Phylogeny of *Rosellinia capetribulensis* sp. nov. and its allies (*Xylariaceae*)

J. Bahl[1]
R. Jeewon
K.D. Hyde
Centre for Research in Fungal Diversity, Department of Ecology & Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong S.A.R., P.R. China

Abstract: A new *Rosellinia* species, *R. capetribulensis* isolated from *Calamus* sp. in Australia is described. *R. capetribulensis* is characterized by perithecia immersed within a carbonaceous stroma surrounded by subiculum-like hyphae, asci with large, barrel-shaped amyloid apical apparatus and large dark brown spores. Morphologically, *R. capetribulensis* appears to be similar to *R. bunodes, R. markhamiae* and *R. megalospora*. To gain further insights into the phylogeny of this new taxon we analyzed the ITS-5.8S rDNA using maximum parsimony and likelihood methods. In addition, a morphological dataset also was analyzed phylogenetically to investigate possible affinities. ITS rDNA based phylogenies reveal that *R. capetribulensis* is closely related to other *Rosellinia* species showing closest affinity to *R. arcuata, R. necatrix* and *R. pepo*. However, analysis of *R. capetribulensis* forms an unsupported branch sister to these taxa. Results from the morphological matrix indicate a close morphological affinity to members of *Rosellinia* subgenus *Rosellinia*. Despite that ITS rDNA and morphological analyses present difficulties in constructing a proper phylogenetic framework among *Rosellinia* and allied genera, there is sufficient evidence to support the establishment of the new taxon in the genus *Rosellinia*. The morphological similarities and differences between *R. capetribulensis* and allied genera such as *Astrocystis* and *Entoleuca* are also briefly discussed.

Key words: *Astrocystis, Entoleuca, Nemania*, phylogeny, rDNA, taxonomy, *Xylariaceae, Xylariales*

INTRODUCTION

The genus *Rosellinia* De Notaris (1844) is an important cosmopolitan genus because its species may cause many plant diseases (Whalley 1996). Members of the genus also are known to be endophytic or saprobic on a variety of plants (Petrini 1992, Smith and Hyde 2001). For over a decade, the

research of the fungi occurring on palms has shown this particular substrate to be a source of fungal diversity (Fröhlich and Hyde 2000, Taylor and Hyde 2003). In continuing studies, we discovered saprobic fungi on fronds of various palm species (i.e., *Archontopheonix, Calamus, Livistona*) in Northern Queensland and revealed a number of unique fungi. We describe a new species in the genus *Rosellinia* from *Calamus* sp.

Most work on *Rosellinia* has focused on species from different geographical regions. Petrini (1992, 2003) compared *Rosellinia* species from temperate zones and New Zealand. Rogers et al (1987) noted the rarity of *Rosellinia* species in tropical rain forests of North Sulawesi, Indonesia. In studies of fungi from palm hosts, Smith and Hyde (2001) indexed twelve *Rosellinia* species from tropical palm hosts. *Rosellinia* species are not frequently isolated when compared to other xylariacieous fungi recorded from palm leaf litter. Most *Rosellinia* collections have been isolated from deciduous woods or dicotolydenous hosts (e.g. Martin 1967; Rogers 1979; Petrini 1992, 2003).

Taxonomic concepts of *Rosellinia* proposed by Petrini (1992) are followed in this study. Species are delineated based on ascospore morphology. According to Petrini (2003) this is the most stable morphological character. However, character combinations of subiculum, anamorph, stromatal characters and microscopic features of the ascus and spores are used to characterize subgenera and species groups. *Rosellinia* subgenus *Rosellinia* as restricted by Petrini (1992, 2003) is characterized by stromata developing within a false subiculum, usually uniperitheciate, hard ectostroma, entostroma reduced at the base and perithecia mostly detached from stromata wall. Contained within the stromata, asci with apical apparati staining blue in Melzer's reagent and containing unicellular ascospores or with a hyaline dwarf cell, germ slit, cellular appendages and/or mucous sheath may be present. The other subgenera are *Corrugata* and *Calomastia* (Petrini 1992). Petrini and Petrini (2005) outlined this current classification, primarily based on character combinations of spore morphology, anamorph and ascus apical apparatus.

Generic delineations of related genera within the *Xylariaceae* are not well defined. *Amphirosellinia* Y.-M.Ju, JD.Rogers, H.-M.Hsieh & Vasilyeva, *Astrocystis* Berk. & Broome, *Colludiscula* I.Hino & Katum., *Entoleuca* Syd., *Guestia* GJD.Smith & K.D.Hyde, *Halorosellinia* (S. Schatz) Whalley, EGB.Jones,

K.D.Hyde & Læssøe, *Helicogermslita* Lodha & D.Hawksw., *Nemania* SF.Gray and *Stilbohypoxylon* Henn. are closely related to *Rosellinia* (Petrini 2003, Ju et al 2004). A number of characters are shared amongst these genera such as apical ring and spore morphology. The perithecia of these genera are immersed within well-developed stromatic tissue. Various authors have discussed the similarities amongst these genera (Petrini and Rogers 1986; Ju and Rogers 1990; Petrini 1992, 2003; Læssøe and Spooner 1994, Rogers and Ju 1996; Smith et al 2001; Ju et al 2004). In this study we will restrict our investigation to the relationship of *Rosellinia* subgenus *Rosellinia* with *Astrocystis*, *Entoleuca* and *Nemania*. This study also provides some preliminary insights into the natural groupings of these genera based on ITS-5.8S sequence analysis.

