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ORIGINAL ARTICLE

Cyanonectria, a new genus for *Nectria cyanostoma* and its *Fusarium* anamorph

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Abstract The new genus *Cyanonectria* is proposed for *Nectria cyanostoma* (≡ *Cyanonectria cyanostoma* comb. nov.). This genus is characterized by Nectria-like, red perithecia that have a bluish-purple papilla and a *Fusarium* anamorph. DNA sequences (large subunit and internal transcribed spacers of the nuclear rDNA) indicate that *C. cyanostoma* is not closely related to *Nectria* sensu stricto. In addition, the phylogenetic data also show that the closest relatives for *Cyanonectria* also have *Fusarium* anamorphs.

Taxonomic novelties *Cyanonectria* Samuels & Chaverri, *Cyanonectria cyanostoma* (Sacc. & Flageolet) Samuels & Chaverri

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Introduction

Historically, bright-colored, soft-textured, superficial, uniloculate, perithecial ascomycetes have been classified in the genus *Nectria* of the Hypocreales. Recent taxonomic studies have redefined *Nectria*, and many of its species have been distributed among several genera in the Bionectriaceae and Nectriaceae (Rossman et al. 1999). The Bionectriaceae includes Nectria-like species having white to orange perithecial walls that do not change color in KOH or lactic acid; typical members of the Nectriaceae have orange to red perithecial walls that turn dark red in KOH and yellow in lactic acid. In the Nectriaceae, some exceptions include *Albonectria* with white perithecial walls and *Gibberella* with bluish-purple perithecial walls. In these genera, other morphological characteristics such as perithecial wall structure and anamorph indicate their relationship to the Nectriaceae (Rossman et al. 1999). The separation of the Bionectriaceae from the Nectriaceae is supported by molecular sequence data (Castlebury et al. 2004, Rossman et al. 2001).

The genus *Nectria* is now restricted to species having a specific ascomatal wall structure and a *Tubercularia* anamorph among other characteristics (Rossman et al 1999); these species are typically weak parasites of woody plants. To accommodate some of the over 1,000 species described in *Nectria*, new genera have been described and old genera revived or redefined. Some of the common genera in the Nectriaceae that include red Nectria-like fungi are *Calonectria, Cosmospora, Lanatonectria*, *Neocosmospora*, and *Neonectria*.

Fusarium is found only in the Nectriaceae where it is anamorph to species in *Albonectria, Cosmospora*, *Gibberella*, and *Haematonectria*, as well as the anomalous species *'Nectria' atrofusca* (Schwein.) Ellis & Everh. and '*N.' desmazieri* Fuckel 1870 non DeNot. & Becc. 1863. Many of the known teleomorphs of *Fusarium* are in *Gibberella*, and the type species of *Fusarium, F. sambucinum* Fuckel (Leslie and Summerell 2006), is the anamorph of *Gibberella pulicaris* (Fr.: Fr.) Sacc. (Rossman et al. 1999). Thus, *Fusarium* in the strictest sense might refer only to those species having *Gibberella* teleomorphs or to those species for which no teleomorph is known but which are derived from within *Gibberella*. In general, the anamorph of each teleomorph genus is referable to one or more sections of *Fusarium*; thus anamorphs of *Albonectria* belong in *Fusarium* sect. *Spicarioides*, anamorphs of *Cosmospora* belong in sects. *Episphaeria* and *Coccophilum*, anamorphs of *Gibberella* belong in sections *Discolor, Elegans*, *Lateritia* and *Liseola*, and anamorphs of *Haematonectria* belong in sect. *Martiella* (Gerlach and Nirenberg 1982; Rossman et al. 1999).

Cosmospora was reserved by Rossman et al. (1999) for species having 'small,' red, smooth, thin-walled, typically< 20 μm, non- or weakly stromatic species. Many members of the genus have pale yellow-brown, spinulose to tuberculate ascospores, anamorphs in *Fusarium* sects. *Coccophilum* and *Episphaeria*, and are found on other ascomycetes or insects. Other species occur on plant material and are not obviously on other fungi or insects, their ascospores are hyaline and smooth, and anamorphs are species of *Chaetopsina* or *Volutella*, or are acremonium- or verticillium-like. *Cosmospora* is now known to be polyphyletic (Zhang and Zhuang 2006).

Nectria cyanostoma Sacc. & Flageolet (Saccardo 1902; von Höhnel 1919) could be included in *Cosmospora* in the sense of Rossman et al. (1999); it has small, <250-μm-diameter, partially red, superficial, non-stromatic perithecia and, as will be shown below, a *Fusarium* anamorph. This species is rarely reported in the literature. von Höhnel (1919) provided a detailed description of the perithecia of a part of Rehm Ascomycetes exsiccati no. 2165. Although part of the type collection, this number was issued in 1918, more than 16 years after the description of the species (Pfister 1985). Although the portion he examined was immature with no ascospores, von Höhnel (1919) recognized that this was an isotype. We recollected*"N." cyanostoma* in southwestern France in 1994 and several times since then, always from southern France. Relatively common on twigs of *Buxus sempervirens* L., it is not associated with any disease symptoms. The species is highly distinctive because of the bluish-purple coloration of the perithecial apex, the basis for its epithet, '*cyanostoma*.'

The objective of this paper is to redescribe and illustrate *"N." cyanostoma*, describe its anamorph, elucidate its generic placement, and describe a new monotypic genus for this species.

Materials and methods

Teleomorph and anamorph morphological characterization

Herbarium specimens were obtained from U.S. National Fungus Collections (BPI) and collected fresh from the field. For morphological characterization of the teleomorph, the macromorphology of the perithecia was observed and described: distribution of perithecia on the host; perithecium shape, color, and reaction to 3% w/v potassium hydroxide (KOH) and 100% lactic acid; and color of the perithecial apex. To observe internal and microscopic characteristics, the perithecia were rehydrated briefly in KOH, then supported by Tissue-Tek O.C.T. Compound 4583 (Miles, Elkhart, IN, U.S.A.), and sectioned at a thickness of ca. 15 μm with a freezing microtome. Characteristics of asci and ascospores were observed by rehydrating the perithecium in 3% KOH, removing part of the centrum with a fine glass needle, and placing it on a glass slide. Measurements of continuous characters such as length and width were made using the beta 4.0.2 version of Scion Image software (Scion, Frederick, MD, U.S.A.). Continuous measurements are based on 30 measured units, except where noted, and are reported as the extremes (maximum and minimum in parentheses) separated by the mean plus and minus standard deviation.

