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## Neotropical Ascomycetes 13. *Cornipulvina* and *Erythromada*, two new genera from the Caribbean and elsewhere

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Huhndorf, S.M., Miller, A.N., Fernández, F.A. and Lodge, D.J. (2005). Neotropical Ascomycetes 13. *Cornipulvina* and *Erythromada*, two new genera from the Caribbean and elsewhere. *Fungal Diversity* 20: 59-69.

*Cornipulvina ellipsoides* is described as a new genus and species in the family *Boliniaceae*, order *Bolinales* and *Erythromada lanciospora* is described as a new genus and species in the *Sordariomycetidae*. *Cornipulvina* is distinguished by irregular stromata with long rostrate necks and ellipsoid ascospores lacking a germ pore. *Erythromada* differs from similar scolecosporous genera in its superficial, clustered, ovoid ascomata and its nonseptate, wider ascospores. Phylogenetic analyses of nuclear 28S large subunit (LSU) sequences supports the establishment of both genera.

**Keywords:** *Boliniaceae*, Neotropics, *Sordariomycetidae*, systematics

### Introduction

We are surveying terrestrial wood-inhabiting pyrenomycetes in the Neotropics (Huhndorf, 1997; Huhndorf and Fernández, 1998, 2005; Fernández and Huhndorf, 2004, 2005). In this paper we report two taxa as yet unknown to science. One taxon possesses ellipsoid, one-celled ascospores and distinctive superficial stromata while the other has scolecospores and clustered ascomata. New genera are created to accommodate these taxa.

### Material and methods

Ascomata were mounted in water and replaced with lactophenol containing azure A. Measurements were made and images were captured of

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material in both mounting fluids. Ascomata were sectioned at 5  $\mu\text{m}$  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, ANM = A.N. Miller, DJL = D.J. Lodge and GJS = G.J. Samuels. 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 and dimensions given for the substrates are diameters.

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

Methods for DNA extraction, PCR amplification and sequencing of the LSU gene along with procedures for the alignment of LSU sequences have been fully described elsewhere (Huhndorf *et al.*, 2004; Miller and Huhndorf, 2005).

#### ***Phylogenetic analyses***

Portions of the 5' and 3' ends of the LSU gene along with the single spliceosomal intron, which occurred in *Linocarpon appendiculatum*, were excluded from all analyses. Nine ambiguously aligned regions were delimited and recoded as nine unequivocally coded characters using INAASE 2,3b (Luzoni *et al.*, 2000). The remaining unambiguously aligned characters were subjected to a symmetric stepmatrix generated with the program STMatrix 2.2 (François Lutzoni & Stefan Zoller, Dept. of Biology, Duke University). Unequally weighted maximum parsimony analyses were conducted using PAUP\* 4.0b10 (Swofford, 2002) as follows: constant characters were excluded, gaps were treated as missing, 20,000 random-addition replicates were implemented with TBR branch-swapping, MULTREES option was in effect, and zero-length branches were collapsed. Bootstrap support was estimated by performing 2000 bootstrap replicates (Felsenstein, 1985), each consisting of a heuristic search with 100 random-addition replicates using the above settings. Two members of the *Xylariales* were used as outgroup taxa

based on previous analyses (Huhndorf *et al.*, 2004; Miller and Huhndorf, 2004, 2005)

Maximum likelihood analyses were conducted as above using PAUP\* 4.0b10 except constant characters were included, 1000 random-addition replicates were implemented, and TBR branch-swapping was subjected to a reconnection limit of 12. The nucleotide substitution model calculated by Modeltest 3.7 (Posada and Crandell, 1998) was the GTR model (Rodriguez *et al.*, 1990) with unequal base frequencies (freqA = 0.2442, freqC = 0.2145, freqG = 0.3164, freqT = 0.2249), a substitution rate matrix (A $\leftrightarrow$ C = 1.9111, A $\leftrightarrow$ G = 5.1823, A $\leftrightarrow$ T = 2.9437, C $\leftrightarrow$ G = 2.003, C $\leftrightarrow$ T = 15.2693, G $\leftrightarrow$ T = 1.000), a proportion of invariable sites = 0.4869, and a gamma distribution shape parameter = 0.5253.