Petrini (1992, 2003) has made significant contributions to the taxonomy of *Rosellina*. This study also will examine the relationships of *Rosellinia* with other morphologically similar genera using previously published datasets based on numerical taxonomy and ITS-5.8S rDNA sequence data.

MATERIALS AND METHODS

Collection.—The current study is based on collections made during the rainy season (March 2003) from Queensland, Australia. Decayed fronds from various palm species were collected from Daintree National Park, Cape Tribulation (approximately 150 km north of Cairns) and examined for fungi after incubation. Ascoma contents were mounted in sterile water and viewed using an Olympus light microscope. Twenty-five ascospores, 15 asci and 15 paraphyses were measured and means calculated. Rings were tested for an amyloid reaction with Melzer's reagent and presence or absence of a sheath was determined with India ink. Attempts to isolate the fungus in pure culture were unsuccessful. Spores did germinate, but failed to grow.

DNA extraction, PCR amplification and sequence assembly.—DNA was extracted from herbarium specimens using the Chelex method, modified from Walsh et al (1991) and Hirata and Takamatsu (1996). Ascospores were picked up using fine forceps, suspended in 300 μL of 5% Chelex solution (Bio-Rad, Richmond, California), disrupted and content vortexed thoroughly for 1 min. Tubes were incubated at 100 C for 10 min and vortexed again 1 min to allow maximum disruption and incubated for an additional 5 min at 100 C. Contents were centrifuged at a speed of 13 000 g for 2 min and the supernatant was transferred to a new tube. The sample was further incubated at the same temperature, vortexed and centrifuged and supernatant used for amplification reactions.

PCR amplification reactions consisted of 5 μL Promega 10× reaction buffer with $MgCl_2$, 4 μL of 2.5 μM dNTPs, 0.5 μL of 0.01% BSA, 0.5 μL of 1% PVP, 1.5 μL of 10 mM of each flanking primer, 33.7 μL sterile distilled H_2O and 0.3 μL of 5 u of Taq polymerase. Seven to 12 ng of DNA was used for the reaction; the volume of distilled H_2O was adjusted for a final volume of 54 μL. ITS4 and ITS5 primers as defined by White et al (1990) were used. PCR conditions were 3 min hot start (95 C) followed by 45 cycles of 1 min at 95 C, 50 s at 50 C and 1 min at 72 C followed by 10 min extension period at 72 C. PCR product was purified with GFX™ purification kit (27-9602-01, Amersham Biosciences) according to the instructions provided. The purified product was sequenced using an ABI 3730 autosequencer at The Genome Research Centre, The University of Hong Kong using primers ITS4 and ITS5.

Phylogenetic analysis.—Taxa from the *Xylariaceae*, representing the most commonly studied genera, plus additional taxa from the *Amphisphaeriaceae*, were included for various analyses. Multiple alignment of the data was performed using ClustalX 1.83 with a gap penalty of 14.00 and an extension penalty of 5.66. Manual optimization was done where necessary. Ambiguous regions were excluded from analysis unless alignments could be recoded for parsimony analysis (discussed below).

All tree searches were conducted using PAUP* 4b10 (Swofford 2002), unless otherwise stated. Parsimony analysis of the morphological data set published by Petrini (1992) with the addition of the new species *Rosellinia capetribulensis* and *Astrocystis bambusae*, *A. cocoes* and *A. mirabilis*. Analysis for this data set was conducted followed Petrini (1992). Except, taxa with more than 20% data missing were excluded from this analysis.

Maximum parsimony and maximum likelihood analyses were conducted to test the appropriate placement of the new taxa in the genus *Rosellinia* and extract the maximum amount of phylogenetic information from the ITS data set. Weighted parsimony, treating the gaps as a fifth character, and recoding ambiguous regions was conducted using 100 heuristic search replicates using the tree bisection reconnection swapping algorithm with random addition sequence and a random starting tree. INAASE (Lutzoni et al 2000) was used in order to recode the ambiguous regions without violating positional homology. Step matrices to account for transition/transversion (Ti/Tv) ratios were created based on the data set using STMatrix (Lutzoni and Zoller 2001, Dept. of Biology, Duke University, Miadlikowska et al 2002). Four regions were subsequently recoded and were incorporated into the data set. Branching support statistics were generated by bootstrapping the dataset (100 bootstrap replicates of one heuristic search).