Cultures were obtained by isolating asci containing ascospores on cornmeal-dextrose agar [CMD; Difco, cornmeal agar + 2% w/v dextrose supplemented with antibiotics 0.2% each neomycin (neomycin trisulfate salt hydrate; Sigma Chemicals, St. Louis, MO, U.S.A.) and streptomycin (streptomycin sulfate; Sigma)]. Morphological observations of the colonies and anamorph in culture were based on isolates grown on potato-dextrose agar (PDA; Difco) and SNA (low nutrient agar; Nirenberg 1976) for 3 weeks in an incubator at 25°C with alternating 12 h/12 h fluorescent light/darkness.

DNA extraction, polymerase chain reaction (PCR), and sequencing

All isolates used in the molecular analyses are listed in Table 1. Isolates of *Cosmospora coccinea, C. episphaeria, "Nectria" balansae,"N." cyanostoma, Neonectria coccinea, Neo. veuillotiana*, and *Rubrinectria olivacea* (Table 1) were grown in 6-cm-diameter Petri dishes containing PDA. Plates were incubated at 25°C for ca. 1 week. DNA was

Table 1 Isolates and accession numbers used in the phylogenetic analyses

Species (teleomorph/anamorph)	Isolate number	Genbank accession numbers	
		LSU	ITS
Albonectria rigidiuscula (Berk. & Broome) Rossman & Samuels/*Fusarium decemcellullare* Brick	NRRL 13412	U88104[a]	–
Calonectria kyotensis Teresh./*Cylindrocladium floridanum* Sobers & C.P. Seym.	ATCC 22677	U17408[a]	AF261740[a]
Corallomycetella repens (Berk. & M.A. Curtis) Rossman & Samuels/*Rhizostilbella hibisci* (Pat.) Seifert	CBS 313.72T	AY138481[a]	AY138847[a]
Cosmospora coccinea Rabenh./*"Verticillium" olivaceum* W. Gams	CBS 114050	FJ474079	FJ474072
Cosmospora episphaeria (Tode: Fr.) Rossman & Samuels/ *Fusarium aquaeductuum* (Radlk. & Rabenh.) Lagerh. var. *medium* Wollenw.	NRRL 20687	U88100[a]	–
Cosmospora episphaeria/ Fusarium aquaeductuum var. *medium*	GJS 98–160	–	FJ474073
Cosmospora flammea (Tul. & C. Tul.) Rossman & Samuels/*Fusarium coccophilum* (Desm.) Wollenw. & Reink.	NRRL 20441	U88103[a]	–
Cosmospora vilior (Starbäck) Rossman & Samuels/*Acremonium berkeleyanum* (P. Karst.) W. Gams	ATCC 16217	U57348[a]	U57673[a]
Cyanonectria cyanostoma (Sacc. & Flageolet) Samuels & Chaverri/*Fusarium* sp.	CBS 101734	FJ474081	FJ474076
Fusarium acutatum Nirenberg & O'Donnell	CBS 402.97	AY213704[a]	AY213653[a]
Fusarium lichenicola (Speg.) Sacc. & Trotter	NRRL 34123	DQ236687[a]	DQ094645[a]
Fusarium oxysporum Schltdl.: Fr.	NRRL 22902	U34537[a]	U34566[a]
Fusarium proliferatum (Matsush.) Nirenberg ex Gerlach & Nirenberg	NRRL 31071	AF291060[a]	AF291061[a]
Gibberella subglutinans (E.T. Edwards) P.E. Nelson, et al./*Fusarium subglutinans* (Wollenw. & Reinking) P.E. Nelson, Toussoun & Marasas	NRRL 22016	U34530[a]	U34559[a]
Gibberella moniliformis Wineland/*Fusarium verticillioides* (Sacc.) Nirenberg	NRRL 43697	–	EF453174[a]
Gibberella moniliformis/Fusarium verticillioides	NRRL 22172	AY249379[a]	–
Gibberella pulicaris (Fr.) Sacc./*Fusarium sambucinum* Fuckel	NRRL 20765	X65475[a]	X65482[a]
Haematonectria haematococca (Berk. & Broome) Samuels & Nirenberg/*Fusarium solani* (Mart.) Sacc.	NRRL 22157	AF178390[a]	AF178421[a]
Haematonectria illudens (Berk.) Samuels & Nirenberg/*Fusarium illudens* C. Booth	NRRL 22090	AF178362[a]	AF178393[a]
Hypocrea lutea (Tode) Petch/*Gliocladium viride* Matr. (outgroup)	CBS 102037	U00739[a]	AY737773[a]
Hypocrea rufa (Pers.: Fr.) Fr. /*Trichoderma viride* Pers.: Fr. (outgroup)	CBS 114374	AY489726[a]	X93980[a]
Lanatonectria flavolanata (Berk. & Broome) Samuels & Rossman/*Actinostilbe* sp.	CCFC 216608	AY281098[a]	–
Lanatonectria flocculenta (Henn. & E. Nyman) Samuels & Rossman/*Actinostilbe macalpinei* (Agnihothrudu & Barua) Seifert & Samuels	#76873	DQ119567[a]	–
Leuconectria clusiae (Samuels & Rogerson) Rossman et al./*Gliocephalotrichum bulbilium* J.J. Ellis & Hesseltine	ATCC 22228	AY489732[a]	–
Leuconectria clusiae/Gliocephalotrichum bulbilium	CBS 451.92	–	AF220976[a]
"Nectria" atrofusca (Schwein.) Ellis & Everh. /*Fusarium staphyleae* Samuels & Rogerson	NRRL 22316	AF178392	AF178423
"Nectria" balansae Speg.	AR 4446	FJ474080	FJ474074
Nectria cinnabarina (Tode: Fr.) Fr./*Tubercularia vulgaris* Tode: Fr.	NRRL 20484	L36625	L36626[a]
"Nectria" desmazieri Fuckel/*Fusarium buxicola* Sacc.	NRRL 20474	U88125[a]	–
Nectria pseudotrichia Berk. & M.A. Curtis/*Tubercularia lateritia* (Berk.) Seifert	CBS 102034	U17410[a]	–
Nectricladiella camelliae (Shipton) Crous & C.L. Schoch/*Cylindrocladiella microcylindrica* Crous & D. Victor	CBS 111794	AY793432[a]	–
Nectricladiella camelliae/Cylindrocladiella microcylindrica	CPC 10452	–	AY793453[a]
Neocosmospora vasinfecta E.F. Sm./*Acremonium*-like	NRRL 22468	DQ236360[a]	DQ094318[a]
Neonectria coccinea (Pers.: Fr.) Rossman & Samuels/*Cylindrocarpon candidum* (Link) Wollenw.	CBS 237.29	AY677327[a]	–
Neonectria coccinea/Cylindrocarpon candidum	CBS 291.81	–	FJ474075
Neonectria radicicola (Gerlach & L. Nilsson) Mantiri & Samuels/*Cylindrocarpon destructans* (Zinssm.) Scholten	CBS 156.47	AY677320[a]	AY677272[a]
Neonectria ramulariae Wollenw./*Cylindrocarpon obtusiusculum* (Sacc.) U. Braun	CBS 151.29	AY677333[a]	AY677291[a]
Neonectria sp./*Cylindrocarpon cylindroides* Wollenw.	CCFC 226722	AY283551[a]	–
Neonectria sp./*Cylindrocarpon cylindroides*	CBS 503.67	–	AY677261[a]
Neonectria veuillotiana (Sacc. & Roum.) Samuels & Mantiri/*Cylindrocarpon candidulum* (Sacc.) Wollenw.	GJS 98–145	FJ474082	FJ474077
Pseudonectria rousseliana (Mont.) Wollenw./*Volutella buxi* (DC.: Fr.) Berk.	CBS 114049	U17416[a]	–
Rubrinectria olivacea (Seaver) Rossman & Samuels/*Nalanthamala* sp.	CBS 120617	FJ474083	FJ474078

