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FULL PAPER

Dolabra nepheliae on rambutan and lychee represents a novel lineage of phytopathogenic Eurotiomycetes

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Abstract Rambutan (*Nephelium lappaceum*) and lychee (*Litchi chinensis*) are tropical trees in the Sapindaceae that produce delicious edible fruits and are increasingly cultivated in tropical regions. These trees are afflicted with a stem canker disease associated with the ascomycete *Dolabra nepheliae*. Previously known from Asia and Australia, this fungus was recently reported from Hawaii and Puerto Rico. The sexual and asexual states of *Dolabra nepheliae* are redescribed and illustrated. In addition, the ITS and large subunit of the nuclear ribosomal DNA plus fragments from the genes *RPB2*, *TEF1*, and the mitochondrial small ribosomal subunit were sequenced for three isolates of *D. nepheliae* and compared with other sequences of ascomycetes. It was determined that *D. nepheliae* represents a new lineage within the Eurotiomycetes allied with *Phaeomoniella chlamydospora*, the causal agent of Petri grapevine decline.

Keywords Canker · *Litchi* · *Nephelium* · *Phaeomoniella chlamydospora* · Sapindaceae · Systematics

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Introduction

Rambutan (*Nephelium lappaceum* L.) and lychee (*Litchi chinensis* Sonn.) are tropical trees in the Sapindaceae that produce delicious edible fruits. Although these plants originated in Asia, they have been widely cultivated as garden fruit trees throughout the tropics and are now being developed as crop plants for commercial exploitation. Rambutan flourishes from sea level to 600 m in tropical, humid regions having evenly distributed rainfall (Morton 1987; Tindall 1994). Depending on the location, rambutan can produce up to two crops a year (Laksmi et al. 1987). Recently, production of rambutan and lychee has increased in Australia as well as in Hawaii and Puerto Rico where improved plant germplasm for disease resistance and increased fruit production is being investigated. In Hawaii, about 1 million kg of these tropical specialty fruits valued at $4.5 million were grown in 2007, with longan, lychee, and rambutan garnering higher farm prices compared to other fruit crops in this niche market of exotic fruit (NASS-USDA 2008).

A number of fungal diseases attack both *Nephelium lappaceum* and *Litchi chinensis*. One of these diseases is a stem canker disease associated with the ascomycete *Dolabra nepheliae* C. Booth & W.P. Ting (Booth and Ting 1964). *Dolabra nepheliae* was originally described from Malaysia, has been reported from Australia (Janick and Paul 2008), and was recently reported from Hawaii and

Puerto Rico (Rossman et al. 2007). An asexual state was described by Zalasky et al. (1971). With the increased planting of rambutan as a specialty crop, it is likely that this fungus will spread. Using DNA sequence data, the phylogenetic placement of this species was determined. In addition, a redescription and illustrations of the sexual and asexual state of *Dolabra nepheliae* are provided.

Materials and methods

Morphological methods

Fresh specimens of cankers with ascomata from Hawaii and Puerto Rico were obtained as air-dried collections. Three isolates were grown from single ascospores plated on Difco corn meal agar (CM) supplemented with 0.2% dextrose and antibiotics (2 mg/ml neomycin and streptomycin). Germinated spores were transferred to Difco potato dextrose agar (PDA) and CM plates. All isolates were maintained on CM slants at 4°C. Living cultures were deposited in the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands. The original specimens from which isolates were obtained were deposited in the U.S. National Fungus Collections (BPI). For microscopic examination, material was rehydrated and mounted in 3% KOH. Fruiting bodies were sectioned at ~10 µm thick using a freezing microtome. Sections were mounted in lactic acid with cotton blue. Observations of microscopic features were made using a Zeiss Axioplan 2 microscope with bright-field illumination. Photographs and measurements of microscopic features were taken using an Olympus Q Color 5 digital camera (Olympus America, Center Valley, PA, USA) and ImagePro software (Media Cybernetics, Silver Spring, MD, USA). Specimens examined are listed following the descriptions. To determine cultural characteristics each isolate was inoculated onto two 90-mm-diameter plastic Petri plates of PDA and allowed to grow for 1 week at 25°C under alternating fluorescent (12 h) and near-ultraviolet (12 h) light. Colony diameters were averaged. Color names are based on Rayner (1970).