Analyses of Bayesian inference were conducted using MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). The GTR model was implemented as above, and four independent runs, each consisting of four MCMC chains, were ran for 10,000,000 generations with trees sampled every 1000<sup>th</sup> generation resulting in 10,000 total trees. The first 1,000 trees, which extended well beyond the burn-in phase in each analysis, were discarded and the remaining 9,000 trees were used to calculate posterior probabilities.

## Results

Maximum likelihood analyses of LSU sequences generated a single most likely tree (Fig. 1). This tree did not differ in topology from the single most parsimonious tree generated in unequally weighted maximum parsimony analyses (data not shown) except for the placement of *Leptospora gregaria*. The establishment of two new genera for these recently discovered taxa is supported by both molecular and morphological data.

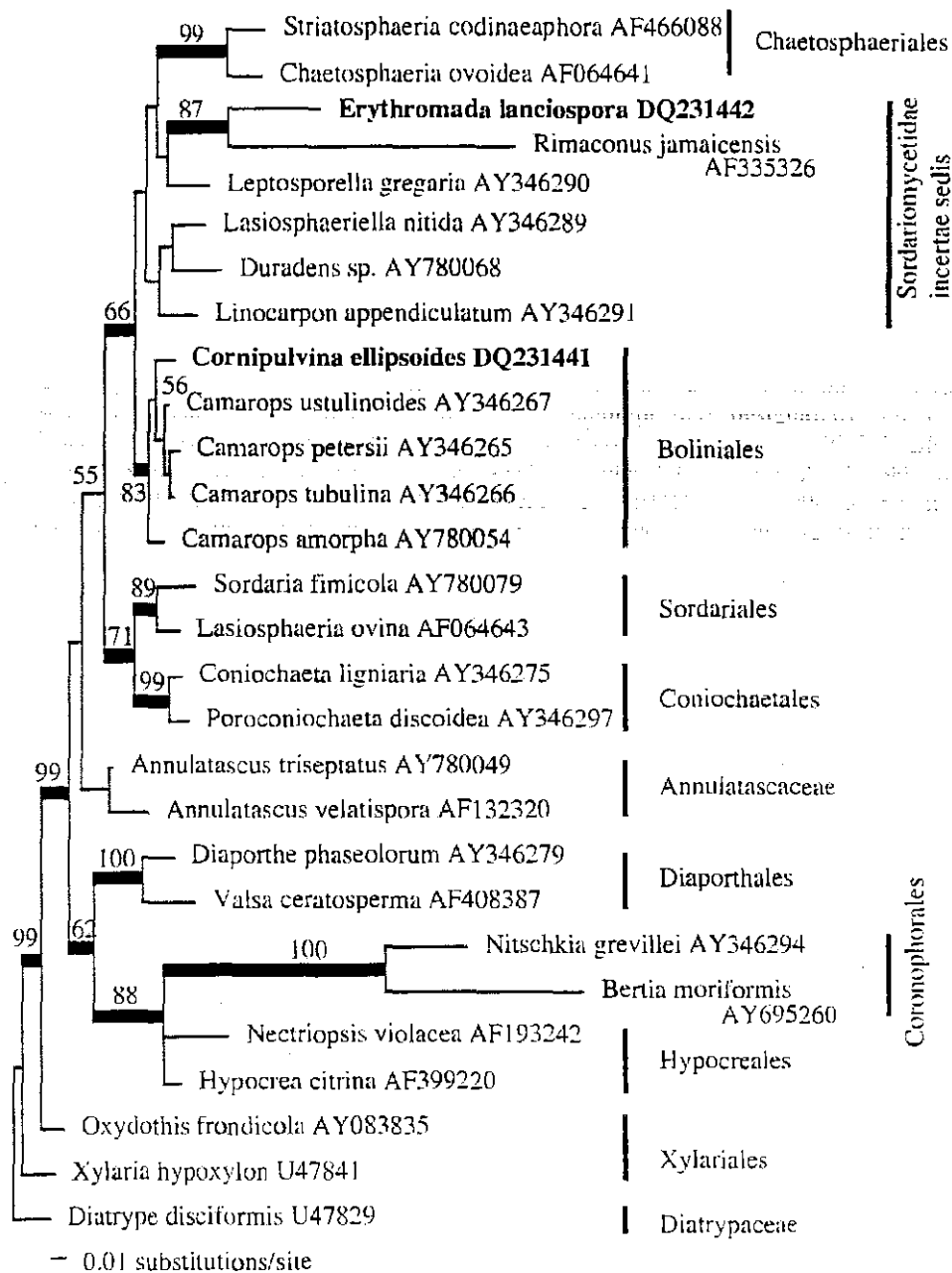
### ***Cornipulvina*** Huhndorf, A.N. Mill., F.A. Fernández & Lodge, **gen. nov.**

