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Teleomorph-anamorph connections: the new pyrenomycetous genus *Carpoligna* and its *Pleurothecium* anamorph

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Abstract: Several collections of a pyrenomycete identified as *Chaetosphaeria* were made from decorticated wood of twigs and branches in Costa Rica, Panama, Puerto Rico and continental USA. Discrete and continuous characters of the ascomata, asci and ascospores in these collections show that they are very similar morphologically and represent populations of the same species. However, culturing of single ascospore isolates from these collections yielded an anamorph unlike the typical phialidic anamorphs of *Chaetosphaeria*. This anamorph fits the description of *Pleurothecium recurvatum*, for which a teleomorph connection has not been yet established. Sequences of the ITS region of the nuclear ribosomal DNA of three collections from Costa Rica, Puerto Rico and USA were compared and found to be 98% similar. Parsimony and maximum likelihood analyses of sequences of the large subunit nuclear-encoded ribosomal DNA from representative taxa of eight ascomycetous orders show this ascomycete as a monophyletic group distinct from *Chaetosphaeria*. Statistical analyses of hypothetical trees based on the most parsimonious and the most likely trees rule out phylogenetic affinities of this pyrenomycete to *Chaetosphaeria*. These analyses suggest possible affinities to the Hypocreales and/or the Microascales. Based on morphology of the anamorph and analyses of ribosomal DNA sequence data, *Carpoligna pleurothecii* gen. et sp. nov. are described. The implications of the phylogenetic relationships of *Carpoligna* in the practical identification of morphologically similar pyrenomycetes are discussed.

Key Words: *Cacumisporium*, *Chaetosphaeria*, ITS, large subunit nrDNA, Hypocreales, Lasiosphaeriaceae, Magnaporthaceae, Microascales, Sordariales, systematics

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INTRODUCTION

The genus *Chaetosphaeria* Tul. comprises a poorly known group of pyrenomycetous ascomycetes, mostly found on decorticated wood on soil. The genus was first described by Tulasne and Tulasne (1863) to circumscribe pyrenomycetes with glabrous ascomata, sometimes surrounded by conidiophores, hyaline ascospores and a conidial state in *Chloridium* Link ex Fr. Saccardo (1883) altered the concept to include pyrenomycetes with setose ascomata and partly colored ascospores. Müller and von Arx (1962) elaborated on the definition of the genus to circumscribe taxa with dark, glabrous, ascomata with two wall layers, thin-walled asci with a simple apical plate, and two or more-celled, hyaline ascospores. A more detailed account on changes in the taxonomic concept of *Chaetosphaeria* is provided by Gams and Holubová-Jechová (1976). Identification of species of *Chaetosphaeria* is particularly challenging because the morphology of the teleomorph is strikingly homogeneous but the morphology of the anamorphs is very diverse. Hence, particular emphasis has been placed on characters of the anamorphs in order to distinguish species in the genus (Gams and Holubová-Jechová 1976, Hughes and Kendrick 1968).

Several collections of a pyrenomycete were made from decorticated wood of twigs and branches in Costa Rica, Panama, Puerto Rico and continental USA. This pyrenomycete fits the few simple morphological characters of the teleomorph of a typical *Chaetosphaeria*. However, in vitro studies yielded an anamorph different from those previously connected to this genus. This anamorph matches the description of *Pleurothecium recurvatum* (Morgan) von Höhnel. An account by Goos (1969b) points out that the genus was erected by F. von Höhnel in 1919, based on *Acrothecium recurvatum* Morgan, and later described by him in 1923. Although *Pleurothecium* has been commonly found on decaying wood in temperate areas (Goos 1969b), it has not been connected to a teleomorph, either by in vitro studies or by association on the substrate.

The goals of this study are to report the connection of a *Chaetosphaeria*-like pyrenomycete to the *Pleurothecium* anamorph and to determine the phylogenetic relationships of this pyrenomycete to *Chae-*

tosphaeria and other representative taxa in various orders of ascomycetes using DNA sequence data.

MATERIALS AND METHODS

In vitro studies and imaging.—Ascomata contents from specimens SMH 2097 from Puerto Rico; SMH 2352, 2492, and 3248 from Costa Rica; SMH 2722 from USA; SMH 3411 and 3599 from Panama were extracted and spread onto water agar plates. After 24 h, germinating single and multiple ascospores were cut out from the medium and transferred to water agar, cornmeal agar and malt-extract agar in 6-cm-diam petri plates, and incubated for 30 d at room temp. Colonies were frequently observed with a dissecting microscope for rate of growth and presence of reproductive structures, particularly anamorphs. Microscopic observations of morphological structures were also made from squash mounts of ascomata. Ascomata were sectioned at 5 μm for light microscopy using the techniques of Huhndorf (1991) and images were captured using bright field (BF), phase contrast (PH) and differential interference microscopy (DIC).

Images of microscopic morphological structures were captured using a Power Computing Powertower Pro 225 Mac/OS system with a Scion LG-3 Framegrabber from a Dage DC-330 video system on a Olympus BH-2 and Wild M5A microscopes. Measurements of asci, ascospores, conidiophores and conidia were made on images captured live with NIH digital image processing software system Scion 1.59 (National Institute of Health, Bethesda, Maryland). Images were processed by using Adobe Photoshop 3.0 (Adobe Systems Incorporated, Mountain View, California).

DNA extraction, PCR amplification, sequencing and sequence alignment.—Isolates were grown in 1.5 mL microcentrifuge tubes containing 1.0 mL of potato dextrose broth (Difco). The broth was decanted and the mycelium was washed and centrifuged twice in deionized sterile water. Total genomic DNA was extracted following the method of Graham et al (1994), except that tissue was not ground in liquid nitrogen but instead was ground directly in the extraction buffer.

The ITS region of the nrDNA was amplified by using reagents in a Repli-pack Reagent set (Boehringer Mannheim Corporation, Indianapolis, Indiana) in the following manner: 1.5 μL of 25 mM $MgCl_2$, 2.5 μL of 10× reaction buffer (100 mM Tris, 500 mM KCl, pH 8.3), 0.5 μL of 8 mM d-NTPs, 1.25 μL each of 20 μM primers ITS1 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. The same protocol was used for amplification of the large subunit nrDNA, except that amounts of reagents were doubled and primers LR0R and LR7 (Moncalvo et al 1993) were used instead, for a total reaction volume of 50 μL.