Nucleic acid extraction and polymerase chain reaction (PCR) amplification

Fungal genomic DNA was isolated by scraping mycelium from PDA plates. This mycelium were subsequently ground up, and the DNA was extracted using the FastDNA kit and the FastPrep instrument from MPI Biochemicals (Irvine, CA, USA). We initially evaluated the following regions: the internal transcribed spacer regions plus the 5.8S nuclear ribosomal RNA (ITS) and the large subunit (28S) for the nuclear ribosome (LSU). DNA amplifications were completed using *Taq* polymerase (GenScript, Piscataway, NJ, USA), with FailSafe PCR 2× PreMix E (Epicentre, San Diego, CA, USA) under the following PCR conditions: 94°C for 2 min; five cycles of 94°C for 40 s, 55°C for 45 s lowering by 0.8°C per cycle and 72°C for 90 s; 30 cycles of 94°C for 30 s, 52°C for 45 s, and 72°C for 120 s, and a final cycle for 10 min at 72°C. The ITS region was amplified with primers ITS4 and ITS5 (White et al. 1990) and LSU amplified with primers LR0R and LR5 (Vilgalys and Hester 1990). To confirm that the cultures were actually *D. nepheliae* and sequences obtained were not the result of contamination, DNA was reisolated twice from these cultures. To provide a confident placement of *Dolabra* within the class Eurotiomycetes, we also amplified a number of gene fragments used by the Assembling the Fungal Tree of Life project (AFTOL) (Schoch et al. 2009). This procedure resulted in DNA sequence data obtained from two protein-coding genes, the translation elongation factor-1 alpha (*TEF1*) and the RNA polymerase II largest subunit (*RPB1*), as well as the mitochondrial small ribosomal subunit (MSSU). Primer sets used for these genes were as follows: *TEF1*–983, 2218R (initially obtained from S. Rehner: http://ocid.nacse.org/research/deephyphae/EF1primer.pdf), *RPB1*–RPB1-Ac, RPB1-Cr (initially obtained from V. Hofstetter), and MSSU–mrSSU1, mrSSU3R (Zoller et al. 1999). Primer sequences are available at the AFTOL website (aftol.org). PCRs for these three genes were performed under conditions described previously (Lutzoni et al. 2004; Schoch et al. 2009).

Phylogenetic analysis

Initial alignments were prepared using the M-Coffee web server (Moretti et al. 2007) and, for the ITS alignment, variable regions were excluded using GBlocks with the following settings: minimum size of blocks = 10, allow gapped positions, and allow 55% flanking sequences. Outgroups were selected according to Geiser et al. (2006). For the ITS data set, phylogenetic trees were obtained by maximum-parsimony analysis with the branch-and-bound search option in PAUP* (Swofford 2002). The LSU data set was analyzed with a heuristic search with 100 random additions in the same program (Fig. 1). In both data sets, alignment gaps were treated as missing data and characters were unordered and of equal weight in all cases. Branches of zero length were collapsed, and all multiple, equally parsimonious trees were saved. Group support was evaluated by 1,000 bootstrap pseudoreplicates. Tree length was calculated and the resulting trees were printed with TreeView v. 1.6.6 (Page 1996) and Treedyn (Chevenet et al. 2006). The complete set of sequences and the related taxa used for comparison are listed in Table 1 with their GenBank Accession numbers.