*Etymology*: *L. cornis* = horned; *pulvinus* = cushion, refers to the stromal structure.

*Stromata* superficialia, forma irregularia, longirostria, textura mollia. *Perithecia* globosa vel subglobosa, monosticha. *Paraphyses* angustae. *Asci* cylindracei, brevi-stipitati, annulo apicali brevi. *Ascospores* ellipsoideo, nonseptatae, hyalinae.

*Typus genericus*: *Cornipulvina ellipsoides* Huhndorf, A.N. Mill., F.A. Fernández & Lodge

*Stromata* superficial, irregular shaped, with long necks, soft texture. *Perithecia* globose or subglobose, monostichous. *Paraphyses* narrow. *Asci* cylindrical, short-stipitate, with short, wide ring. *Ascospores* ellipsoid, nonseptate, hyaline.



**Fig. 1.** Phylogram of the single most likely tree ( $-\ln L = 4979$ ) generated from a maximum likelihood analysis of 1205 bp of the 5' end of nuclear LSU rDNA for 28 ascomycete sequences. Thickened branches indicate Bayesian posterior probabilities  $\geq 95\%$  while numbers above branches refer to maximum parsimony bootstrap values  $\geq 50\%$ . GenBank accession numbers follow taxon names.

***Cornipulvina ellipsoides*** Huhndorf, A.N. Mill., F.A. Fernández & Lodge, **sp. nov.** (Figs. 2-10)

*Etymology* : Refers to the shape of the ascospores.

*Stroma* superficialis, forma irregularis, 1-7 mm diam; longirostris; extus fusca et intus fulva; textura mollis. *Perithecia* subglobosa, 400-500 µm diam, monosticha. Paries perithecii in sectione longitudinali 20-25 µm crassus, unistriatus. *Papilla* longi-cylindracea, 250-275 µm lata, 200-1100 µm alta. *Paraphyses* angustae. *Asci* cylindranei, 55-65 × 5.5-6.5 µm, brevistipitati, annulo apicali brevi, 2-2.5 µm lato, 1-1.5 µm alto. *Ascosporae* ellipsoideae, 7-9 × 3.5-4 µm, nonseptatae, hyalinae, sine vagina vel appendicibus.

*Stroma* superficial, irregular shaped, from 1-7 mm wide, 0.8-0.9 mm high, with projecting long and short necks, outer surface brown to blackish with pale brown ostiolar and neck apices, internally pale brown, texture soft, composed of loosely packed pseudoparenchymatic cells. *Perithecia* subglobose, 400-500 µm diam., monostichous, with long to short necks. *Perithecial wall* in longitudinal section uniformly 20-25 µm thick, 1-layered, composed of polygonal to elongate pseudoparenchymatic cells (8-12 × 2-3.5 µm). *Ascomatal necks* short to elongate, emergent from the stroma, new necks proliferating through locations of old, broken necks, 250-275 µm wide, 200-1100 µm long, ostioles circular, 25-30 µm diam., with paraphyses. *Paraphyses* tapering, narrowing toward the apex, 1.5-2.5 µm wide, sparse, persistent, without gelatinous coating. *Asci* cylindrical, 55-65 × 5.5-6.5 µm, short stalked, numerous, basal and lateral, lining the peripheral wall of the centrum, unitunicate, apex with a short, wide ring, 2-2.5 µm wide, 1-1.5 µm high, with 8 uniseriate ascospores. *Ascospores* ellipsoid, 7-9 × 3.5-4 µm, straight, hyaline, nonseptate, smooth, no germ pore seen, without sheath or appendages.