PCR was performed by using the following thermocycling parameters: initial denaturation temp 95 C for 2 min, followed by 35 cycles of denaturation at 95 C for 1 min, annealing at 50 C for 1 min and extension at 72 C for 1 min. A final extension step of 10 min 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 ITS1 and ITS4 for the ITS region (White et al 1990), and LROR, LR3, LR3R, LR5, LR6 and LR21 for the large subunit nrDNA (Rehner and Samuels 1995, Vilgalys and Hester 1990, Vilgalys and Sun 1994). Sequencing reactions were performed by using the ABI Prism Dye Terminator Cycle Sequencing kit (Perkin-Elmer Corporation). Sequenced products were cleaned using centrisep columns (Princeton Separations, Adelphia, New Jersey), and were run through a polyacrylamide gel using an ABI Prism 377 DNA Sequencer (Applied Biosystems). A total of 22 sequences from the large subunit nrDNA were used: 7 were generated in this study and 15 were obtained from GenBank (TABLE I). Sequences were assembled and aligned by using Sequencher version 3.0 (Gene Codes Corporation). Alignment was checked by eye and corrected manually when necessary.

Phylogenetic analyses.—All phylogenetic analyses were performed using PAUP* 4.0.0d61 compiled for the PPC platform (Swofford 1998). A first maximum parsimony analysis (MP1) was executed as follows: All 16 ambiguous regions of the alignment (deposited in TreeBASE) were excluded, the remaining sites were all unordered and unweighted, gaps were treated as missing, 1000 random-addition sequence replicates were implemented, the branch-swapping algorithm was TBR, the MULPARS option was in effect, and zero-length branches were not collapsed.

The second maximum parsimony analysis (MP2) was identical to MP1 except that gaps were used as a fifth character state, the 16 ambiguously aligned regions were excluded but re-included as 16 unequivocally coded characters. Each of these 16 coded characters was subjected to a specific step matrix derived from pairwise comparisons of sequences (Lutzoni, Wagner, Reeb unpubl). The remaining sites were subjected to a symmetric step matrix (A(-)G=1, C(-)T=1, A(-)C=2, A(-)T=2, C(-)G=2, G(-)T=2, gap(-)A=3, gap(-)C=3, gap(-)G=3, gap(-)T=3). The step matrix was obtained by applying the dinucleotide frequency option in PAUP* on included nucleotide sites only. These frequencies were first converted to percentages of reciprocal changes and then converted to costs of changes using the negative natural logarithm of the frequencies (Felsenstein 1981, Wheeler 1990, Maddison and Maddison 1992). The costs were rounded to integers and then used in the symmetric step matrix. The costs for changes involving a gap were calculated by taking the highest cost for changes among nucleotide (2) and adding 1 to it. The triangle inequality was tested for all step matrices using MacClade 3.05 (Maddison and Maddison 1992).

The Hasegawa-Kishino-Yano (1985) model was used for all maximum likelihood analyses. The base frequencies, the transition/transversion ratio, and the proportion of invariable sites for the maximum likelihood analysis (ML) were estimated using the describe tree option on one of the 21 most parsimonious trees generated by MP1. These parameters were set using the likelihood options in the Analysis

TABLE I. List of taxa used in the molecular analyses. All sequences are for the large subunit nrDNA unless indicated

Taxa	Culture designation	Sequence source	GenBank accession no.
Carpoligna pleurothecii	SMH 2097	Fernandez & Huhndorf	AF064778[a] AF064645
Carpoligna pleurothecii	SMH 2492	Fernandez & Huhndorf	AF064779[a] AF064646
Carpoligna pleurothecii	SMH 2722	Fernandez & Huhndorf	AF064780[a]
Petriella setifera	ATCC 26490	Spatafora	U48421
Microascus trigonosporus	RSA 1942	Spatafora	U47835
Balansia aristidae	J. F. White	Glenn & Bacon	U57677
Neocosmospora diparietospora	ATCC 13214	Rehner & Samuels	U17413
Nectria vilior	ATCC 16217	Glenn & Bacon	U57348
Hypomyces tremellicola	GJS 90-36	Rehner & Samuels	U17427
Hypocrea schweinitzii	CTR 79-225	Spatafora	U47833
Diaporthe phaseolorum	FAU 458	Spatafora	U47830
Chaetosphaeria vermicularioides	SMH 1985	Fernandez & Huhndorf	AF064644
Chaetosphaeria ovoidea	SMH 2605	Fernandez & Huhndorf	AF064641
Chaetomium globosum	ATCC 44699	Spatafora	U47825
Lasiosphaeria ovina	SMH 1538	Fernandez & Huhndorf	AF064643
Cercophora newfieldiana	SMH 2622	Fernandez & Huhndorf	AF064642
Daldina concentrica	ATCC 36659	Spatafora	U47828
Xylaria hypoxylon	ATCC 42768	Spatafora	U47841
Eurotium amstelodami	NRRL 89	Peterson	U29549
Emericella nidulans	NRRL 187	Peterson	U29841
Pleospora herbarum	ATCC 11681	Dong et al	U43476
Leptosphaeria doliolum	ATCC 32813	Dong et al	U43473
Saccharomyces cerevisiae	clone pyl ra3	Bayev et al	J01355

ATCC, American Type Culture Collection, Rockville, Md.; GJS, G. J. Samuels, Systematic Botany and Mycology Laboratory, USDA, Beltsville, Md.; SMH, S. M. Huhndorf, The Field Museum, Chicago, IL.; NRRL, National Center for Agricultural Utilization Research, Peoria, IL; CTR, Clark T. Rogerson, New York Botanical Garden, N.Y.; FAU, F. A. Uecker, Systematic Botany and Mycology Laboratory, USDA, Beltsville, Md.; RSA, Rancho Santa Ana culture collection, Claremont, CA.

[a] ITS sequences.

menu of PAUP*. The likelihood of this topology was calculated for 1 to 4 rate categories using PAUP* describe tree option. The four likelihood values were plotted against the number of rate categories. The point of inflection was used to determine the optimal number of rate categories and estimated shape parameter of the gamma distribution. These two values were set using the likelihood options. The heuristic search consisted of 100 random addition sequences, saving optimal trees from each replicate, even if they were not optimal overall, TBR branch-swapping algorithm, and MULPARS option in effect.

The support for the internodes of the most-parsimonious trees was estimated by 1000 bootstrap replicates (Felsenstein 1985) with a heuristic search with one random-addition sequence for each bootstrap replicate. The collapse zero-length branches option was in effect, and all nonparsimony-informative characters were excluded. The support for the internodes of the maximum likelihood tree was estimated with 570 bootstrap replicates using the same parameter settings as for the ML analysis, but removing the nonvariable sites.

All best topologies from MP1, MP2 and ML analyses, as well as hypothetical phylogenetic relationships of *Carpoligna* to other pyrenomycetous orders were compared using the Kishino and Hasegawa test (Kishino and Hasegawa 1989) and the Wilcoxon signed ranks test (Templeton 1983) as implemented in PAUP* 4.0.0d61. In order to recreate hypothetical trees, *Carpoligna* was repositioned in both the most parsimonious MP2 tree and on the most likely ML tree by using MacClade 3.05 (Maddison and Maddison 1992).