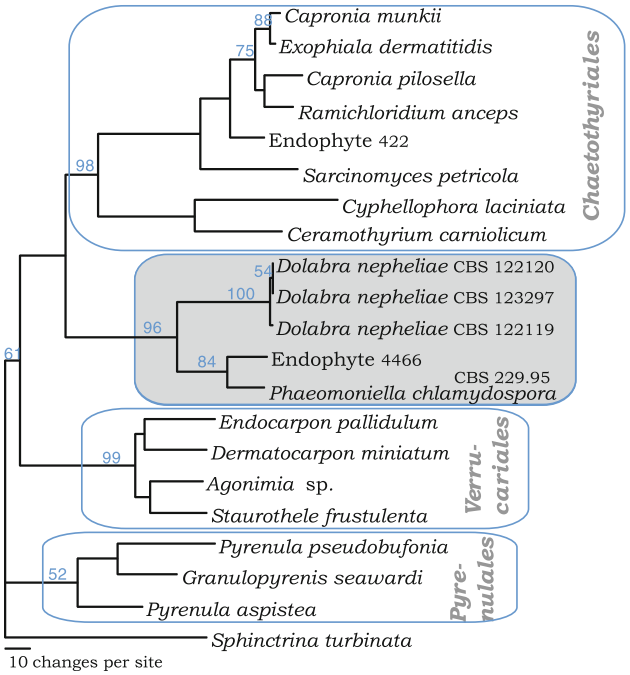


Fig. 1 Phylogenetic placement of *Dolabra nepheliae* based on the large subunit (LSU) nrDNA. One of six most parsimonious trees (742 steps) with LSU sequences exemplifying the major related groups in the Eurotiomycetes. Bootstrap values from 1,000 pseudoreplicates are shown on the nodes

Additional alignments were completed by adding the newly generated sequence to initial alignment files generated by WASABI (Kauff et al. 2007) by using the 'consensus align' option using MUSCLE (Edgar 2004) in Geneious Pro version 4.6.5 (Biomatters, Auckland, New Zealand). A supermatrix was obtained from four genes (LSU, *TEF1*, *RPB1*, MSSU) consisting of 40% missing data. *Geoglossum nigritum* was used as an outgroup following Gueidan et al. (2008); this was analyzed with maximum likelihood in RAxML v 7.0.4 (Stamatakis 2006) applying unique model parameters for each gene. The dataset was thus partitioned according to each gene and separate codons (eight partitions) as in Schoch et al. (2009). The result was a general time-reversible model (GTR) applied for DNA sequences with a discrete gamma distribution and four rate classes. A tree was obtained by simultaneously running a fast bootstrap search of 1,000 pseudoreplicates followed by a search for the most likely tree under functional setting 'a'. We also did 100 successive searches in RAxML under the GTR model with gamma rate distribution and starting each search from a randomized tree. The resultant most likely tree (log likelihood, –42731.850035) is shown in Fig. 2 with bootstrap values plotted onto the nodes. This data set was also analyzed in PAUP* by doing 1,000 bootstrap pseudoreplicates and 10 heuristic random additions per replicate. Parsimony bootstrap values for clades shown in the RAxML tree were added to the nodes. A maximum parsimony ran with 1,000 random additions in 5 trees with a length of 9,545 steps (not shown). Alignments have been deposited in TreeBASE as S2590.

Results

Sequencing and analyses

ITS sequences for the three isolates of *Dolabra nepheliae* from Hawaii and Puerto Rico and from the two related host genera, *Litchi* and *Nephelium*, were compared and showed that these were the same species (data not shown). The ITS sequences were compared with sequences in the GenBank database using BLAST analyses. The results suggested that these isolates were most closely related to species in the Chaetothyriales, Verrucariales, the grape pathogen *Phaeomoniella chlamydosporia*, and several unnamed environmental isolates in the Eurotiomycetes. The closest relationship was with *Phaeomoniella chlamydospora*, the causal agent of Petri grapevine decline (Crous and Gams 2000; Dupont et al. 2000; Groenewald et al. 2001) and fungal Endophyte 4466 obtained from an environmental study of *Picea mariana* (Higgins et al. 2007). An ITS data set moderately supported shared ancestry with these sequences (data not shown).

To determine more accurately the phylogenetic placement of *Dolabra nepheliae*, we compared three LSU sequences of *D. nepheliae* with a range of taxa and environmental isolates using maximum parsimony as shown in Fig. 1. Although these results indicate that *D. nepheliae* falls within the Eurotiomycetes, this species does not group within any of the major orders Chaetothyriales, Pyrenulales, or Verrucariales. Rather, *D. nepheliae* forms a separate clade with *Phaeomoniella chlamydospora* and Endophyte 4466 mentioned above (Hoffman and Arnold 2008).