ATCC American Type Culture Collection, Manassas, VA, USA, *CBS* Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, *CCFC* Canadian Collection of Fungal Collections, Ottawa, ON, Canada, *NRRL ARS* Culture Collection, Peoria, IL, USA

[a] Sequences obtained from GenBank

extracted from mycelium and conidia harvested from the surface of the agar. The PowerPlant™ DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, U.S.A.) was used to extract DNA from the samples. Other sequences used in the analyses were obtained from Genbank (Table 1).

Partial large subunit (LSU) sequences and complete internal transcribed spacers (ITS) 1 and 2 sequences including 5.8S of the nuclear ribosomal DNA were used in the analyses. The primers used for LSU were LROR for the forward direction and LR5 for the reverse direction (Vilgalys and Hester 1990); and for ITS were ITS 1 or 5 for the forward direction and ITS 4 for the reverse direction (White et al. 1990). Each 50-µL PCR reaction consisted of 25 µL Promega Master Mix 2X (Promega, Madison, WI, U.S.A.), 2.5 µL ITS 1 or 5, 2.5 µL LR5, 1 µL of the DNA template, and 19 µL of sterile RNAase-free water. PCR reactions were run in an Eppendorf Mastercycler *ep* using the following parameters: (1) 94°C for 5 min, (2) 35 cycles of 94°C for 1 min, 53°C for 1 min, and 72°C for 1 min, and (3) 72°C for 5 min. PCR products were cleaned using ExoSAP-IT® (USB, Cleveland, OH, U.S.A.) following the manufacturers instructions. Clean PCR products were sequenced using ITS 1 or 5, ITS 4, LROR, and LR5 primers at the DNA Sequencing Facility (Center for Agricultural Biotechnology, University of Maryland, College Park, MD, U.S.A.). Sequences were assembled and edited with Sequencher 4.9 (Gene Codes, Madison, WI, U.S.A.). Sequences have been deposited in GenBank (Table 1).

Phylogenetic analyses

ITS and LSU sequences were aligned with ClustalX 2.0.7 (Thompson et al. 1997). The alignment was improved with Seaview 2.4 (Galtier et al. 1996). Maximum parsimony (MP), maximum likelihood (ML), and Bayesian (BI) analyses were done with all sequences, first ITS and LSU separately, and then with the combined data sets. MP analyses were conducted with PAUP* version b10 (Swofford 2002) using a heuristic search, starting trees obtained by stepwise addition using a simple addition sequence, a TBR branch swapping algorithm, and the MulTrees option ON. Gaps were considered as missing characters. Bootstrap analysis was performed with 1,000 replicates, using a full heuristic search. A 50% majority rule consensus tree was constructed in PAUP*. ML analysis was carried out with DNAML as implemented in PHYLIP 3.67 (Felsenstein 1989). BI was done using MrBayes 3.1.2 (Huelsenbeck et al. 2001, 2002). jMODELTEST (Posada 2008; Posada and Buckley 2004) was used to select the models of nucleotide substitution for ITS and LSU. jMODELTEST calculates the likelihood parameters with PhyML (Guindon and Gascuel 2003). The number of substitution schemes was set to 11, base frequencies +F, rate variation +I and +G, and the base tree for likelihood calculations was set to "ML optimized." A total of 88 models were compared. Once the likelihood scores were calculated, the models were selected according to the Akaike information criterion (AIC). Under the AIC settings, the AICc (corrected for smaller samples) was selected, parameter importances were calculated, and models were averaged, with a 100% confidence interval. After jMODELTEST was run, likelihood settings were set to ITS: GTR + G (Lset base=0.2284 0.2951 0.2454 0.2311; nst=6; rmat=1.3198 1.8489 2.1014 1.0599 3.5141 1.0000; rates = gamma; shape=0.2380; ncat=4; pinvar=0) and LSU: TIM1+I + G (Lset base=0.2390 0.2213 0.3031 0.2367; nst=6; rmat=1.0000 2.3727 0.6274 0.6274 7.7614 1.0000; rates = gamma; shape=0.3070; ncat=4; pinvar= 0.5520). The resulting ML tree was plotted with DRAW GRAM as implemented in PHYLIP. The ML tree was then imported into PAUP and bootstrap analysis was run with 1,000 replicates using the "fast heuristic search." For the BI analysis, four chains and 5,000,000 Markov Chain Montecarlo generations were run and the current tree was saved to a file every 100 generations. Once stability was reached both in terms of likelihood scores and parameter estimation, the first 5,000 trees were discarded ("burn in"). The remaining trees ("post-burn in") were pooled and a 50% majority-rule consensus tree was obtained with PAUP*. Because the ITS and LSU tree topologies were not in conflict using a reciprocal 70% BP threshold (Mason-Gamer and Kellogg 1996; Reeb et al. 2004), only a combined phylogeny is presented (Fig. 1).