For the maximum likelihood search Mrmodeltest version 2.0 (Nylander 2004, http://www.ebc.uu.se/systzoo/staff/nylander.html) was used to determine the appropriate evolutionary model for likelihood search. Bayesian searches were conducted on the program MrBayes 3.0b4 (http://morphbank.ebc.uu.se/mrbayes/, Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). Node support was determined by conducting a Bayesian tree search and presented as posterior probabilities. The apriori assump-

tions of the appropriate evolutionary model as determined by Mrmodeltest 2.0 (Nylander 2004) were maintained for the Bayesian as well as maximum likelihood analyses. Maximum likelihood (ML) trees were saved and compared using likelihood optimality criteria with trees generated by parsimony analyses. Trees were statistically compared using Kishino-Hasegawa test (KH test) (Kishino and Hasegawa 1989) and nonparametric Templeton Test (Templeton 1983) as implemented in PAUP.

RESULTS

Rosellinia capetribulensis J. Bahl, R. Jeewon, & K.D. Hyde. sp. nov.   FIGS. 1–8

Etymology. In reference to the collection locale, Cape Tribulation, Queensland, Australia.

Stroma nigra, superficialia niger, globosa (0.82 mm, n = 15), per subiculum perdurans, atrobrunneus (FIGS. 1–2). Asci, clavate, breviter pedicellati, unitunicati, octosporim 270–290 × 45–65 μm (280.3 × 54.2 μm, n = 15), apparatu apicali bene evoluto, iodo colorem caeruleum ducens (ca. 21.3 × 14.1 μm, n = 15) (FIGS. 3–6). Ascosporae fusiformia atrobrunneus germinali longitudinali per totam 103–155 × 15–22.5 μm (124 × 17 μm, n = 25) (FIGS. 7 and 8). Paraphyses numerosae, filiformes.
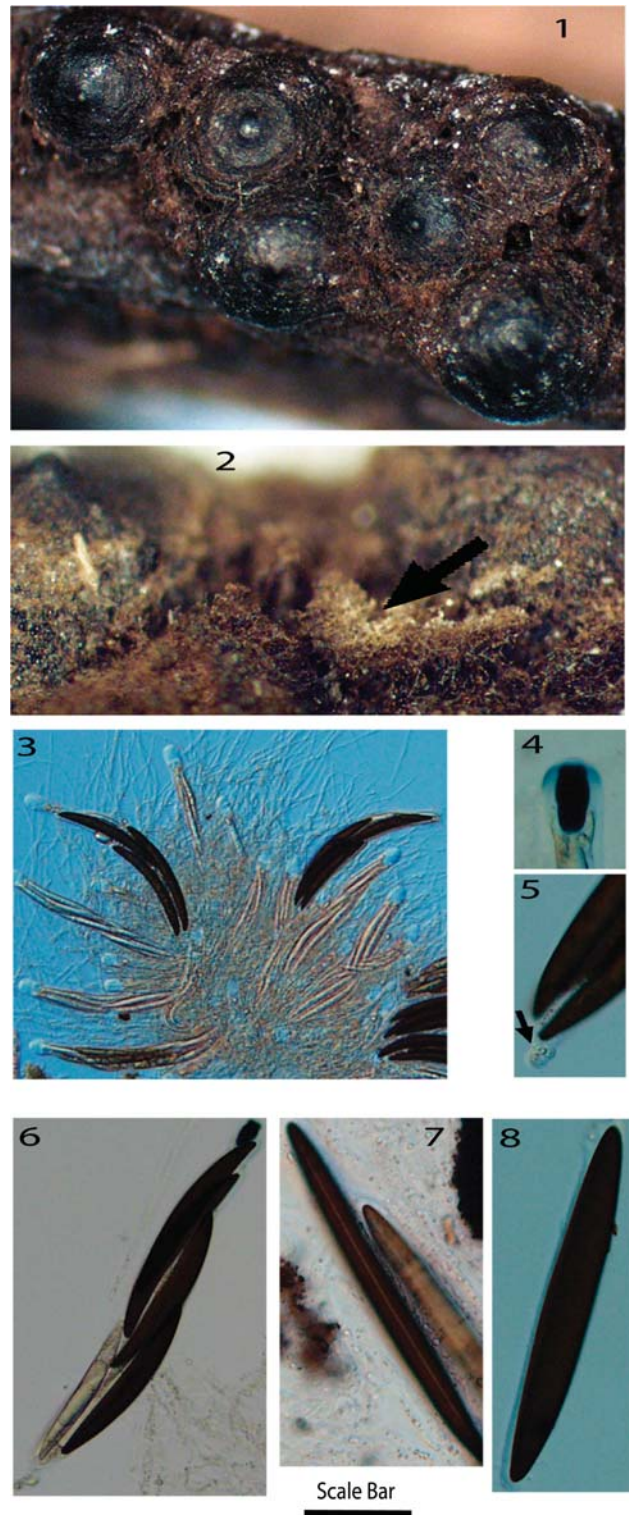
Ascomata on palm rachides appear as superficial black domes with a central ostiole surrounded by persistent dark brown to black superficial hyphae (subiculum-like) within a well-developed carbonaceous stroma (0.82 mm, n = 15) (FIGS. 1 and 2). Peridium easily separated from the stroma in fresh material. *Paraphyses* persistent at maturity, hypha-like, tapering slightly toward the rounded apex, ca. 4.75 μm at the base and 2.5 μm at tip. Asci 270–290 × 45–65 μm (280.3 × 54.2 μm, n = 15), 8-spored, clavate, short pedicellate, apically rounded, with a large non-refractive, amyloid, barrel-shaped, subapical apparatus (ca. 21.3 × 14.1 μm, n = 15) (FIGS. 3–6). Ascospores 103–155 × 15–22.5 μm (124 × 17 μm, n = 25), overlapping bi-seriate, fusiform, tapering slightly at the apices to rounded ends, dark brown to black at maturity, unicellular, smooth, with a germ slit running the entire length of the spore, possessing a thin, inconspicuous sheath (FIGS. 7 and 8). Gene reference nuclear encoded ribosomal DNA sequence of ITS-5.8s sequence (GenBank accession no. AY862570).