To further test the placement of *D. nepheliae* with gene targets used by the AFTOL project, we amplified three additional gene fragments (*TEF1*, *RPB1*, MSSU) and combined these in a supermatrix with LSU and similar gene sequences produced by AFTOL. We also included a subset of rock-inhabiting fungi in the Eurotiomycetes from Gueidan et al. (2008). Following Wiens (2006), we included taxa in our supermatrix even though considerable data were missing (40%). The resultant phylogeny is shown in Fig. 2. It is congruent with two recent studies in the Ascomycota and Eurotiomycetes, specifically those by Schoch et al. (2009) and Gueidan et al. (2008). This phylogeny groups together *D. nepheliae* with *Phaeomoniella chlamydospora* and Endophyte 4466 with strong support outside the known orders of the Eurotiomycetes. The exact

Table 1 Taxa, isolate numbers, and GenBank sequence numbers used in the phylogenetic analyses

Taxon	Isolate number	ITS	LSU	MSSU	RPB1	TEF1
Chaetothyriomycetidae						
Chaetothyriales						
Capronia pilosella	AFTOL 657		DQ823099			
Ceramothyrium carniolicum	AFTOL 1063		EF413628		EF413629	EF413629
Cyphellophora laciniata	AFTOL 1033		EF413619			
Exophiala dermatitidis	AFTOL 668		DQ823100		DQ840555	DQ840566
Exophiala pisciphila	AFTOL 669		DQ823101		DQ840556	DQ840567
Exophiala salmonis	AFTOL 671		EF413609	FJ225745	EF413610	EF413612
Ramichloridium anceps	AFTOL 659		DQ823102	FJ225752	DQ840557	DQ840568
Sarcinomyces petricola	AFTOL 2195		FJ176893			
Coryneliales						
Caliciopsis orientalis	AFTOL 1911		DQ470987	FJ190654	DQ471185	DQ471111
Caliciopsis pinea	AFTOL 1869		DQ678097	FJ190653		DQ677937
Pyrenulales						
Anthracothecium nanum	AFTOL 1649		FJ358271		FJ358403	
Granulopyrenis seawardi	AFTOL 2013		EF411062			
Lithothelium septemseptatum	AFTOL 12		AY584638	AY584620		
Pyrenula aspistea	AFTOL 2012		EF411063			EF411069
Pyrenula pseudobufonia	AFTOL 387		AY640962	AY584720	DQ840558	
Pyrgillus javanicus	AFTOL 342		DQ823103	FJ225774	DQ842010	
Verrucariales						
Agonimia sp.	AFTOL 684		DQ782913		DQ782853	DQ782917
Dermatocarpon miniatum	AFTOL 91		AY584644	AY584616	DQ782821	DQ782893
Endocarpon pallidulum	AFTOL 661		DQ823097	FJ225674	DQ840552	
Polyblastia melaspora	AFTOL 1356		EF413601		EF413602	
Staurothele frustulenta	AFTOL 697		DQ823098	FJ225702	DQ840553	
Wahlenbergiella mucosa	AFTOL 2264			FJ225720	EF689804	
Eurotiomycetidae						
Eurotiales						
Aspergillus fumigatus	AFTOL 1079				Genome	Genome
Aspergillus nidulans	AFTOL 1080				Genome	Genome
Eupenicillium javanicum	AFTOL 429		EF413621	FJ225778		
Eupenicillium limosum	AFTOL 2014		EF411064			EF411070
Monascus purpureus	AFTOL 426		DQ782908	FJ225780	DQ842012	
Penicillium freii	AFTOL 378		AY640958	AY584712		
Onygenales						
Coccidioides immitis	AFTOL 1084		Genome		Genome	Genome
Ajellomyces capsulatum (anamorph *Histoplasma capsulatum*)	AFTOL 1083		Genome		Genome	Genome
Spiromastix warcupii			DQ782909	FJ225794	EF413613	DQ782900
Mycocaliciomycetidae						
Mycocaliciales						
Sphinctrina turbinata	AFTOL 1721		EF413632	FJ713611		
Incertae sedis						
Dactylospora haliotrepha	AFTOL 758		FJ176855			
Dactylospora mangrovei	AFTOL 2108		FJ176890			FJ238411
Dolabra nepheliae	CBS 123297	GU345749	GU332515			
Dolabra nepheliae	CBS 122119		GU332516	GU332518	GU332520	GU332522
Dolabra nepheliae	CBS 122120		GU332517	GU332519	GU332521	GU332523