RESULTS

Morphological data.—Conidiophores and conidia produced on water agar and cornmeal agar plates were observed and identified as those of *Pleurothecium recurvatum*. No teleomorph has been previously connected to *Pleurothecium* and a new genus is described to accommodate this pyrenomycete.

Carpoligna F. A. Fernández et S. M. Huhndorf, gen. nov.

Ascomata dispersa vel congregata, superficialia, globosa

vel obpyriformia, papillata vel breviter rostrata, ostiolata; pagina aspera vel hirsuta; subiculum tomentosum copiosum. Paries unistratus, pseudoparenchymaticus, e cellulis multangularibus vel elongatis compositus. Paraphyses sparsae, simplicium, fatiscentium. Asci cylindracei vel clavati, brevistipitati, unitunicati, parietibus tenuibus, cum vel sine annulo, octospori. Ascosporae fusiformes, septatae.

Ascomata globose to obpyriform, scattered to gregarious, superficial to partly-immersed in the substratum, papillate, sometimes with a short beak. *Ascomatal wall* 1-layered, composed of polygonal to elongate pseudoparenchymatic cells. *Paraphyses* sparse, simple, not interspersed among asci, disintegrating. *Asci* cylindrical-clavate, unitunicate, thin-walled, with or without an apical ring, with 8 ascospores. *Ascospores* fusiform, septate.

Etymology. Carpo from the Greek meaning fruit and *ligna,* a modification of the Latin *lignum* meaning wood.

Type species. Carpoligna pleurothecii.

Carpoligna pleurothecii F. A. Fernández et S. M. Huhndorf, sp. nov. FIGS. 1–23

Ascomata dispersa vel congregata, superficialia, globosa vel obpyriformia, 198–238 μm diametro, 247–257 μm alta, papillata vel breviter rostrata, ostiolata; pagina ad apicem setosa. Paries superficie e textura angulari compositus, 12–22 μm crassus, unistratus, pseudoparenchymaticus, e cellulis multangularibus vel elongatis compositus. Paraphyses sparsae, simplicium, fatiscentium. Asci cylindracei vel clavati, 75–108 × 10–14 μm, brevistipitati, unitunicati, parietibus tenuibus, ad apicem rotundatis, cum annulo, octospori, ascosporae ad apicem biseriatae ad basim uniseriatae. Ascosporae fusiformes, 17–25 × 4–6 μm, hyalinae, triseptatae.

Ascomata globose to subglobose, dark brown, 198–238 μm in diameter, 247–257 μm in height, mostly separate, scattered to gregarious, superficial to partly-immersed in the substratum, papillate, surface setose, especially at the apex; setae sparse to numerous, straight or slightly curved, thick-walled, multiseptate, with acute apices. *Ascomatal wall* of textura angularis in surface view, composed of polygonal to elongate pseudoparenchymatic cells, 12–22 μm thick. *Ascomatal apex* papillate, short, bluntly conical. *Paraphyses* sparse, simple, not interspersed among asci, disintegrating. *Asci* cylindro-clavate, sometimes short-stalked, 75–108 × 10–14 μm, unitunicate, thin-walled, rounded apex, a distinctive apical ring sometimes visible, with 8 ascospores irregularly biseriate above and uniseriate below. *Ascospores* fusiform, with rounded end cells, 17–25 × 4–6 μm, hyaline, three-septate, a large central, refractive guttule sometimes present in each cell.

Colonies on malt-extract agar fast growing, mostly immersed, brown-greenish on reverse, superficial hyphal growth restricted, white in color, no conidiophores or conidia produced. Colonies on water agar fast growing, hyaline to light brown, mostly immersed. *Conidiophores* single, dark brown, discrete, multiseptate, 62–170 × 3.5–5 μm, with two or more rhizoids at the base. *Conidia* produced holoblastically in succession on repeated sympodial proliferations of the conidiogenous cell, distinctive teeth with blunted ends remaining after conidia detachment; hyaline, cylindrical-fusiform, 17–26 × 5.5–8 μm, sometimes inequilateral, three-septate, sometimes constricted, apical end rounded, with a basal hilum and accumulating in droplets on conidiophores.

TYPE. UNITED STATES. ILLINOIS: Ogle Co., Lorado Taft Field Camp, 09/29/1996, on wood pieces, *S.M. Huhndorf 2722 & F. Fernández* (holotype, F).

Anamorph. Pleurothecium recurvatum (Morgan) von Höhnel.

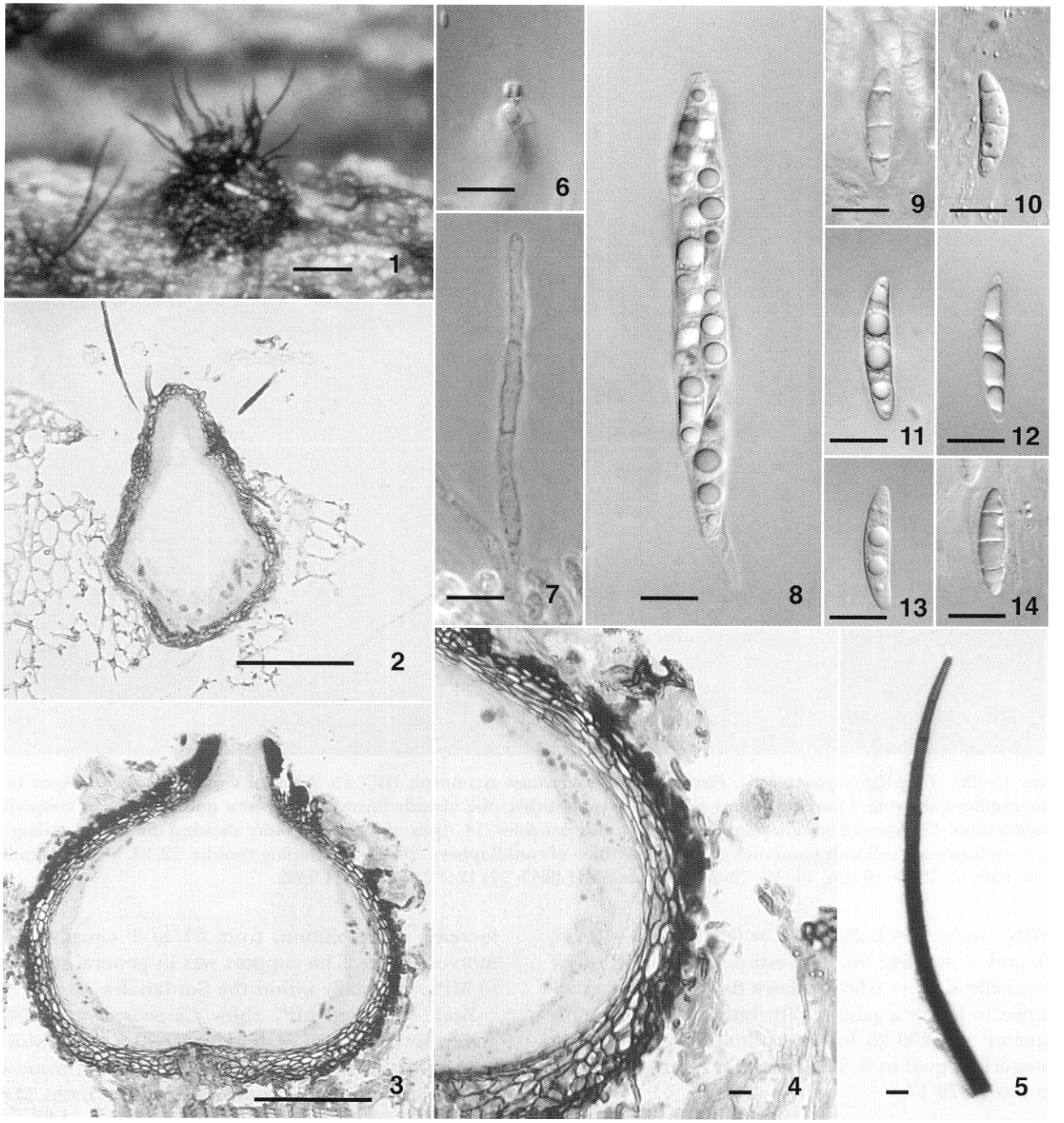
Etymology. pleurothecii refers to the anamorph.

