raciborskii* mistakenly led us to believe the anamorph was *Conioscypha*. Recent work has shown *Conioscypha* to be related to *Ascotaiwania* Sivan. & H.S. Chang and *Carpoligna* F.A. Fern. & Huhndorf and unrelated to *Chaetosphaeria* species (Réblová and Seifert, 2004).

Elongate conidiophores are seen in the anamorphs of *C. lapaziana* and *C. panamensis* and occasionally in *C. ellisii*, *C. raciborskii* and *C. rubicunda*. More often in these latter three species, the phialides form directly on the hyphae without forming distinct conidiophores, again resembling *Conioscypha*. The conidia of *C. panamensis* are ellipsoid but remain hyaline and those of *C. lapaziana* differs in having a horizontal orientation on the conidiogenous cell. The conidia of *C. ellisii*, *C. raciborskii* and *C. rubicunda* are globose and in *C. rubicunda* and some collections of *C. raciborskii* also differ in having elongate hyaline appendages (Figs. 86, 101-103). The appendaged conidia resemble those found in *Bahusutrabeija angularis* V.G. Rao & de Hoog and *B. globosa* Bhat & Kendrick. That genus is also characterised by having elongate conidiophores with or without conspicuous collarettes on the conidiogenous cells collarettes (Rao and de Hoog, 1986; Tsui *et al.*, 2001).

In three of the isolates of *C. ellisii* (SMH3807, SMH3824, SMH3860) no anamorph formed in culture. The other three isolates of *C. ellisii* (SMH2758, SMH2519, SMH3809) and two isolates of *C. raciborskii* (SMH2036, SMH2132) formed both *Craspedodidymum*-like and *Chloridium*-like synanamorphs. Two isolates of *C. raciborskii* (SMH2017, SMH3119) and *C. rubicunda* formed only *Craspedodidymum*-like anamorphs in culture. *Chaetosphaeria panamensis* and *C. lapaziana* also formed a *Craspedodidymum*-like anamorph, differing from the other ones in the

morphology of the conidium and conidiogenous cell. The failure to produce a particular anamorph in culture should be interpreted with caution. Even isolates from the same specimen vary in their ability to produce anamorphs in culture.

Some of these *Chaetosphaeria* species probably have widespread distribution patterns. *Chaetosphaeria raciborskii* is abundant and was frequently encountered in our tropical collecting sites and also commonly found in New Zealand (T. Atkinson, pers. comm.; Samuels, pers. comm.). *Chaetosphaeria lapaziana* was also somewhat common and could possibly have a wider distribution than is currently known. *Chaetosphaeria ellisii* is currently known from temperate, North American collections only. It is possible that European collections might fall within this species, however, no European specimens were examined for this project. *Chaetosphaeria rubicunda* is currently known only from tropical Costa Rican and Puerto Rican collections. A species like *C. panamensis* is either rare or more probably cryptic since culture information is necessary to identify its unique anamorphic morphology. Further collecting is needed to discern possible distribution patterns.

Despite the abundance in nature of *C. ellisii*, *C. raciborskii* and *C. lapaziana*, we have not uncovered a lengthy nomenclatural history that would indicate they have been frequently encountered. However an exhaustive search has not been made and likely epithets and collections might be buried under other scolecosporous genera. Two genera were checked as possible synonyms for *C. raciborskii* were *Acerbiella* Sacc. and *Leptospora* Penz. & Sacc. *Acerbiella macrospora* (Rick) Sacc. & D. Sacc., the type of that genus, is a synonym of *Tubeufia clintonii* (Peck) Barr (lectotype designated here: Fungi Rickiani #12012, S. Leopoldo, leg. Rick (PACA!), consisting of two pieces of decorticated wood with abundant setose perithecia). An additional collection filed as *A. macrospora* at PACA is not this species but a species of *Rosellinia*. *Leptospora* also is not related to *C. raciborskii* but instead is a distinct unitunicate genus with scolecosporous ascospores. It is being redescribed elsewhere (Huhndorf *et al.*, unpublished).

Although analyses of the ITS nrDNA sequence data have provided some insight into the phylogenetic relationships in this complex group, analyses of additional collections would be needed to further resolve their placement.

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