Results

Phylogenetic analyses

Sequencing and alignment of ITS and LSU resulted in 638 and 676 bp, respectively, including indels and missing characters. 121 ambiguously-aligned sites in the ITS alignment were excluded from the analyses. The ITS alignment had 325 constant characters, 69 variable parsimony-uninformative characters, and 123 parsimony-informative characters. The parsimony tree scores for ITS were: consistency index (CI) = 0.557, retention index (RI) = 0.634, and homoplasy index (HI) = 0.443. The LSU alignment resulted in 540 constant characters, 32 variable parsimony-uninformative characters, and 104 parsimony-informative characters. The parsimony tree scores for LSU were: CI=0.425, RI=0.646, and HI=0.575. In the combined analyses of ITS and LSU, 865 characters were constant, 101 parsimony-uninformative, and 227 parsimony-informative. The heuristic search for the most parsimonious trees resulted in 448 trees with 913 steps in length (CI=0.483; RI=0.621; HI=0.517). The ML and BI analy-

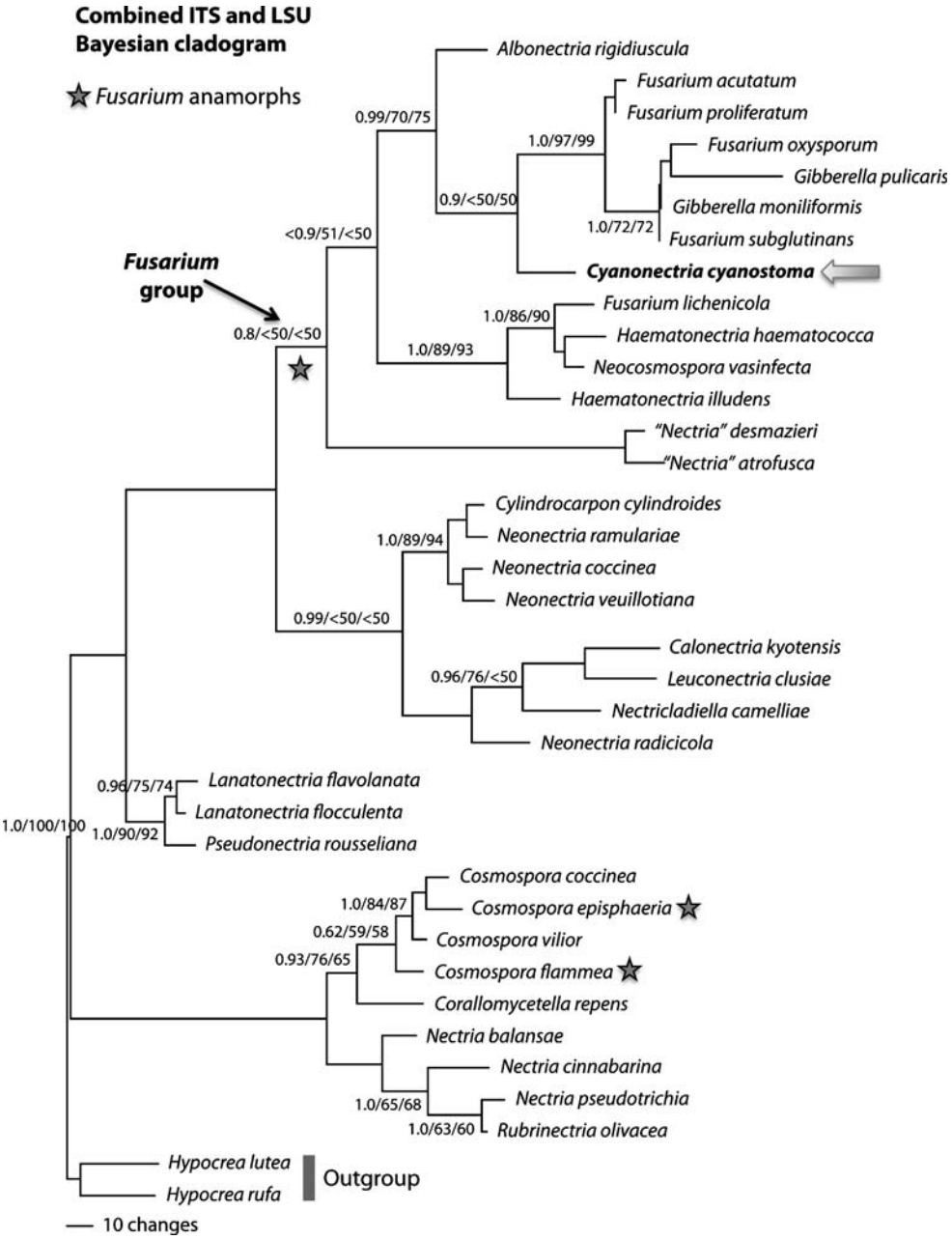

Fig. 1 Combined ITS and LSU Bayesian cladogram (Ln −6,173.158). BI posterior probabilities/ML bootstrap/MP bootstrap indicated at branches. All nodes were present in the 50% majority rule consensus tree. Species with *Fusarium* anamorphs indicated with a *star*. For abbreviations, see text