Habitat. Decorticated wood of twigs and branches.

Known distribution. Costa Rica, Panama, Puerto Rico, and continental United States (Illinois). There are collections of *Pleurothecium* reported from Canada, the United States and England (Goos, 1969b).

Additional specimens examined. COSTA RICA. SAN JOSE: San Gerardo de Dota, Albergue de Montagne, Savegre, trail to waterfall along Río Savegre, 9°32′38″ N, 83°48′51″ W, 2150 m, 05/11/1996, on a 12.5-cm-diam log, *S. M. Huhndorf 2352 & F. Fernández*; Sendero la Catarata, 9°32′38″N, 83°48′51″W, 2150 m, 05/13/1996, on a 15-cm-diam log, *S. M. Huhndorf 2431 & F. Fernández*; SAN JOSE: Dist. Acosita, circa de Palmichal, Río San Pablo, 9°50′23″N, 84°10′34″W, 1500 m, 05/17/1996, on a 5-cm-diam branch, *S. M. Huhndorf 2492 & F. Fernández*; GUANACASTE: Liberia, Sector Santa María, Estación Biológica, trail to el Bosque Encantado, 10°45′53″N, 85°18′12″W, 750 m, 6/26/1997, on wood fragment, *S. M. Huhndorf 3248.* PANAMA. Panama canal, Barro Colorado Island National Monument, Fausto trail, 9°10′N, 79;°50′W, 50–150 m, 9/15/1997, on a 7.5-cm-diam branch, *S. M. Huhndorf 3411 & F. Fernández*; Shannon trail, 9/22/1997, on a 2-cm-diam branch, *S. M. Huhndorf 3581 & F. Fernández*; Shannon trail, 9/23/1997, on wood of *Cecropia, S. M. Huhndorf 3595 & F. Fernández*; Shannon trail, 9/23/1997, on a 10-cm-diam branch, *S.M. Huhndorf 3599 & F. Fernández.* UNITED STATES. PUERTO RICO: Luquillo Mts., El Verde Research Area, 16-ha Grid, 14.03, 18°19′27″N, 65°48′54″W, 01/29/1996, on a 30-cm-diam log, *S. M. Huhndorf 2097.*

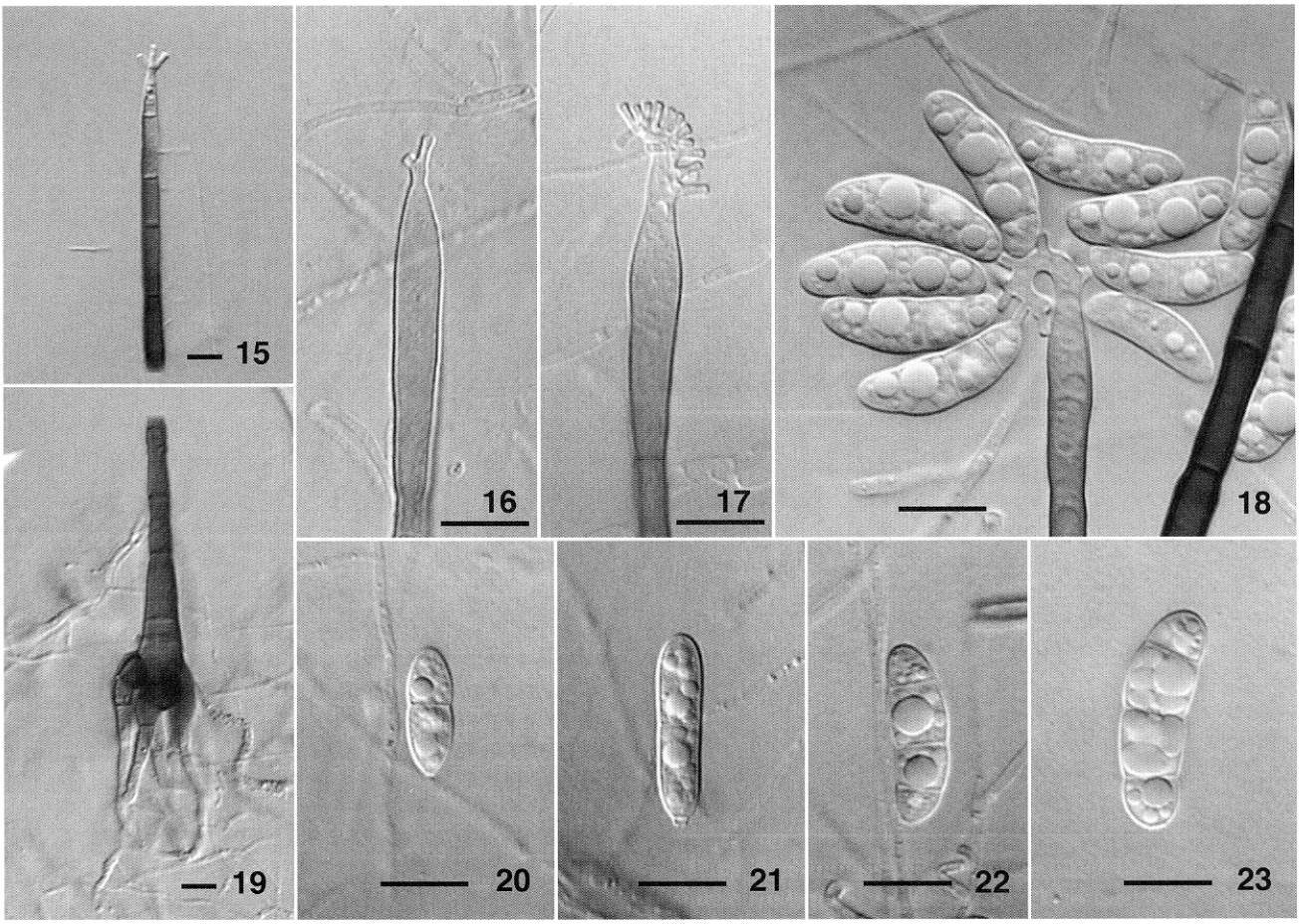
Molecular data.—ITS sequences are 541 bp. The average similarity is 98% among these sequences obtained from three collections of *Carpoligna.* Pair-wise comparisons among the three sequences show six differences (4 indels and 2 transitions) between isolates SMH 2097 (Puerto Rico) and SMH 2492 (Costa Rica); six differences (5 indels and 1 transition) be-