*Anamorph* not seen.

*Habitat*: On decorticated wood.

*Known distribution*: Brazil, Puerto Rico, Venezuela.

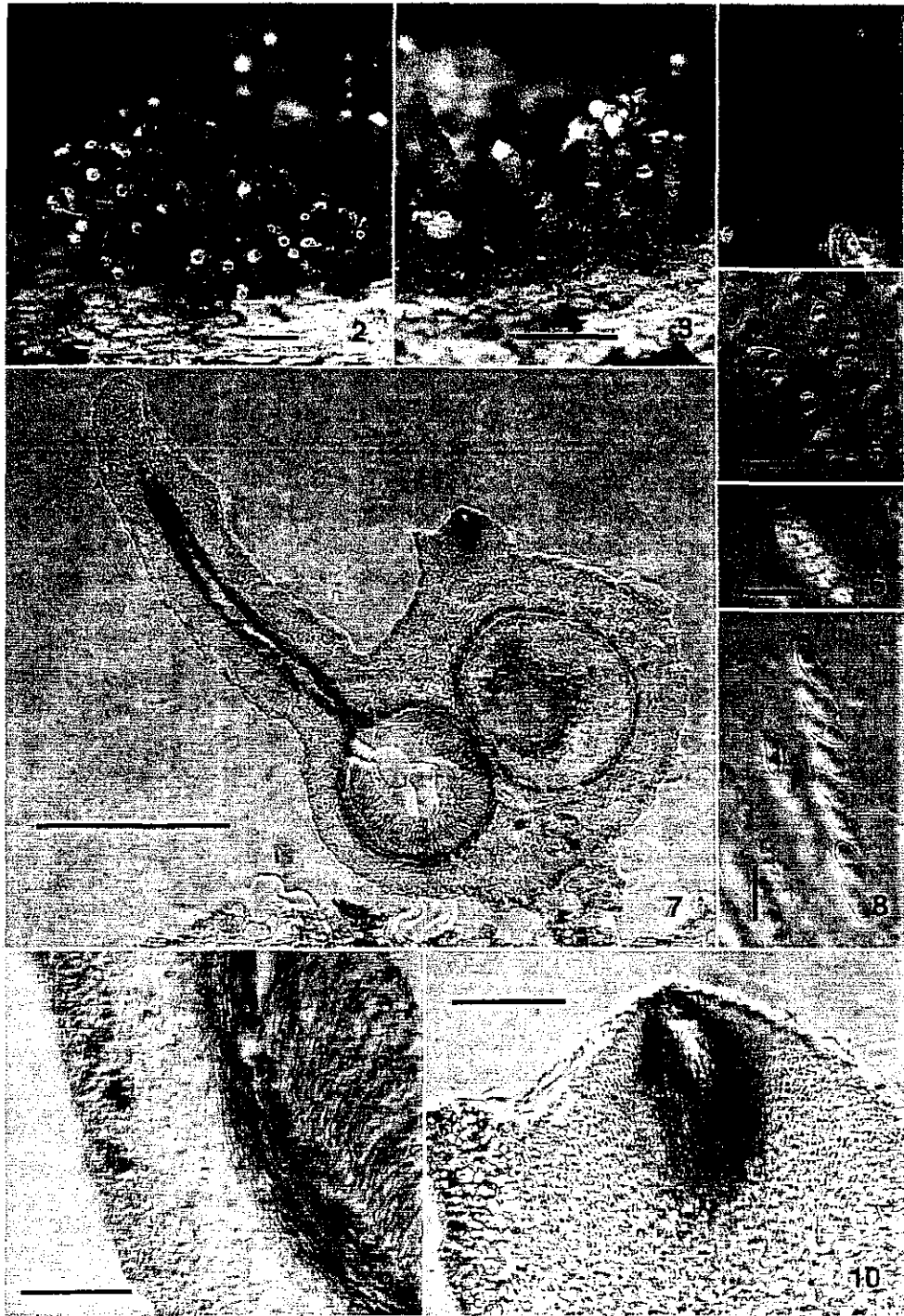
*Material examined*: BRAZIL. GJS1083 (BPI, F, NY). Puerto Rico: Luquillo Mts, Bisley Watershed 3, elev 220 m, [18.3167, -65], on 16 cm decorticated log; 8-V-1995, SMH, DJL, SMH1378 (F, **holotype here designated**), VENEZUELA. EDO. ARAGUA: Parque Nacional Henri Pittier, Rancho Grande Biological Station, trail to Guacamayo, ca 10°21'N, 67°41'W, elev 1250-1400 m, on decorticated log, 4-XII-1990, GJS, SMH, B. Hein, GJS7934 (BPI, F, NY).