Anamorph. Unknown.

Habitat. Saprobic on decaying rachis of *Calamus* sp.

Specimens examined. Australia. Queensland: Cape Tribulation, Daintree National Park, decaying rachis of *Calamus* sp., March 2003, J. Bahl CCT008 (Holotype HKU[M] no 17499).

Phylogenetic analysis. The morphological data set

FIGS. 1–8. *Rosellinia capetribulensis* (from holotype). 1 Ascoma as seen on host (*Calamus* sp.). 2. Superficial hyphae (arrow). 3. Asci and paraphyses. 4. Asci. 5. Apical apparatus (stained in Melzer's reagent). 6. Ascus base showing the pedicel (arrow). 7. Ascospore showing thin sheath. 8. Ascospore with germslit. Bar: 1 = 0.1 mm, 2 = 0.25 mm, 3 = 90 μm, 4 = 40 μm, 5–8 = 25 μm.
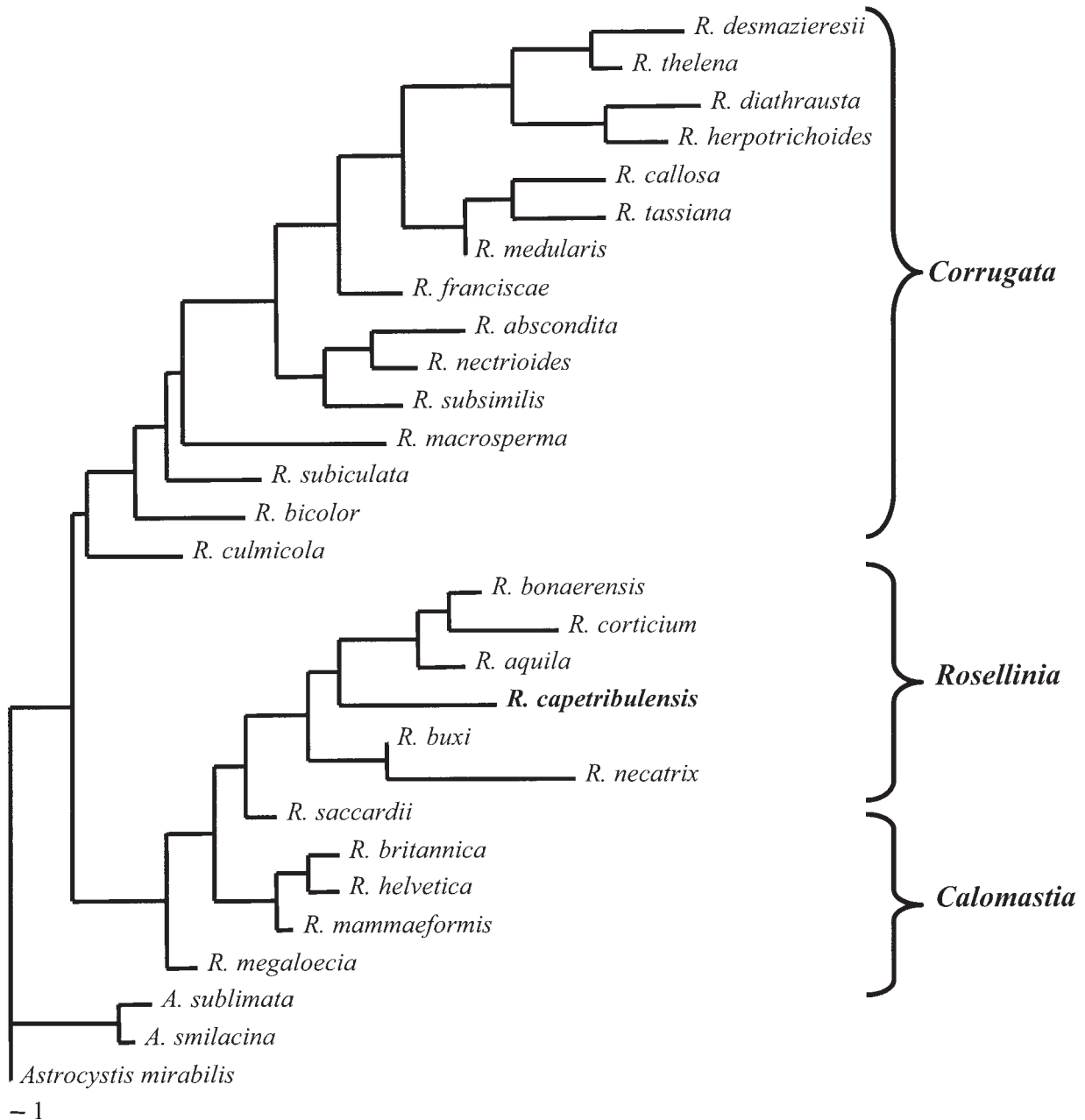
R. desmazieresii
R. thelena
R. diathrausta
R. herpotrichoides
R. callosa
R. tassiana
R. medularis
R. franciscae
R. abscondita
R. nectrioides
R. subsimilis
R. macrosperma
R. subiculata
R. bicolor
R. culmicola