FIGS. 1–14. *Carpoligna pleurothecii*. 1. Ascoma on substrate. 2, 3. Longitudinal sections through ascomata BF. 4. Longitudinal section through ascomal wall BF. 5. Ascomal seta BF. 6. Apical ring of ascus DIC. 7. Paraphysis PH. 8. Ascus DIC. 9–14 Ascospores DIC. Scale bars: 1–3 = 100 μm; 4–14 = 10 μm. 1, 2, 9, 14 from SMH 2352; 3–8, 11, 13 from HOLOTYPE SMH 2722; 10 from SMH 2097; 12 from SMH 2492.

tween isolates SMH 2097 and SMH 2722 (USA) and five differences (4 indels and 1 transition) between isolates SMH 2492 and SMH 2722.

The size of the final data matrix for the large subunit nrDNA was 22 sequences by 898 sites. A total of 16 ambiguously aligned regions were delimited resulting in the exclusion of 157 sites from MP1, MP2 and ML analyses. Of the remaining 741 characters, 506 were constant and 64 variable characters were parsimony-uninformative for a total of 171 putative parsimony-informative characters. Base frequencies for the 741 included sites from the large subunit



FIGS. 15–23. *Carpoligna pleurothecii, Pleurothecium recurvatum* anamorph DIC. 15. Apex of conidiophore. 16. Apex of a conidiophore showing a conidiogenous cell with two denticles: one already formed and a new one forming by sympodial proliferation. 17. Apex of conidiophore with numerous denticles. 18. Apex of a conidiophore showing the typical coiling of the conidiogenous cell with numerous denticles. 19. Base of conidiophore. 20, 21. Developing conidia. 22, 23. Mature conidia. Scale bars: 15–23 = 10 μm. 15, 16, 19–21, 23 from SMH 2097; 17, 18, 22 from SMH 2492.

nrDNA were: A = 0.258 82, C = 0.239 02, G = 0.259 20, and T = 0.242 96. The estimated proportion of invariable sites = 0.5635. Rates for variable sites assumed to follow a gamma distribution with shape parameter = 1.260 25 for an optimal number of rate categories equal to 3. The transition/transversion ratio was 1.870 17.

Maximum parsimony analysis 1 (MP1).—The equally weighted parsimony analysis generated 21 equally most-parsimonious trees found in two islands of trees. One island of 9 trees was hit 834 times and the second island of 12 trees was hit 166 times. Most topological differences were found within the Hypocreales and Sordariales (FIG. 24A).

Maximum parsimony analysis 2 (MP2).—The unequally weighted parsimony analysis revealed one most-parsimonious tree (FIG. 24B). The inclusion of ambiguously aligned regions as coded characters and the use of step matrices on all characters explain the increase in resolution, from 21 to 1 equally parsimonious trees. The support was in general superior to MP1, especially within the Sordariales.

Both MP1 and MP2 show *Carpoligna* as a sister group to the Microascales-Hypocreales clade with a bootstrap value of 76% in MP2. However, some of the equally most parsimonious trees from MP1 showed *Carpoligna* as sister group to the Microascales, or to the Xylariales-Diaporthales-Sordariales-Microascales-Hypocreales clade (not shown). Except for the rooting differences between MP1 and MP2, Pleosporales versus Eurotiales, respectively, all discrepancies between the two maximum parsimony analyses occurred in weakly supported regions of the trees (FIGS. 24A, B).

Maximum likelihood analysis (ML).—All 100 random-addition sequences using maximum likelihood as the optimization criterion converged on one most likely tree (-ln likelihood = 3696.46478, FIG. 24C). The