***Erythromada*** Huhndorf, A.N. Mill., F.A. Fernández & Lodge, **gen. nov.**

*Etymology*: L. *Erythro* = red; *omada* = group, refers to the color and clustered habit of the ascomata.

*Ascomata* obpyriformia vel ovoidea, superficialia, nonpapillata. *Paraphyses* angustae. *Asci* falcati, brevi-stipitati, annulo apicali alto. *Ascosporae* filiformae. nonseptatae, hyalinae.

*Typus genericus*: *Erythromada lanciospora* Huhndorf, A.N. Mill., F.A. Fernández & Lodge.



*Ascomata* obpyriform to ovoid; superficial; clustered, reddish-brown, nonpapillate. *Paraphyses* narrow. *Asci* cylindrical, short-stipitate, with large, cylindrical ring. *Ascospores* filiform, nonseptate, hyaline.

***Erythromada lanciospora*** Huhndorf, A.N. Mill., F.A. Fernández & Lodge, **sp. nov.** (Figs. 11-26)

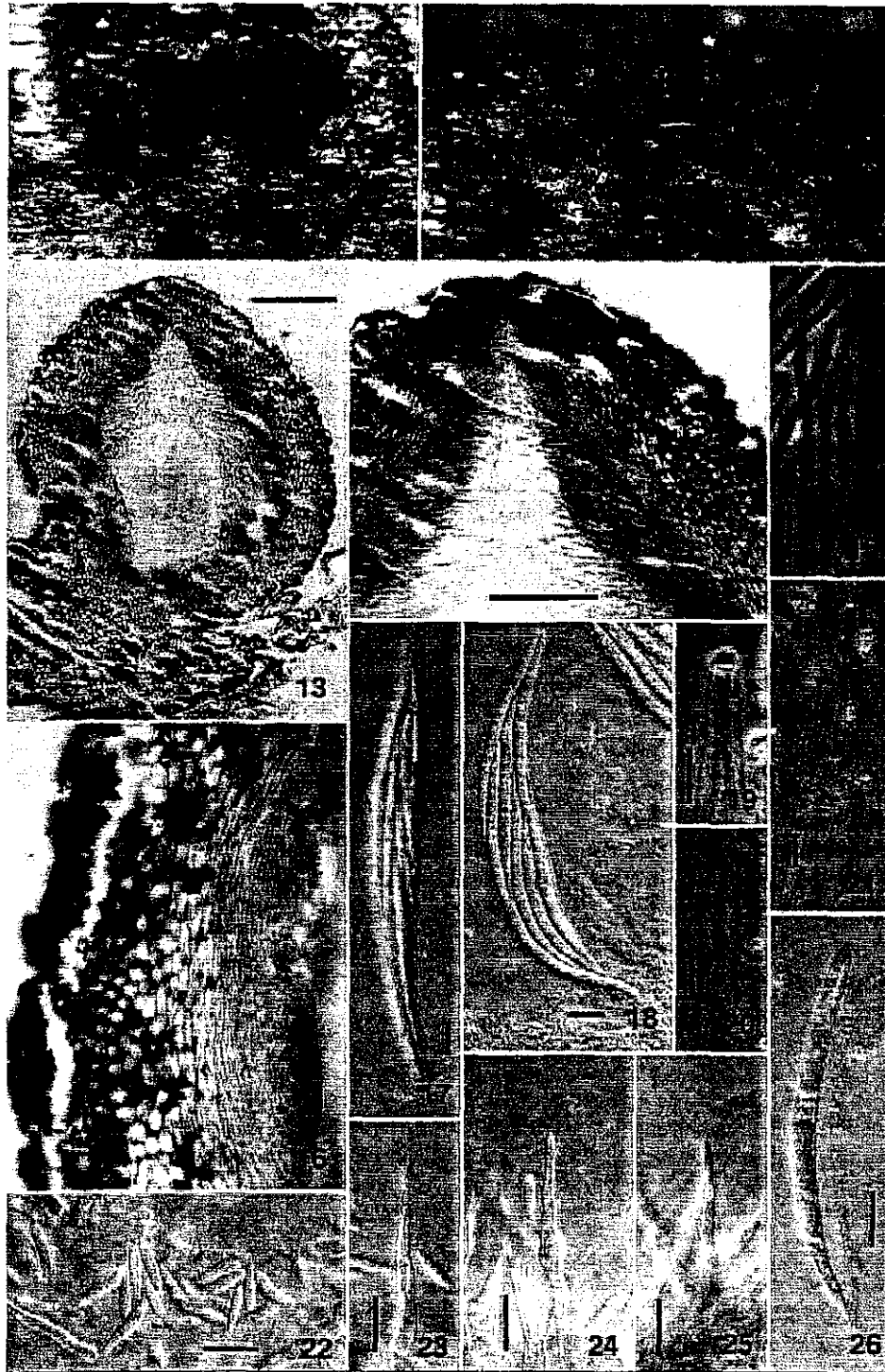
*Etymology*: *lanciospora* = spore armed with a lance or point