Corrugata

R. bonaerensis
R. corticium
R. aquila
R. capetribulensis
R. buxi
R. necatrix

Rosellinia

R. saccardii
R. britannica
R. helvetica
R. mammaeformis
R. megaloecia

Calomastia

A. sublimata
A. smilacina
Astrocystis mirabilis

− 1

FIG. 9. One of two equally parsimonious trees generated using 31 morphological characters.

consisted of 31 characters from 35 taxa. All gaps were treated as missing and the analysis resulted in two equally parsimonious trees (FIG. 9). Both trees showed *R. capetribulensis* nested within *Rosellinia* subgenus *Rosellinia.* Apomorphic characters of each subgenus are addressed in detail in Petrini (1992). Amongst the subgenus *Rosellinia, R. capetribulensis* shares the following apomorphic characters: globose stromata, papillate ostiole, with cracks in the stromata, a large apical apparatus, with long and wide ascospores. Of these characters, the apical

apparatus and spore length and width are unique amongst the taxa used in this analysis.

The ITS data set consisted of 722 characters from 24 taxa, of which 280 were excluded. Four regions were recoded using INNASE (Lutzoni et al 2000) and included for parsimony analysis. Phylogeny obtained from parsimony analysis (gapmode = newstate; Ti/Tv approximately 2 : 3 and 4 recoded regions) is chosen to represent the relationships of *Rosellinia capetribulensis* with *Rosellinia* subgenus *Rosellinia* and allies. Parsimony informative characters were approximately

34%. The search resulted in a single tree (FIG. 10, TreeBase accession no. SN2160). Phylogeny under these criteria/parameters gave better bootstrap support for the groupings described. Bayesian values greater than 95% are shown on corresponding nodes. *Rosellinia capetribulensis* showed close affinities to other *Rosellinia* species (referred as clade A in tree). However, it formed an unsupported sister branch that was basal to this clade. Clade A includes *Entoleuca mammata*, which groups closely with *R. aquila*, the type of *Rosellinia*. Related genera *Nemania* and *Astrocystis* formed monophyletic clades respectively. However, there was little statistical support between these genera and Clade A.

Maximum likelihood searches resulted in a single tree while Bayesian searches using four chains saved 10 001 trees for each Bayesian search. The first 1000 trees saved were eliminated as the burn in number for the chains was determined to be within the first 600 trees sampled. Statistical tests showed no significant differences between search treatments (results not shown). Variation of node support was the major difference between trees.

DISCUSSION

Justification for the new species.—Characters that define *Rosellinia capetribulensis* are: presence of a subiculum, shape and size of the stroma, the large, well-developed barrel-shaped, amyloid ascal apical apparatus, and large fusiform ascospores with a narrow sheath and a germ slit running entire length of the ascospore. These characters agree well with the taxonomic concept of Rosellinia (Ju and Rogers 1990; Petrini 1992, 2003; Læssøe and Spooner 1994; Smith and Hyde 2001). The rings are well developed, especially in taxa with large ascospores (Petrini 2003). Spore characters are most important in differentiating species (Petrini 2003), and there are few Rosellinia species that could be confused with *R. gigantispora*. Morphologically *R. gigantispora* is most similar to *R. bunodes* (Berk. and Broome) Sacc., 1882 (González and Rogers 1995) and *R. megalospora* (Saccas 1956). However, the ascospores of *R. bunodes* taper to fine apices (ca. 1.5–2 μm) and have a germ slit much shorter than its spore length compared to the relatively blunt spore ends in *R. capetribulensis* and the full-length germ slit. Furthermore, in *R. capetribulensis* the ascus is clavate compared to the cylindrical the ascus of *R. bunodes*. The ascomata in *R. capetribulensis* also lack brown scale ornamentations present on ascomata of *R. bunodes*. Although the ascospores in *R. bunodes* and *R. capetribulensis* overlap in length, the spores of *R. bunodes* are 5–12 μm wide, whereas those in *R. capetribulensis* are 15–22.5 μm wide. *R. capetribulensis* and *R. megalospora* share a number of morphological characters and confusion between these taxa could arise. Primarily, the perithecia of *R. capetribulensis* are easily separated from the stroma in fresh material. The stroma of *R. megalospora* is densely gregarious and subglobose to pyriform, whereas *R. capetribulensis* is globose and sparse and sometimes in groups. The asci of both taxa are large to accommodate the large spores within and both have rounded apices and short pedicels. However, the shape of the ascus in *R. capetribulensis* is clearly clavate, whereas the asci of *R. megalospora* are cylindrical to cylindric-clavate. The dimensions of the asci are 185–230 × 24–32 μm in *R. megalospora* compared to 270–290 × 45–65 μm in *R. gigantispora*. The apical apparatus of both genera are large and amyloid and are similar in dimensions. However, the apical apparatus of *R. megalospora* yellows with age while that of *R. capetribulensis* remains hyaline at maturity. However, the apical ring of *R. megalospora* appears to show high variability in shape and dimensions at different developmental stages. Furthermore, the shape of the apical ring, although similar, appears to taper at the base whereas the apical ring of *R. capetribulensis* has similar width at the apex and base. The spores show similar morphology. The apices of ascospores in *R. megalospora* taper to finer ends compared to *R. gigantispora*, which have blunt ends. Spores of *R. capetribulensis* are 124 × 17 μm compared to 76.5 × 11.6 μm for taxa of *R. megalospora*. In addition, presence of a mucilagenous sheath does not seem to be a characterization of ascospores from *R. megalospora*.