Table 1 continued

Taxon	Isolate number	ITS	LSU	MSSU	RPB1	TEF1
Fungal endophyte	4466		DQ979444			
Fungal endophyte	422		EF420091			
Phaeomoniella chlamydospora (as *Phaeoacremonium chlamydosporum*)	CBS 229.95		AF353609			
Rock isolates (taken from Gueidan et al. 2008)						
TRN488			FJ358262	FJ225766	FJ358394	
TRN475			FJ358260	FJ225764	FJ358392	
TRN107			FJ358253	FJ225758	FJ358386	
TRN214			FJ358256	FJ225761	FJ358389	
TRN508			FJ358265	FJ225770	FJ358398	
A14			FJ358268			
TRN242			FJ358257	FJ225762	FJ358390	
A95			FJ358269		FJ358401	
Outgroup (Geoglossomycetes)						
Geoglossum nigritum	AFTOL 56		AY544650	AY544740	DQ471115	DQ471044

ITS internal transcribed spacer, *LSU* large subunit, *MSSU* mitochondrial small ribosomal subunit

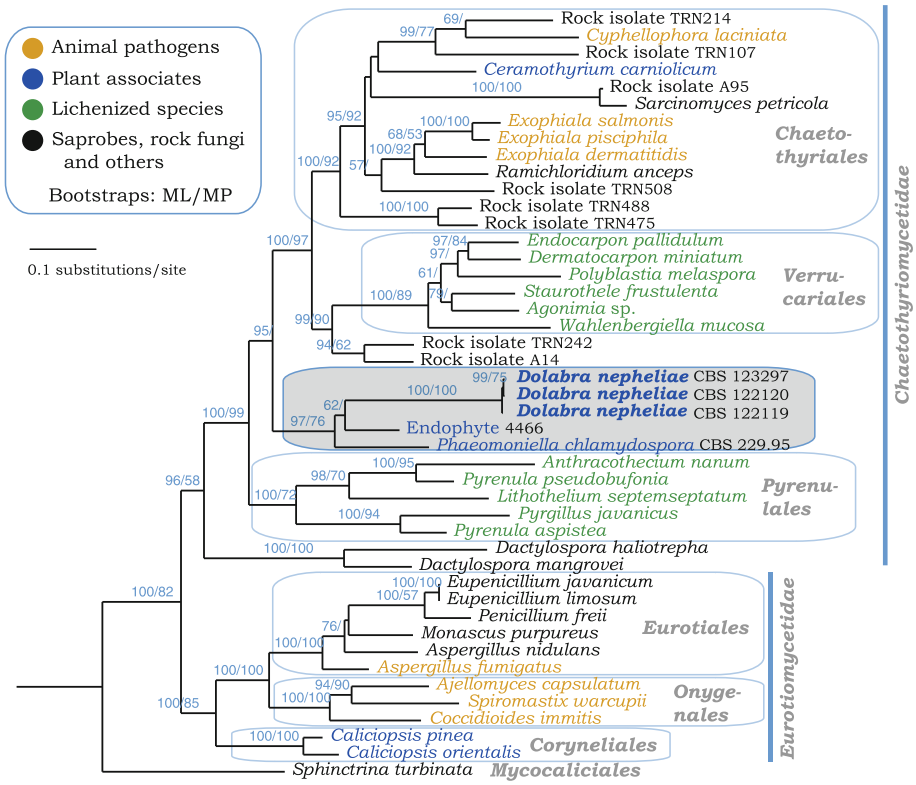


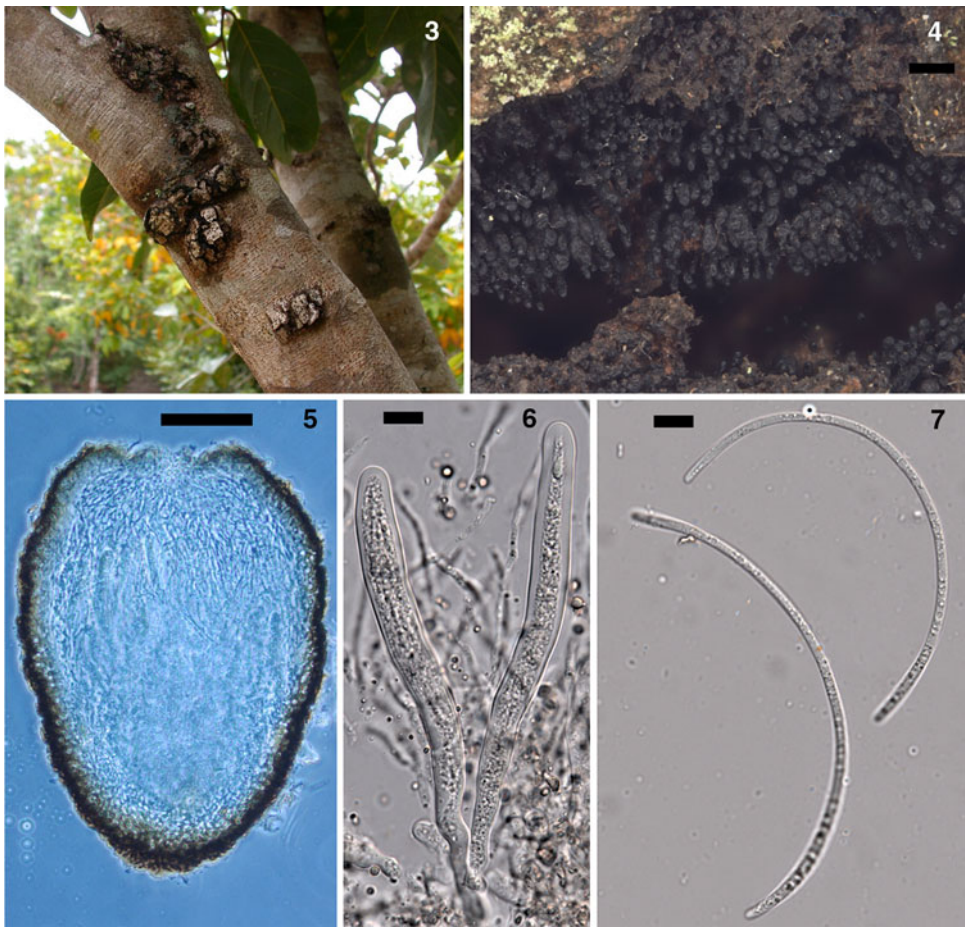
Fig. 2 A maximum-likelihood tree obtained with RAxML from four genes—LSU nrDNA, *RPB2*, *TEF1*, mitochondrial small ribosomal subunit (MSSU)—data from representative lineage in Eurotiomycetes. Orders and subclasses are indicated, and bootstrap values (*ML*, maximum likelihood; *MP*, maximum parsimony) are plotted on the *nodes*. Bootstrap values below 50% are not shown. The outgroup branch with *Geoglossum nigritum* is not shown in the figure

placement of this group was in conflict between the maximum-parsimony and maximum-likelihood analyses. *Dolabra nepheliae* was sister to the Pyrenulales with poor bootstrap support in the parsimony analyses (55% bootstrap, not shown), possibly the result of long branch attraction, and sister to both the Chaetothyriales and Verrucariales using maximum likelihood (95% bootstrap).

Taxonomy

Dolabra nepheliae C. Booth & W. P. Ting, Trans Br Mycol Soc 47:237 Figs. 3–11

Associated with corky bark symptoms, small to large irregular patches of raised bark on main trunk and lateral branches. Symptoms similar on root stock. Cankers on



Figs. 3–7 *Dolabra nepheliae*. **3** Cankers on woody trunks of *Nephelium lappaceum* caused by *D.nepheliae*. **4** Ascomata developing within fissures of cankers on *N.lappaceum*. **5** Transverse section of immature ascoma showing ascomatal wall and centrum filled with pseudoparaphyses (section by G. Samuels). **6** Bitunicate asci and pseudoparaphyses. **7** Ascospores. *Bars* **4** 300 μm; **5** 100 μm; **6**, **7** 10 μm