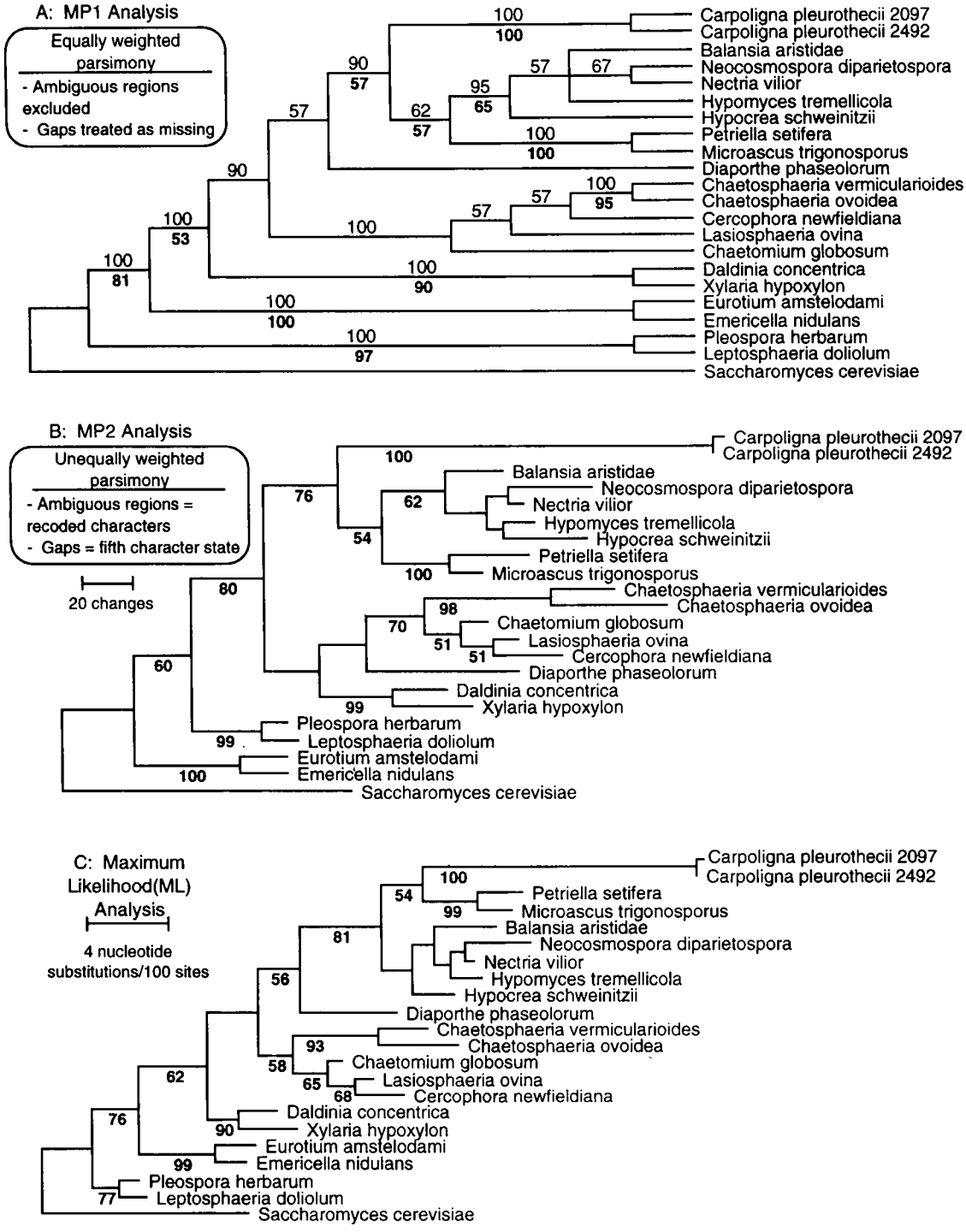


FIG. 24. Phylogenetic relationships of *Carpoligna* to representative taxa of 8 orders of ascomycetes. For all trees, bootstrap values ≤ 50% are not shown. A. Majority rule consensus tree of 21 equally most-parsimonious trees generated by the MP1 analysis. Tree length = 554 steps, consistency index (CI, excluding uninformative characters) = 0.54, rescaled consistency index (RC) = 0.39. Numbers above each internode represent the percentage of these 21 trees which share these specific binary partitions. The numbers below internodes (and in bold) are the percentages of 1000 bootstrap replicates supporting specific binary partitions. B. Single most parsimonious tree resulting from the MP2 analysis. Tree length = 1261 steps, consistency index (CI, excluding uninformative characters) = 0.60, rescaled consistency index (RC) = 0.46. The numbers associated with internodes are the percentages of 1000 bootstrap replicates supporting specific binary partitions. C. Most likely tree (-ln likelihood = 3696.464) generated by the ML analysis. The numbers associated with internodes are the percentages of 570 bootstrap replicates supporting specific binary partitions.

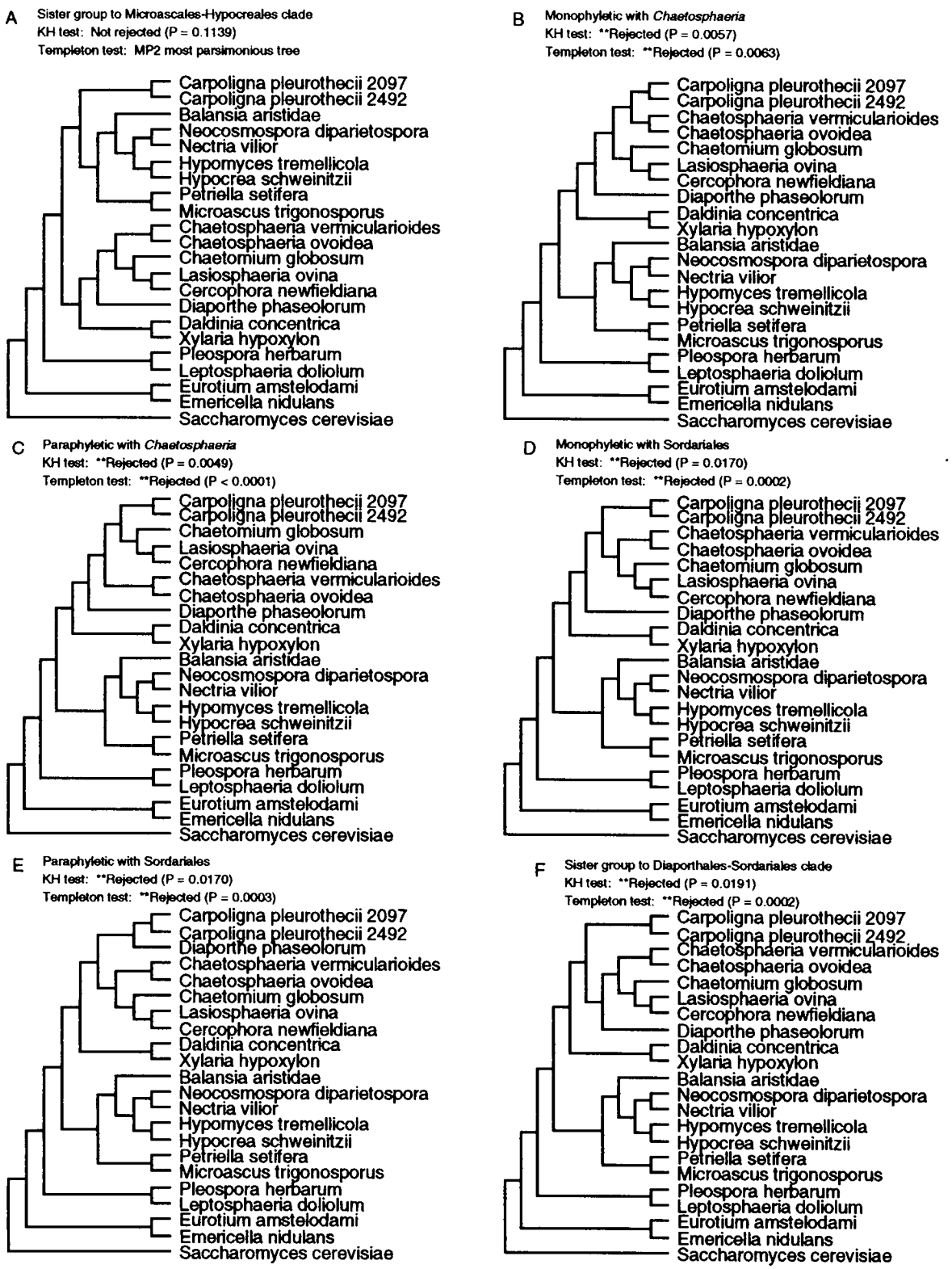
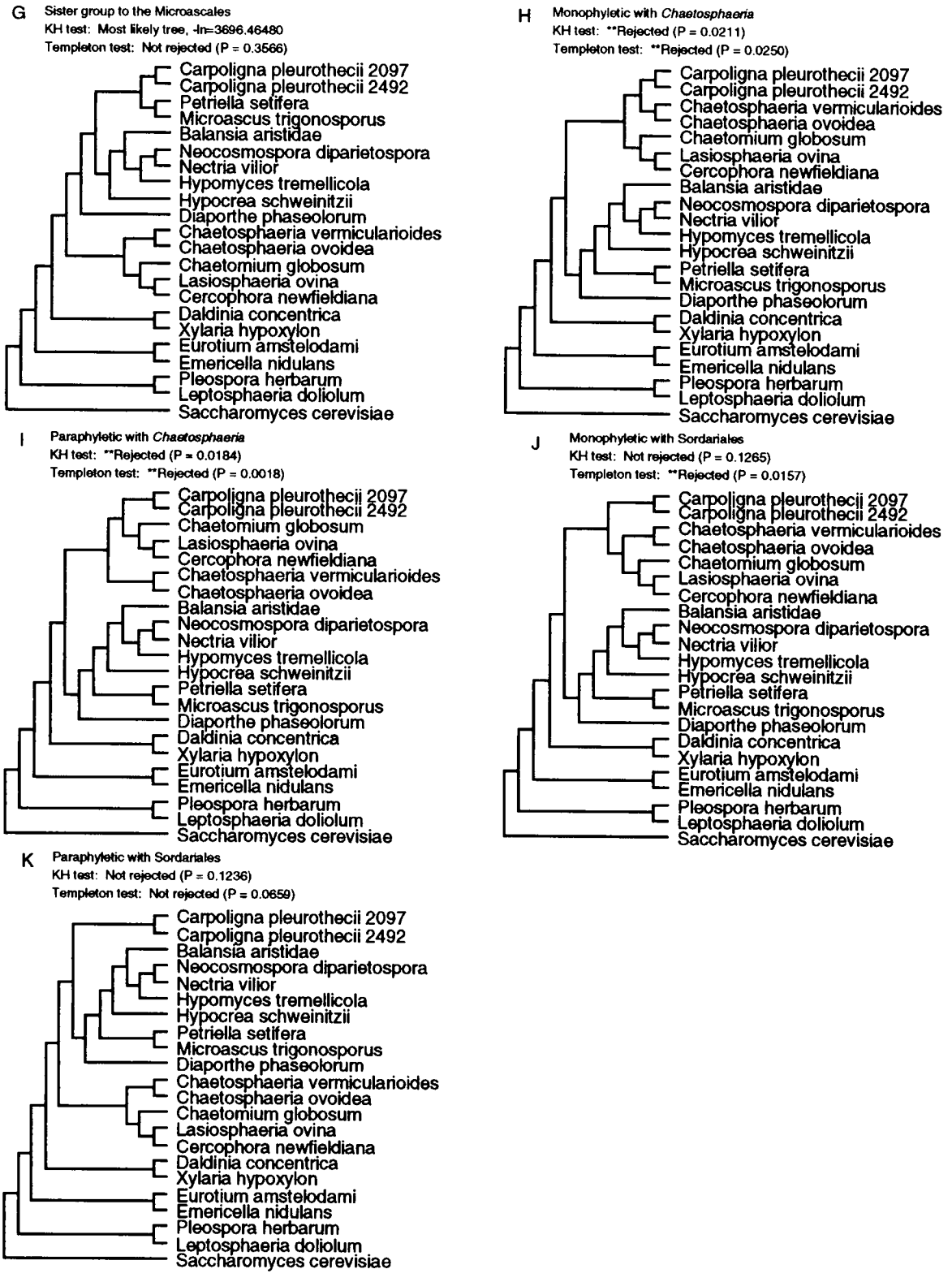