The apical ring of *Rosellinia capetribulensis* is exceptionally large and typically barrel-shaped. The apical ring of *Rosellinia* subgenus *Rosellinia* is typically taller than broad. Shape of the ring varies slightly from cuboid to barrel shaped to slightly urn shaped. The large well-developed ring most likely facilitates the ejection of the large spores (Rogers 1979, Petrini 2003). The pedicel is rather short in *R. capetribulensis* as compared to other *Rosellinia* species (Smith and Hyde 2001, Petrini 2003)

Analysis of numerical data shows that *R. capetribulensis* is nested within the subgenus *Rosellinia* as proposed by Petrini (1992). Analyses of ITS-5.8s rDNA data concurs that this is the best placement amongst taxa sampled. Further investigations into the ITS-5.8s data set (parsimony, model based maximum likelihood) revealed little further information regarding the generic affinities of these taxa. These are explored and reviewed below.
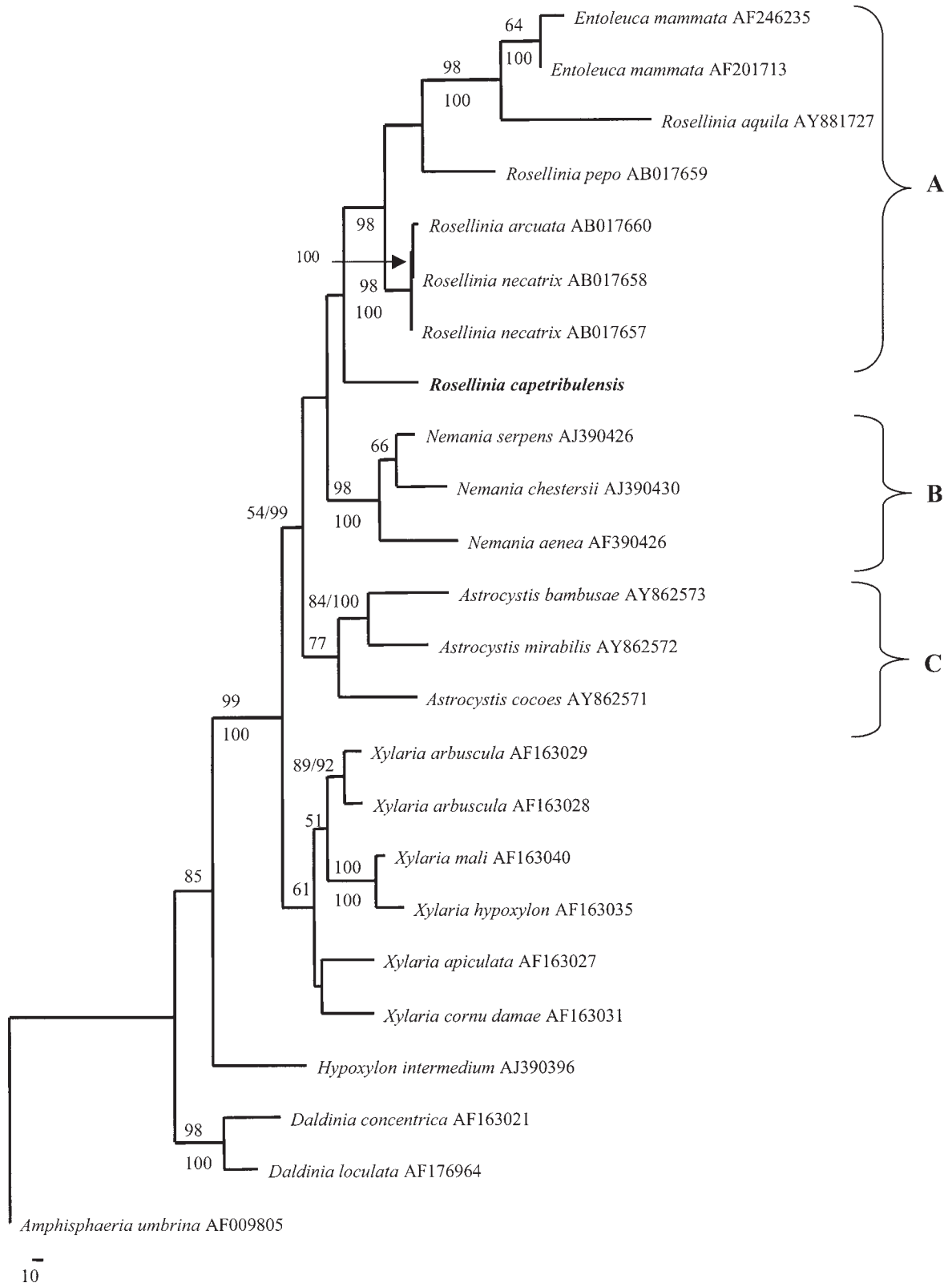
FIG. 10. Tree generated using optimality criteria based on 442 aligned characters of the ITS-5.8s regions from 24 taxa; TL = 1480.90, CI = 0.597, RI = 0.603. Numbers represent Bootstrap (value) support (above branches)/Bayesian posterior probabilities (value) (below branches or following a backslash [/]) >50 % and >90 % respectively.