woody branches, slightly roughened to irregularly globose, extending from surface up to 1 cm, with deep fissures in which ascomata develop. Ascomata crowded, aggregated, lining fissures, superficial, dark brown to black, ovoid to elongate with irregularly schizogenous, ostiolar opening, collapsed laterally with apex sunken when dried, surface smooth to slightly roughened, 350–750 μm high × 225–375 wide, with long stalked base. Ascomatal wall gelatinous, of two regions: outer region black, of thick-walled cells; inner region of hyaline, thin-walled cells. Pseudoparaphyses abundant, hyaline, unbranched, septate, extending beyond asci to upper region of ascomata. Asci bitunicate, narrowly clavate, 100–130 × 10–14 μm. Ascospores long cylindrical, curved, with rounded ends, 96–136 × 2.5–3.5 μm, hyaline.

Pycnidia dark brown to black, ovoid, scattered to clustered, 110–150 × 110–125 μm diameter, smooth. Pycnidial wall of two regions: outer region of brown to black, thick-walled cells; inner region of hyaline cells, 2–8 rows; opening by an irregular slit, occasionally opening completely to appear discoid. Conidiophores hyaline, septate, short, formed from inner cells of the pycnidial wall. Conidiogenous cells holoblastic, hyaline, smooth, cylindrical 2.0–3.4 × 8.3–10.7 μm. Conidia long fusiform, slightly curved to lunate, 35–76 × 1.9–4.2 μm, (2–4) 5-septate, smooth, hyaline, base slightly truncate, apex rounded. Pycnidia developing toward center of colony after 1 week on PDA, appearing same as pycnidia on substratum.

Growth on PDA after 1 week in 25°C: colony 0.8–2.0 cm diameter, with heaped mycelium, white at margin, becoming slightly darker or dark green (dull green) toward center when pycnidia produced; reverse olivaceous buff, becoming olivaceous toward center. After 2 weeks on PDA, 1.8–2.6 cm diameter, appearing similar to after 1 week, white at margin, reverse olivaceous buff.

Hosts: *Litchi chinensis* Sonn. (lychee) and *Nephelium lappaceum* L. (rambutan), Sapindaceae. Also reported on *Nephelium mutabile* Blume (pulasan) (Zalasky et al. 1971).

Distribution: Australia (Janick and Paul 2008), Malaysia (type locality, Booth and Ting 1964), USA: Hawaii, Puerto Rico (Rossman et al. 2007).

Type specimens examined: MALAYSIA: Selangor, Petaling Jaya, on bark of *Nephelium lappaceum*, 18 October 1962, W.P. Ting (IMI 96355 Holotype); ibid., 25 September 1962 (IMI 95969 Topotype).

Additional specimens examined: USA: Hawaii, Hilo, in USDA greenhouse, on *Nephelium lappaceum*, causing cankers, 18 June 2007, Lisa Keith, det. and isol. Amy

Rossman AR 4422 = CBS 122119 (BPI 878189); Hawaii, Island of Kauai, Kilauea, on *Litchi chinensis*, 19 November 1984, George Wong and Chuck Hodges (BPI 626373, IMI 293693); Puerto Rico: Mayaguez, in USDA greenhouse, on small twigs of *L. chinensis*, May 2007, Ricardo Goenaga, det. and isol. Amy Rossman AR 4421 = CBS 123297 (BPI 878188); Mayaguez, in USDA greenhouse, on *N. lappaceum*, causing cankers, April 2007, Ricardo Goenaga, det. and isol. Amy Rossman AR 4426 = CBS 122120 (BPI 878190). In addition, the fungus was observed in 2001 associated with galls on twigs and branches of rambutan trees grown in a commercial orchard in the Papaikou district of the island of Hawaii.

Notes: The description presented in the original publication and the type specimens agree with observations of the more recently collected specimens from Hawaii and Puerto Rico. The specimens from Hawaii and Puerto Rico demonstrate the extensive cankers and deep fissures caused by this fungus that result in a serious disease. The type specimens showed only superficial wounding of the upper layers of tissue. The ascomatal and pycnidial wall structure is described here in detail for the first time.