*Ascomata* obpyriformia vel ovoidea, 400-550  $\mu\text{m}$  diametro, 500-700  $\mu\text{m}$  alto, nonpapillata, pagina ascomatum glabrata. Paries ascomatis superficialis textura angularis-globosa, in sectione longitudinali 70-90  $\mu\text{m}$  crassus, bistriatus. *Papilla* rotundata, periphysibus induta. *Paraphyses* angustae abundae. *Asci* falcati, 120-185  $\times$  15-25  $\mu\text{m}$ , brevi-stipitati, octospori, tetraseriati, annulo apicali alto. *Ascosporae* filiformae, acuminatae, (60-) 70-100 (-110)  $\times$  3.5-5.2  $\mu\text{m}$ , nonseptatae, hyalinae, sine vagina vel appendicibus.

*Ascomata* obpyriform to ovoid, 400-550  $\mu\text{m}$  diam., 500-700  $\mu\text{m}$  high, not collapsing when dried; superficial, numerous, clustered on a basal stroma, nonpapillate, surface glabrous, slightly roughened, red brown appearing striate at the apex. *Ascomatal wall* of *textura angularis-globosa* in surface view; in longitudinal section 70-90  $\mu\text{m}$  thick at the sides, thicker at the base where it is part of the basal stroma; 2-layered, inner layer composed of thick-walled pseudoparenchymatic to scleroplectenchymatic cells (6-12  $\times$  3-7  $\mu\text{m}$ ), elongate, compressed and flattened towards the centrum, outer layer composed of polygonal to isodiametric and intermittently melanized cells. *Ascomatal apex* broadly rounded, ostiole circular, 30-60  $\mu\text{m}$  wide, periphysate. *Paraphyses* tapering, 4.5-7.5  $\mu\text{m}$  wide at the base, narrowing toward the apex, 1.5-3.5  $\mu\text{m}$  wide, abundant; persistent, without gelatinous coating. *Asci* curved fusiform to widely falcate (narrower at the apex and base, wide in the middle); 120-185  $\times$  15-25  $\mu\text{m}$ , short-staked, numerous, basal and lateral, lining the peripheral wall of the centrum, unitunicate, apex with a large, cylindrical ring, 2.5-3.5  $\mu\text{m}$  wide, 1.7-2.8  $\mu\text{m}$  high, with 8 tetraseriate ascospores. *Ascospores* long fusiform to falcate, apical end pointed, basal end tapering and pointed, (60-) 70-100 (-110)  $\times$  3.5-5.2  $\mu\text{m}$ , slightly curved, hyaline, smooth, nonseptate, without sheath or appendages.