ses of the combined ITS and LSU data produced one tree each with a Ln likelihood=−71,34.8324 and −6,173.158, respectively. The topologies of the MP, ML, and BI trees are congruent. BP analyses of the combined MP and ML trees show low support for most nodes (Fig. 1). There is high support (>70%) for 13 clades in the MP tree and 14 clades in the ML tree. In the BI analyses, posterior probabilities were ≥0.90 in 20 clades. All clades in the MP tree were present in the 50% majority rule consensus tree; all consensus indices were 100%.

In the MP, ML, and BI analyses, *"Nectria" cyanostoma* is not contained in any of the other known genera/clades with *Fusarium* anamorphs, i.e., *Albonectria, Cosmospora, Gibberella*, and *Haematonectria* (Fig. 1). The clade that contains *"N." cyanostoma* and other genera with fast-growing *Fusarium* anamorphs (i.e., *A. rigidiuscula, F. acutatum, F. proliferatum, F. oxysporum, F. subglutinans, G. moniliformis*, and *G. pulicaris*), excluding *"N." desmazieri* and *"N." atrofusca*, and *Haematonectria* spp., is supported by 75% MP and 70% ML bootstrap), and 0.99 BI posterior probabilities, and is present in the 50% majority rule consensus tree. Thus, the closest relatives of *"N." cyanostoma* are *Albonectria* and *Gibberella*. Given the unique position of *"N." cyanostoma* among these genera having *Fusarium* anamorphs, a new genus, *Cyanonectria*, is established for this species as described below.

From the phylogenetic analyses, it can also be inferred that *Cyanonectria cyanostoma* is not closely related to *Nectria* or *Cosmospora* sensu stricto, two genera morphologically similar to *Cyanonectria* (Fig. 1).

Morphological analyses

Isotype specimens of *Nectria cyanostoma* were issued as Rehm, Ascomycetes exsiccati 2165. A portion of that exsiccate number is preserved as BPI 551652, which we designate here as lectotype. Subsequent collections, cited below, agree in all regards with this lectotype specimen. No anamorph was seen on the lectotype or other specimens. A recently collected specimen, BPI 748307, was grown in pure culture from single ascospores. In order to establish the link between the perithecia, as represented by the lectotype collection, and the *Fusarium* anamorph, we designate BPI 748307 as epitype of *Nectria cyanostoma*. An ex-epitype culture has been deposited in the Centraalbureau voor Schimmelcultures as CBS 101734.

Cyanonectria cyanostoma is known only from southwestern and south-central France and appears to be restricted to twigs of *Buxus sempervirens*. Perithecia of *C. cyanostoma* form in fissures of bark of *B. sempervirens*, not associated with any fungal host. Perithecia of *C. cyanostoma* are superficial on a minute, basal stroma. The perithecial venter is red, becoming darker red in KOH and yellow in lactic acid. The apex is large, broadly rounded and bluish-purple (Fig. 2a,b,d); it becomes darker in KOH and reddish in lactic acid (Fig. 2c,e). The bluish-purple pigmentation may extend partially below the base of the apex. The perithecial wall of *C. cyanostoma* is 15–20 μm wide and comprises two regions (Fig. 2f.). Asci are narrowly clavate (Fig. 2h) and have a conspicuous apical ring (Fig. 2i). Ascospores are equally 2-celled, pale yellow-brown, and finely warted (Fig. 2j).

A *Fusarium* anamorph developed in cultures derived from single ascospores of one collection of *C. cyanostoma* (BPI 748307). Cultures were slow-growing, reaching a diameter of 4 cm on PDA and CMD and 3 cm on SNA after nearly 3 weeks at 25°C. No pigment was observed on any medium. Macroconidia were produced sparsely from pionnotal stromata and had a more or less distinctly pedicellate foot-cell. Microconidia were not observed. Colonies grown on PDA st 25°C were white to cream and entirely slimy with no visible aerial mycelium.

Taxonomy

Cyanonectria cyanostoma is not *Nectria* sensu stricto. Species of *Nectria* have a wide, pseudoparenchymatous perithecial wall; they form on stromata and are usually weak parasites of woody plants. Their anamorphs often form from the same stroma as the perithecia; they are sporodochial, pycnidial or synnematal and are classified in *Tubercularia* (Seifert 1985; Rossman 1989; Rossman et al. 1999).

The morphology of *Cyanonectria cyanostoma* suggests *Cosmospora*. Typical of *Cosmospora*, the perithecial wall of *Cy. cyanostoma* is <20 μm wide, its asci have a distinct apical ring, and the ascospores are pale yellow-brown and finely warted. The main morphological distinction between *Cy. cyanostoma* and species of *Cosmospora* is the unusual 'dual' perithecial pigmentation of the perithecial apex. Perithecia of *Cosmospora* species are uniformly red in KOH and yellow in lactic acid. A second distinction is that *Cosmospora* species typically are fungicolous, with a few species being entomopathogens. The *Fusarium* anamorph of *Cy. cyanostoma* hardly differs from the sect. *Eupionnotes* anamorphs of the many members of *Cosmospora* (e.g., *C. episphaeria/C. purtonii*), although in these species colonies tend to be pink or salmon and overall slimy from conidial production. In *Cy. cyanostoma* only, macroconidia form, and these tend to be somewhat broader than found in many members of *Cosmospora*.

The phylogenetic analyses exclude *Cy. cyanostoma* from *Nectria* sensu stricto, *Cosmospora*, and all other *Fusarium* lineages (Fig. 1). Its closest relatives are members of *Albonectria* and *Gibberella*, both of which have *Fusarium* anamorphs.