Phylogeny from the ITS sequence data contain three major clades (A, B and C). Clade A is characterized by *Entoleuca mammata* and *R. aquila, R. arcuata, R. necatrix, R. pepo* with *R. capetribulensis* forming an unsupported sister branch. This clade is supported by high bootstrap/ Bayesian posterior probabilities. In all phylogenetic analyses, ITS sequences alone do not appear to be reliable in resolving interspecific relationships. However, analysis does indicate that *Entoleuca mammata* should be reassigned to *Rosellinia* subgenus *Rosellinia*. Morphologically, this is supported by the carbonaceous stroma, amyloid, apical apparatus which is taller than broad, pigmented ascospores with a straight germ slit and anamorph type. However, stromata of *Rosellinia* species are most frequently unitheciate and sometimes fused with neighboring stromata, but *Entoleuca* has multitheciate stromata. Currently, there are more than 100 *Rosellinia* species with a global distribution (Kirk et al 2001), and molecular data available is minimal. Analyses of the data including species available which do not fall into the subgenus *Rosellinia* failed to provide further information on the monophyly of the subgenus. *Rosellinia desmazieri* typifies the subgenus *Currugata* as proposed by Petrini (1992). However, inclusion into the analysis was uninformative (results not shown). Clades B and C are defined by the monophyletic groupings of *Nemania* and *Astrocystis* respectively. The larger clade consisting of *Astrocystis, Entoleuca, Nemania* and *Rosellinia* share a number of morphological similarities and are discussed in detail below.

Based on ITS data, *R. capetribulensis* shows affinity to members of a complex of genera that taxonomically are disputed (i.e. is *Astrocystis* a synonym of *Rosellinia*), but relationships are not statistically supported. Many of the genera grouped within this clade have at one time or another been assigned to *Rosellinia*. Common taxonomic characters and morphological overlap of the complex does not exclude the possibility of a common evolutionary ancestor.

Rosellinia versus *Astrocystis*. *Astrocystis* and *Rosellinia* are morphologically similar genera. However, differences do exist (Læssøe and Spooner 1994, Petrini 2003). Læssøe and Spooner (1994) considered differences in ascus apparatus, ascus pedicel length and the type of the anamorph to be most significant in generic delineation. The apical apparatus in *Astrocystis* is relatively small with parallel or slightly tapering sides. However, *Rosellinia* species have relatively massive and barrel-shaped apical apparati (Læssøe and Spooner 1994). The most striking difference between *Astrocystis* and *Rosellinia* is the anamorph type. In *Astrocystis* the anamorph has been recognized as the form genus *Acanthodochium*

(Samuels et al 1987, Rogers 2000), whereas *Dematophora* and *Geniculosporium* anamorphs have been assigned to *Rosellinia* (Rogers 1979; Petrini; 1992, 2003; Læssøe and Spooner 1994; Ju and Rogers 1990). Another difference is the presence or absence of a subiculum (Ju and Rogers 1990; Læssøe and Spooner, 1994). We follow the definition for a subiculum provided by Læssøe and Spooner (1994) where in *Rosellinia* species, it is actually a "false subiculum" where the ascomata are borne directly on the host tissue but surrounded by or embedded within subiculum-like hyphae rather than developing directly upon a mat of hyphae. Ju and Rogers (1990) believed that the presence or absence of a subiculum is an adaptation to the substrate and therefore not important in the generic delineation, and considered *Astrocystis* and *Rosellinia* congeneric. This relied heavily on the argument that *Astrocystis bambusae* and *A. mirabilis* belonged within *Rosellinia* regardless of different developmental and anamorphic characters (Ju and Rogers 1990, Rogers 2000). Phylogenetically, based on analysis of ITS, *Astrocystis* species forms a monophyletic clade, that is strongly supported (Clade C, 77% bootstrap support). Due to developmental and anamorphic differences as well as the topology of the *Rosellinia*-complex we prefer to maintain the delineation of *Astrocystis* and *Rosellinia*.