*Anamorph*: phialidic, from culture (culture from SMH4377 subsequently died). Not seen on the substrate. *Conidiophores* on CMA mononematous, branched, hyaline to pale brown, septate. *Conidiogenous cell* a phialide, cylindrical, narrowing to the apex, 25-35  $\times$  1.5-3.5  $\mu\text{m}$ , with a single apical collarete, 2-2.5  $\mu\text{m}$  wide, 1-2.5  $\mu\text{m}$  deep, not proliferating percurrently. *Conidia* hyaline, cylindrical to clavate, rounded at the apex with a truncate base, nonseptate, 9-13  $\times$  2-2.5  $\mu\text{m}$ .

**Figs. 2-10**, *Cornipulvina ellipsoides*. **2, 3**. Stromata on the substrate. **4**. Paraphyses. **5**. Ascospores. **6**. Ascus apex showing ring. **7**. Longitudinal section through stromata. **8**. Ascus. **9**. Longitudinal section through stromal and perithecial wall. **10**. Longitudinal section through apex of perithecial neck. Figs. 2, 3 by photomacrography; Figs. 4, 6, 7, 10 by PH; Figs. 5, 8, 9 by DIC. All figures from holotype SMH1378. Bars: 2, 3 = 1 mm; 7 = 500  $\mu\text{m}$ ; 9, 10 = 50  $\mu\text{m}$ ; 4-6, 8 = 10  $\mu\text{m}$ .



**Habitat:** On dead wood and bark.

**Known distribution:** Costa Rica, Ecuador, French Guiana, Puerto Rico.

**Material examined:** COSTA RICA, Puntarenas Prov., Santa Elena Cloud Forest Reserve, Monteverde, elev 1190 m, [10.7081, -85.0453]. 13-VII-2001, on bark of 57 cm branch, SMH, FAF, ANM, M.P. DaRin, SMH4487; Reserva Biologica Bosque Nuboso Monteverde, Centro Cientifico Tropical, Sendero Roble, elev 1536 m, [10.3058, -84.7933], 4-XI-2003, on wood fragment, SMH, FAF, SMH4922. ECUADOR, Orellana Prov., Yasuni National Park, Bariso trail, [-,6713, -77.4005], 7-III-2001, on 5 cm branch, FAF, ANM, R. Briones, SMH4377 (F). FRENCH GUIANA, Paul Isnard Area, ca. 150 km S of St. Laurent du Maroni, Citron, Mt. Decou Decou, 04°70'N, 53°90'W, 11, 12-III-1986, on woody vine, GJS, P. Searwar, GJS4200 (NY). PUERTO RICO, Luquillo Mts., Luquillo Exp. Forest, El Verde Research Area, 16-ha Grid, base quadrat 07.04.14, 18°19'28"N, 65°48'59"W, [18.3167, -65.8167], elev 382 m, 18-VI-1995, on 50 cm upper trunk of *Nectandra turbacensis* (Kunth) Nees (*Lauraceae*), S.M. Huhndorf, SMH1526 (F, **holotype here designated**); trail to Rio Sonadora, 23 Nov. 1991, on slowly dying branch of *Guarea guidonia*, DJL, PR707 (BPI); El Verde Research Area, 16-ha Grid, [18.3167, -65.8167], elev 350 to 425 m, 25-IX-1995, on 50 cm log, SMH, SMH1557; 27-IX-1995, on 30 cm log, SMH1605; 13-I-1996, on 50 cm log, SMH1871; 25-I-1996, on 50 cm log, SMH2033; 25-I-1996, on 60 cm log, SMH2047 (F). VENEZUELA, Edo. Merida, Parque Nac. Sierra Nevada, above Tabay, Qda. Coromoto, La Mucuy, 08°36'N, 71°02'W, 2300 m, 9,17-XI-1990, GJS, B. Hein, SMH, T. Iturriaga, G. Rodriguez, M. Herrera, GJS6780, (BPI)