Cyanonectria cyanostoma differs from *Albonectria* in perithecial pigmentation and morphology as well as anamorph and biology. Although placed in the Nectriaceae, the three species of *Albonectria* have white to pale yellow perithecia with relatively thick walls, >25 um wide, are often strongly warted, and occur on a well-developed stroma. Unlike the one-septate ascospores of *C. cyanostoma*, the ascospores of species of *Albonectria* are multiseptate, ranging from 3-septate in *A. albosuccinea* (Pat.) Rossman & Samuels and *A. verrucosa* (Pat.) Rossman & Samuels to 5- to 9-septate in *A. rigidiuscula*. The best-known and most common species of *Albonectria is A. rigidiuscula/F. decemcellulare;* it is fast-growing and causes diseases of woody plants especially in tropical and subtropical regions. *Cyanonectria cyanostoma* is limited to *Buxus* in France and does not appear to be pathogenic.

While *Cyanonectria cyanostoma* produces bicolored perithecia that are scarlet red with a bluish-purple apex, species of *Gibberella* have thick-walled perithecia that are entirely bluish-purple, often distinctly warted. *Gibberella* is a relatively large genus with about 50 described species, and even more species of *Fusarium* without known teleomorphs, including *F. oxysporum*, belong to this clade (Fig. 1; Nirenberg and O'Donnell 1998; O'Donnell et al. 1998; O'Donnell 2000; Tan and Niessen 2003; among

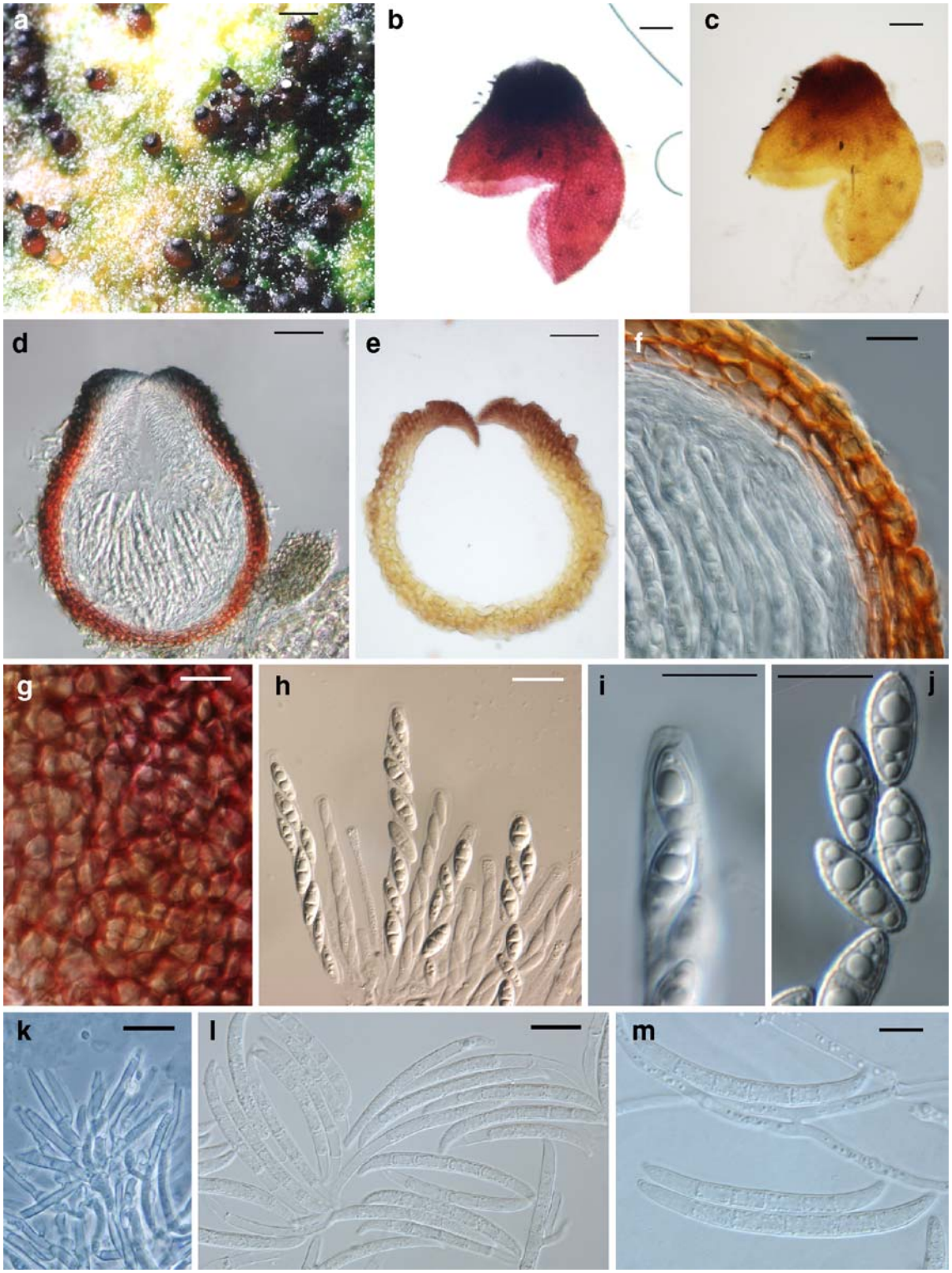

Fig. 2 *Cyanonectria cyanostoma*. **a** Habit of perithecia on bark of *Buxus*. **b**,**c** Crush mounts of perithecia in 3% KOH (**b**) and lactic acid (**c**). **d**,**e** Sections of perithecia in 3% KOH (**d**) and lactic acid (**e**). Note the reddish discoloration of the perithecial apex in **c** and **e**. **f** Median longitudinal section of lateral perithecial wall in 3% KOH. **g** Cells at the surface of the perithecium in face view. **h,i** Asci. Refractive ring in ascus apex seen in **i. j** Ascospores. Note the pale yellow-brown coloration and the fine roughening of the spore wall. **k–m** Anamorph from SNA. **k** Fasciculate phialides. **l,m** Conidia. **a–c,f** from CBS 101734; **d,e,g,i,j** from BPI 878716; **h** from BPI 878714. *Scale bars* **a**=250 μm, **b,c**=50 μm, **d,e**=100 μm, **f–m**=10 μm

others). Most species of *Gibberella* have 3-septate ascospores although 1-septate species are known (Samuels et al. 2001). The *Fusarium* anamorphs are typically fast-growing and include a number of virulent plant pathogens. Although some similarities exist between *Gibberella* and *C. cyanostoma*, the latter is distinct in perithecial color and structure, ascospore septation, characteristics of the anamorph, and pathogenicity.