Entoleuca *and* Nemania. Ju and Rogers (1990) did not accept *Astrocystis* as a valid genus and synonymized it with *Rosellinia*. Although subsequent authors did not agree (Petrini 1992, Læssøe and Spooner 1994), Rogers and Ju (1996) maintained this synonymy in their examination of *Entoleuca*. They believed that the type of *Astrocystis, A. mirabilis* had the same developmental type as *Rosellinia*. Petrini (1992), however, stated that the developmental type found in *Astrocystis mirabilis* is not seen in any species of *Rosellinia*. Rogers and Ju (1996) also discussed the differences between *Astrocystis* and *Entoleuca* reporting that a subiculum is lacking in the development of *Entoleuca* and therefore it may be similar to *Astrocystis*. The main difference between *Astrocystis* and *Entoleuca* is that, in *Entoleuca* the stroma develops below the bark of the host and is initiated by specialized coremoid pegs that break through the overlying bark, whereas in *Astrocystis* the ascoma develops below the anamorph and then displaces it (Rogers and Berbee 1965, Rogers and Ju 1996). Furthermore, in *Entoleuca mammata* (described in detail as *Hypoxylon pruinatum*) the anamorph develops on a sub-epidermal subiculum and not after ascomata development (Rogers and Berbee 1965). Differences between *Entoleuca* and *Rosellinia* are less clear. The teleomorphic stage of both genera is initiated below the surface of the host and the

anamorph forms on a subiculum. However, the specialized coremoid pegs have not been described in *Rosellinia* and the importance of the relative position of the ascomata to the host surface (erumpent becoming superficial versus erumpent) is not clear.

We included the type of *Rosellinia* (*R. aquila*) and *Entoleuca mammata*, which has previously been included in *Hypoxylon*. However, we were unable to obtain a culture of *E. callimorpha*, the type of the genus. In FIG. 10 *Rosellinia pepo* and *R. aquila* group with *Entoleuca mammata* and other *Rosellinia* species with 98% Bayesian posterior probability support. This indicates that *Entoleuca mammata* may be a species of *Rosellinia*. However, the multiperitheciate nature of *E. mammata* is considered an important character in excluding it from *Rosellinia*, as *Rosellinia* species are typically uniperitheciate (Petrini 1992, Rogers and Ju 1996). If *E. mammata* is synonymous with *Rosellinia* this suggests that the uniperitheciate definition of *Rosellinia* needs to be revised and that stromatal morphology of the genus might be more diverse than previously thought.

Confusion surrounds the validity of *Entoleuca*. Læssøe and Spooner (1994) did not accept *Entoleuca* as a valid genus and synonymized it with *Hypoxylon*. Based on comments by Miller (1961) and Martin (1967) they considered the type of the genus, *Entoleuca callimorpha* to be a synonym of *Hypoxylon mammatum* (= *E. mammata*). However, they did not examine the type material of *E. callimorpha* and suggested that *H. mammatum* (= *E. mammata*) was better placed within *Rosellinia*. Rogers and Ju (1996) resurrected *Entoleuca* based on examination of *E. callimorpha*, considered the species to be distinct from *H. mammatum* and transferred it to *Entoleuca*. *Entoleuca* bears a number of similarities with *Nemania* and *Rosellinia*. Developmentally, these genera are characterized by white stromatal tissue that surrounds the perithecia within the stromata. This tissue later darkens or deteriorates during maturity in many species (Rogers and Ju 1996). Furthermore these genera share a common anamorph type. The inclusion of *E. callimorpha* in future studies should clarify the relationship of this genus with others of the *Rosellinia*-complex.

Apical ring morphology appears to be of limited use in determining relationships between various other xylariaceous fungi (Læssøe and Spooner 1994, Smith and Hyde 2001). However, the apical rings of *Astrocystis, Entoleuca, Nemania* and *Rosellinia* all have rings that are typically greater in length than width. The apical ring of members of *Rosellinia* may be useful when associated with other characters. *Astrocystis* and *Nemania* have apical rings that closely resemble each other. However, *Rosellinia* tends to have apical rings that are similar in shape and dimensions to those of *Xylaria* when compared to the rings of *Astrocystis* and *Nemania* (Rogers and Samuels 1986, Læssøe and Spooner 1994, Smith and Hyde 2001). Furthermore, the apical ring of *Entoleuca mammata* is massive and more tall than broad (Rogers and Ju 1996). This character frequently occurs in species of *Rosellinia*.

Limited success in delineating genera within the *Xylariaceae* has been achieved by phylogenetic analysis using large and small subunits of the nuclear encoded ribosomal DNA (Smith et al 2003). The combination of multiple genes from different loci and morphology is needed to clarify relationships within this family. Further studies using a polyphasic approach and including data from secondary metabolites may help clarify the natural groupings of the genus *Rosellinia* and the putative monophyly of the genus with relation to other related taxa.

LITERATURE CITED

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