## Discussion

Both of these taxa were first recognized from Puerto Rican collections and subsequently found in other neotropical areas. Based on morphological characters the taxonomic position of both genera was not readily apparent. *Cornipulvina* has stromata and long necks that suggested an affiliation with the *Thyridiaceae*. The ascospores of *Erythromada* resembled *Rimaconus jamaicensis* Huhndorf, F.A. Fernández: J.E. Taylor, & K.D. Hyde (Huhndorf *et al.*, 2001) or *Duradens* Samuels & Rogerson (Samuels and Rogerson, 1990) but the ascomata are superficial not immersed to erumpent, as in these other genera. The analyses of LSU sequences shows that *Cornipulvina* is related to taxa in the *Boliniales* while *Erythromada* clustered in a large unsupported group of *Sordariomycetidae* that is near taxa in the *Chaetosphaeriales* (Fig. 1).

*Cornipulvina* has a soft-textured stroma similar to some members of *Camarops* and *Apiocamarops*, but differs in its monostichous perithecia with very long, superficial necks. Immersed long necks are often found in members

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Figs. 11-26. *Erythromada lanciospora*. 11, 12. Ascomata on the substrate. 13. Longitudinal section through ascomata. 14. Longitudinal section through apex of ascomatal neck. 15, 21. Paraphyses. 16. Longitudinal section through ascomatal wall. 17, 18. Asci. 19, 20. Ascus apices showing rings. 22. Conidia from CMA. 23-25. Conidiophores from CMA. 26. Ascospore. Figs. 11, 12 by photomacrography; Figs. 13, 14, 16-18, 12-26 by DIC; Figs. 15, 19-21 by PH. Figs. 11-16, 18, 19, 26 from SMH1526; Figs. 17, 20-25 from SMH4377. Bars: 11, 12 = 1 mm; 13 = 100 µm; 14 = 50 µm; 15-26 = 10 µm.

of the *Boliniaceae* with large stromata where the perithecia are polystichous. Ascospores in *Cornipulvina* are one-celled ellipsoid in shape, similar to some members in the family but not noticeably flattened and also differing in lacking an apparent germ pore.

Several scolecosporous taxa occur in the large unsupported Sordariomycetidae group that contains *Erythromada*. Most closely related, with strong bootstrap support is *Rimaconus jamaicensis*. The overall group also contains *Leptosporella gregaria* Penz. & Sacc., *Duradens* sp. and *Linocarpon appendiculatum* K.D. Hyde but their relationships to *Erythromada* are unclear (Fig. 1). All five taxa have elongate, hyaline ascospores but *L. gregaria* and *L. appendiculatum* differ in spores that are narrower while *Duradens* has wider spores and *Rimaconus* has wider, septate spores. *Erythromada* differs from all of these taxa by having superficial, clustered, ovoid ascomata rather than erumpent, conical ones. Also in the group is *Lasiosphaeriella nitida* Huhndorf & F.A. Fernández which has superficial, clustered, ovoid ascomata but differs from the other taxa in having wide, allantoid ascospores (Huhndorf and Fernández, 1999).

## Acknowledgements

This material is based upon work supported by the National Science Foundation under Grant Nos. 0118695 and 0072684. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. Fieldwork was additionally supported by the National Research Council Resident Research Associate Post-doctoral Program in cooperation with the USDA Forest Service, Forest Products Laboratory, Madison, WI, an Explorers Club Research Grant to ANM, and NGS grant No. 6914-00 awarded to FF. We are grateful to Drs. J. Thompson and J. Zimmerman for assistance in Puerto Rico, Dr. Julieta Carranza (Universidad de Costa Rica), Milagro Mata and Loengrin Umaña (INBio) in Costa Rica, and R. Briones (PUCE) and David Suarez (QCNE) in Ecuador. We thank BPI and NY for loan of specimens. Many thanks to Margaret Barr for helpful discussions on the taxonomic placement of *Cornipulvina* and other contentious pyrenomycetes.

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(Received 10 June 2005; accepted 20 September 2005)



Huhndorf, S.M.; Miller, A.N.; Fernández, F.A.; Lodge, D. J. 2005. Neotropical Ascomycetes 13. Cornipulvina and Erythromada, two new genera from the Caribbean and elsewhere. *Fungal Diversity* 20: 59-69.