Haematonectria, which includes *Neocosmospora vasinfecta*, comprises teleomorphs of *Fusarium* sect. *Martiella*, the *F. solani* complex (O'Donnell 2000). Many of these fusaria do not produce a teleomorph. Species of *Haematonectria*, especially *H. haematococca*, occur as plant pathogens, and the ascomata are common in tropical regions especially on newly-killed woody plants. Typical perithecia are entirely scarlet red, superficially warted, and relatively thick-walled, 65–95 μm wide. Unlike *C. cyanostoma*, the perithecial surface is coarsely warted, the warts are formed of thick-walled, angular cells, and in some species the warts can become quite large, over 100 μm tall. The ascospores of species of *Haematonectria* are 1-septate, striate, and become pale yellow-brown. *Cyanonectria cyanostoma* differs from *Haematonectria* in the two-color perithecia, relatively smooth perithecial surface, smooth ascospores that remain hyaline with age, and a *Fusarium* anamorph not in the *F. solani* complex.

The bicolored perithecia of *C. cyanostoma* set it apart from all known ascomycetes. The bluish-purple papilla that becomes red in lactic acid parallels the lactic acid reaction seen in *Gibberella*, whereas the red perithecial venter that becomes yellow in lactic acid is typical of most members of the Nectriaceae. In light of the phylogenetic and phenotypic differences, *Cyanonectria cyanostoma* cannot be accommodated in any of the *Fusarium* lineages nor any known teleomorph genus. Accordingly, we propose the new genus *Cyanonectria* for it.

Cyanonectria Samuels & Chaverri, gen. nov.

Perithecia rubra sed ad apicem cyanea, superficiale, non vel haud stromatica, peridium laterale anguste. Ascosporae flavo-brunneae. Anamorphosis *Fusarium*.

Typus generis

Cyanonectria cyanostoma (Sacc. & Flageolet) Samuels & Chaverri Ascomata typically gregarious, superficial, with or without a minute basal stroma, pyriform; venter red to reddish-brown when dry, dark-red in KOH, yellow in lactic acid; smooth to slightly scaly, apical region bluish-purple, darker in KOH, red in lactic acid. Perithecial wall ≤20 μm wide. Asci unitunicate, cylindrical or broadly cylindrical. Ascospores pale yellow-brown, ornamented. Anamorph *Fusarium*.

Cyanonectria cyanostoma (Sacc. & Flageolet) Samuels & Chaverri, comb. nov. Fig. 2a–m ≡ *Nectria cyanostoma* Sacc. & Flageolet, Rendi Congr. Bot. Palermo 1902: 53. 1902.

Anamorph: Fusarium sp.

Ascomata gregarious, rarely solitary, with or without a minute basal stroma, mycelium not visible, superficial or slightly seated in bark, pyriform, 204–320 μm high, 164–266 μm wide (n=28), laterally collapsed when dry, smooth, rarely with concolouous scales, red to reddish-brown when dry, dark-red in KOH, yellow in lactic acid, with a broadly rounded, "knobby", bluish-purple, slightly constricted apex and apical disc 90–110 μm diam; apical disk darker in KOH, reddish or reddish brown in lactic acid. Cells at surface of perithecial wall circular, 9–22 μm diam, walls ca. 2 μm thick; minute pores common between lumina of adjacent cells. Perithecial wall, including scales, 15–20 μm wide, comprising two intergrading regions. Outer region including scales, 10–15 μm wide, cells circular or tangentially flattened, 7–10 μm long, 3–5 μm wide, walls ca. 2 μm thick, pigmented. Inner region narrow, of tangentially flattened, compressed, thin-walled, hyaline cells lining perithecial cavity. Perithecial apex formed of files of vertically elongated cells originating from inner region of perithecial wall and attaining same length, terminating in wide, more or less clavate, cells ca. 5 μm long, walls 2–3 μm thick, pigmented; files become progressively narrower toward ostiole and merging with periphyses. Asci cylindrical or broadly cylindrical, (35)50–80(97) × (5)7–10 (13) μm (n=109), apex thickened, with an inconspicuous apical pore, eight-spored, ascospores overlapping uniseriate or biseriate above and uniseriate below. Ascospores ellipsoidal, (5.5)10.0–13.0(15.0) × (2.2)3.5–4.7(6.2) μm (n=174), equally two-celled, not or slightly constricted at septum, pale yellow-brown, finely warted.

Characteristics in culture Colonies on SNA 3 weeks, ca. 3 cm diam, white; colony margin highly dissected; aerial mycelium scant, mycelium mostly immersed. Conidia forming sparsely in white pionnotes. Colonies grown on CMD and PDA ca. 4 cm diam, white; colony on CMD similar to SNA but with more conidial production; colony on PDA producing more aerial mycelium, conidia not observed. Conidiogenous cells on SNA develop in fascicles, cylindrical, 20–25 μm long, 3.5–4.0 μm wide. Conidia mainly straight with a slightly hooked tip cell and a more or less pronounced pedicellate foot cell, (1)5–6(7)-

septate. 5-septate: (50)55–69(75) × (5.0)5.7–7.2(8.5) µm (n=29); 6-septate: (58)63–71(76) × (5.2)6.2–7.7(8.0) µm (n=29).