FIG. 25. Statistical comparisons of the most parsimonious MP2 tree (tree A) and the most likely tree (tree G) to hypothetical relationships of *Carpoligna* derived from their topologies (trees B–F and H–K, respectively). Comparisons of tree A to the remaining 10 trees (B–K) were tested using the Templeton method (1983) whereas comparisons of tree G to the other ten trees were tested using the Kishino and Hasegawa method (1989). The first line above each tree indicates the



specific hypothesis tested within the context of this study. The second line shows the result of the Kishino and Hasegawa test when each specific tree is compared to tree G. The third line shows the result of the Templeton test when each specific tree is compared to tree A. Two asterisks indicate that the null hypothesis was rejected at the $P \leq 0.05$ level.

maximum likelihood tree is most similar to the trees generated by the MP1 analysis (FIG. 24A). This is because both of the analyses were performed with the least amount of data, i.e., without the 16 recoded characters derived from the ambiguously aligned regions. In MP2, these regions were unequivocally coded and included in the data matrix as 16 additional characters, 12 of which were parsimony-informative. Each of these coded characters was subjected to a specific step matrix (Lutzoni, Wagner, Reeb unpubl). The inclusion of these 16 coded regions provided a more symmetric topology compared to MP1 and ML, and higher bootstrap support. Because more informative data is included in MP2, the most parsimonious tree resulting from this analysis is our best estimate of the relationship for these selected species of ascomycetes. Without these sixteen additional characters, the MP2 analysis generated six equally parsimonious trees (not shown) that were more similar to the ML than to the MP1 topologies. Interestingly, *Carpoligna* is supported by a very long internode in both the MP2 and the ML trees; which probably reflect a significant increase in the rate of nucleotide substitution when compared to most of the other taxa included in this study.

Phylogenetic relationships of Carpoligna.—Two hypothetical relationships for *Carpoligna* result from the molecular analyses. The ML analysis shows *Carpoligna* to be the sister group to the Microascales (FIG. 24C), whereas the MP1 and the MP2 trees show *Carpoligna* as a sister group to the Hypocreales-Microascales clade (FIGS. 24A, B). The latter hypothesis is supported by higher bootstrap values (ML = 57%; MP1 = 57%, MP2 = 76%). However, none of the best trees generated by ML, MP1 and MP2 were found to be significantly worse than the most likely tree generated by the ML analysis and the most parsimonious tree generated by MP2 (partial results shown in FIGS. 25A, G). Therefore, we cannot reject either hypothesis at the 0.05 level of statistical significance.

Both the Kishino and Hasegawa, and the Templeton tests, always rejected the monophyly and paraphyly of *Carpoligna* with *Chaetosphaeria* at the 0.05 level (FIGS. 25B, C, H, I). While the Templeton test consistently rejected the hypothesis of *Carpoligna* forming a monophyletic group with the Sordariales (FIGS. 25D, J), the Kishino and Hasegawa test did not (FIG. 25J). The potential paraphyletic relationship of *Carpoligna* with the Sordariales was rejected by both tests when using a tree (FIG. 25E) resulting from a modification of the most parsimonious tree (FIG. 25A), but both tests did not reject this hypothesis when using a tree (FIG. 25K) resulting from a modification of the most likely tree (FIG. 25G). Finally, the hypothesis that *Carpoligna* is a sister group to the Diaporthales-Sordariales was rejected by both tests (FIG. 25F).

DISCUSSION

The lack of distinctive morphological characters in the teleomorph of *Carpoligna* makes it difficult to distinguish this taxon from other morphologically simple pyrenomycetes, such as species of *Chaetosphaeria,* which are also commonly found on decaying wood. *Carpoligna* resembles typical *Chaetosphaeria* species, such as *C. ovoidea* (Fr.) Constant., K. Holm & L. Holm in the simple characteristics of the teleomorph: (i) globose to subglobose ascomata with a single-layered wall of pseudoparenchymatic cells, (ii) asci with small, apical ring, and (iii) equal-sized, hyaline, fusiform, three-septate ascospores. Comparisons of the wall structure of the ascoma in *Carpoligna* and in Chaetosphaeria innumera Berk. & Br. ex Tul. (Booth 1957) show no significant differences. However, the morphological characteristics of the anamorph produced by *Carpoligna* are readily distinguishable from the typical phialidic anamorphs of *Chaetosphaeria* species. *Chaetosphaeria* species produce a wide array of anamorphs, such as *Menispora* Persoon, the anamorph of *C. ovoidea, Zanclospora* Hughes & Kendrick, the anamorph of *C. brevispora* Shoemaker, or the *Chalara* (Corda) Rabenhorst anamorph of *C. chalaroides* Hol.-Jech., to name just a few. All are related by their phialidic conidiogenesis. *Pleurothecium recurvatum* produces conidia holoblastically, which makes it distinctly different from these other anamorphs.

The close relationships of *Pleurothecium* are, however, not evident and have been a matter of speculation. *Pleurothecium* has been confused with *Cacumisporium* Preuss because of the superficial similarities of their conidiophores and conidia, although they exhibit quite different conidial ontogenies (Goos 1969a, b). A close relationship of *Cacumisporium* to the typical phialidic anamorphs of species of *Chaetosphaeria* has been previously suggested based on conidiogenesis (Gams and Holubová-Jechová 1976). Based on conidiogenesis, the relationships of *Pleurothecium* must be searched for elsewhere.

The general pattern of holoblastic conidiogenesis with sympodial proliferation and denticles observed in the *Pleurothecium* anamorph of *Carpoligna* is reminescent of the conidiogenesis observed in taxa currently placed in the Magnaporthaceae (Cannon 1994), and formerly placed in the Hyponectriaceae (Barr 1990). For example, the *Pyricularia* Sacc. and *Nakatea* Hara anamorphs of *Magnaporthe grisea* (He-

bert) Barr, and *Magnaporthe salvinii* (Cattaneo) Krause & Webster produce holoblastic conidia on sympodially proliferating conidiophores (Hebert 1971, Krause and Webster 1972). Taxa remaining in the Hyponectriaceae such as *Monographella* Petrak and *Pseudomassaria* Jacz. also produce anamorphs with similar conidiogenesis to that of *Carpoligna* (Barr 1990).

Paraphyses in *Carpoligna* are very similar to those described in *M. salvinii* by Krause and Webster (1972) who propose the term *reliquiae* for the distinctive bands of tissue left behind after asci grow through the pseudoparenchymatous centrum. Similar structures, described as either pseudoparaphyses or apical paraphyses, were described from *Buergenerula spartinae* Kohlmeyer and Gessner (Kohlmeyer and Gessner 1976), a genus also placed in the Magnaporthaceae. The fact that taxa in the Magnaporthaceae are parasitic and/or pathogenic on plants does not necesarily negate phylogenetic affinities to a pyrenomycete with a lignicolous saprobic nutritional habit such as *Carpoligna*. In fact, the use of sequence data and phylogenetic analysis can help shed light on phylogenetic relationships beyond those provided by nutritional habit and, in turn, help bridge the existing phylogenetic disconnection of some plant pathogenic pyrenomycetes from the saprobic pyrenomycetes.

Carpoligna might also have phylogenetic affinities to the Trichosphaeriaceae. This family as circumscribed by Barr (1990) include morphologically simple pyrenomycetes which are saprobic or hypersaprobic on wood and other substrates. They also produce poorly known anamorphs with holoblastic, sympodially proliferating, denticulate conidiogenous cells (Barr 1990). However, the Trichosphaeriaceae also remains a potential home family for the genus *Chaetosphaeria* if excluded from the Lasiosphaeriaceae. This question will remain until the phylogenetic relationships between *Chaetosphaeria* and *Trichosphaeria* Fuckel are resolved.

Another family, the Niessliaceae, also include morphologically simple pyrenomycetes which are saprobic on herbaceous and woody substrates (Barr 1990). However, collabent ascomata, which are diagnostic in this family, are absent in *Carpoligna*. Likewise, paraphyses are absent in this family, which is not the case in *Carpoligna*. Large subunit nrDNA sequence data for representative taxa of these three families were not included in our analyses because either cultures or collections for these taxa were not available at the time of the analyses.

The phylogenetic analyses of large subunit nrDNA data conflicts with the idea of a close phylogenetic relationship between *Carpoligna* and *Chaetosphaeria*, as suggested by the morphological similarities of their teleomorphs. In this context of high morphological similarity between *Carpoligna* and *Chaetosphaeria*, the high degree of DNA divergence between *Carpoligna* and all the other taxa included in this study was unexpected. The degree of divergence recorded in the large subunit nrDNA of *Carpoligna* is much higher than among most orders included in this study. The cause(s) for such a drastic acceleration in rates of nucleotide substitution in the *Carpoligna* lineage is not known yet.

Phylogenetic relationships among pyrenomycetous orders using the large subunit nrDNA in this study are concordant with previously reported phylogenies based on the small subunit nrDNA (Spatafora and Blackwell 1994a, Glenn et al 1996). Sequence data from the large subunit nrDNA in this study show low bootstrap support for a close phylogenetic relationship between the Hypocreales and the Microascales although previous studies of the small subunit nrDNA have shown close phylogenetic relationships between these two orders (Spatafora and Blackwell 1994b, Glenn et al 1996). Interestingly, analyses in this study indicate that phylogenetic relationships of *Carpoligna* to the Microascales or the Hypocreales cannot be ruled out. However, these relationships still remain unclear. Inclusion of sequence data for other taxa in these orders as well as in the Magnaporthaceae, Hyponectriaceae and Trichosphaeriaceae might help clarify these relationships.

Continuing studies are currently being carried out to identify and generate molecular data for poorly represented pyrenomycetous taxa. These data, and their analyses, will most likely provide novel insights into the phylogeny of this important group of ascomycetes.

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