Distribution Southwestern France.

Hosts *Buxus sempervirens* and *Buxus* sp.

Type specimen: France, St. Romain near Rigny, on bark of dry branch of *Buxus sempervirens*, Flageolet n. 32 (PAD). Also, issued as Rehm, Ascomycetes exsiccati 2165 with data: France, Rigny s. Arrouse, on branches of *Buxus sempervirens*, coll. Abbé Flageolet (BPI 551652; LECTOTYPE designated herewith).

Additional specimens examined France, Pyrenées Atlantiques, Isle de Sauveterre de Bearn 64, on *Buxus sempervirens*, Mar 1994, F. Candoussau FC 282 (BPI 737807); same locality, alt. 100 m, on bark of *Buxus* sp., 25 Oct 1998, G.J. Samuels & F. Candoussau (EPITYPE specimen designated herewith: BPI 748307, ex epitype culture CBS 101734=GJS 98–127); Ariège, Rimont, Las Muros, ruisseau de Peyrau, along a little stream, on bark and wood of *Buxus sempervirens*, 11 Sep 1999, J. Fournier JF 99259 (BPI 878714); same locality and host plant, alt. 400 m, 24 Sep 2001, J. Fournier JF 01080 (BPI 878715); Belesta, Forêt Domaniale, mixed fir forest, alt. 900 m, on wood and bark of *Buxus sempervirens*, 29 Sep 2000, J. Fournier JF 00231 (BPI 878716);

Discussion

Based on the phylogenetic analyses of ITS and LSU nrDNA sequences and the morphological comparison to similar taxa, *Cyanonectria cyanostoma* is found to represent a new genus of the Nectriaceae, *Cyanonectria*. Although some clades in the ITS and LSU phylogenetic tree (Fig. 1) are not well supported, two conclusions can be made confidently: 1) *C. cyanostoma* is distinct and separate from the *Albonectria, Gibberella*, and *Haematonectria* clades; and (2) *Cy. cyanostoma* is distinct from *Cosmospora* and *Nectria*.

Cosmospora sensu stricto based on the type species, *C. coccinea*, is more closely related to *Corallomycetella* and *Nectria* s. str., than to *Cy. cyanostoma* (Fig. 1). The closest relatives of *Cy. cyanostoma* are *Albonectria, Gibberella*, and *Haematonectria*. However, *C. cyanostoma* does not group with any of these genera. *Haematonectria*, including *Neocosmospora vasinfecta*, forms a well-supported clade (see also O'Donnell 2000). *Gibberella*, including *F. oxysporum* and other species of *Fusarium* without known teleomorphs also form a clade.

Nowhere else in the Nectriaceae are bicolored perithecia found in which a differential reaction to KOH and lactic acid is manifested, the bluish-purple perithecial apex being typical of *Gibberella* and the red perithecial venter typical of most other genera of the Nectriaceae. The *Fusarium* anamorph could belong to either genus, but it is slimy on PDA and thus more typical of *Cosmospora*. Abbé Flageolet (in Saccardo 1903) wrote to Saccardo that in his garden he had found *Lisea buxi* (Fuckel) Sacc. (≡ *Gibberella buxi* Fuckel, anamorph *Fusarium lateritium* var. *buxi* C. Booth) and *"Nectria" desmazieri* (anamorph *Fusarium buxicola)* as well as *Cyanonectria cyanostoma* and an associated *Fusarium*. Saccardo (1903) considered the *Fusarium* to be indistinguishable from the anamorph of the *Gibberella* and that the bicolored perithecia of *Cyanonectria cyanostoma* offered an excellent example of hybridization between the *Gibberella* and *"N." desmazieri*. Saccardo said that *Cyanonectria cyanostoma* would open the way to new research because it was an actual case of hybridization, not just a suggestion of hybridization. While we do not think that *C. cyanostoma* is a hybrid, its phylogenetic proximity to *Gibberella* suggests that it could have diverged early from that lineage.

Two additional species, *"Nectria" atrofusca/Fusarium staphyleae* and *"N." desmazieri/F. buxicola* were described in *Nectria* but are unlike *Nectria* sensu stricto and have *Fusarium* anamorphic states. *"Nectria" atrofusca* is the cause of twig blight of bladder nut (*Staphylea trifolia* L.) reported from Iowa and the eastern United States (Samuels and Rogerson 1984). The perithecia are purple-red becoming dark brown at maturity, not changing color in KOH or lactic acid. Samuels and Rogerson (1984) noted the dissimilarity in perithecial morphology of this species to other members of the Nectriaceae including those having *Fusarium* anamorphs. This unique placement was confirmed by the sequence data analyzed here (Fig. 1). *"Nectria" desmazieri* as *F. buxicola* is associated with premature leaf fall in *Buxus* and occurs throughout temperate North America and Europe (Booth 1971).

Fusarium is an iconic hyphomycete genus; easily recognized by its peculiar phragmosporous conidia that typically have a foot-shaped basal cell. Moreover, because of the many diseases caused by *Fusarium* species, the genus is deeply integrated into the phytopathology literature. Despite the fact that all fusaria are members of the Nectriaceae, the apomorphic status of the genus ends at the family. As we have noted, several genera of the Nectriaceae, some monotypic such as *Cyanonectria*, have *Fusarium* anamorphs. The *Fusarium* morphology is not monophyletic. A monophyletic *Fusarium* would exclude

Cosmospora. Future taxonomic revisions of *Fusarium* will have to account for this phylogenetic diversity at the generic level. Debate in the systematics community continues as to the merits of adopting just a single name for an ascomycete genus or species, as opposed to the current practice of naming sexual and asexual morphs separately. Among the several sections of *Fusarium* there are often marked biological differences that combine with less obvious morphological characters to define segregate genera. Whether *Fusarium* is dismantled into newly revived and described segregate genera, or whether they are referred to by the corresponding teleomorph name, the genus name *Fusarium* in its current broad sense has limited phylogenetic significance.

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