Discussion

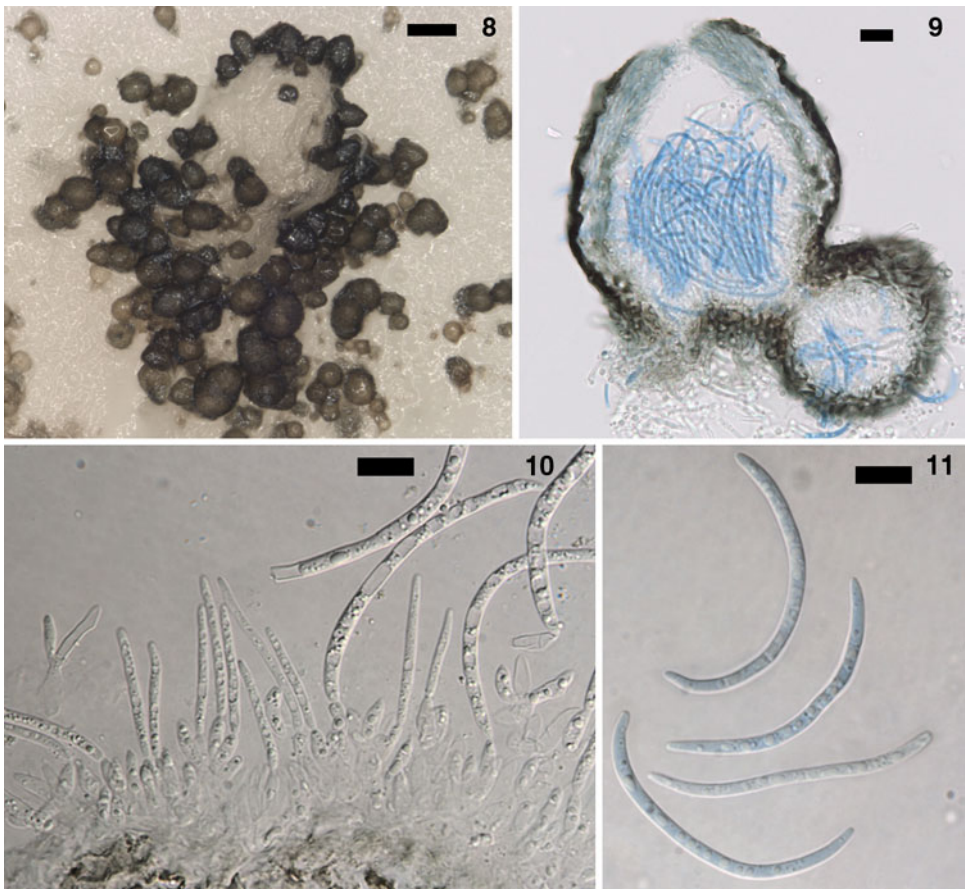
Dolabra nepheliae is distinctive in producing numerous, crowded, large, elongated fruiting bodies on well-developed cankers on the stems of the woody plant hosts, species of *Litchi* and *Nephelium* in the Sapindaceae. This fungus was originally described from Malaysia on cultivated *Nephelium lappaceum* and is also reported on pulasan (*N. mutabile*) and from Australia (Zalasky et al. 1971; Janick and Paul 2008) with specimens apparently deposited in KLA, although these could not be located. The specimen previously collected in 1984 by George Wong and Chuck Hodges on *Litchi chinensis* suggests that this fungus has been in Hawaii for some time, most likely on plants grown in residential gardens. This fungus is now also known from Puerto Rico (Rossman et al. 2007), possibly imported from Hawaii on plant germplasm.

The disease appears on the bark of older branches and stems causing the formation of corky tissue; new growth is not initially affected by the disease. Symptoms of the disease extend from older to younger tissue as the fungus grows out from the main trunk to the stems and eventually the twigs. In severe cases, dieback of entire branches may occur. The disease progresses slowly, and it may take years for a tree to be severely affected, even in cases in which new growth such as chupons or new branches appear to be free of disease. Trees do not die from the disease, but severe cases show reduced tree growth. A field survey at the Waiakea Agriculture Experiment Station in Hilo, Hawaii, where 55 accessions are grown, did not result in a pattern of symptom development, indicating that location and variety do not appear to have any effect (K. Nishijima, personal communication). Rambutan and litchi trees grown in a semiarid environment do not show symptoms of the disease even several years after planting.

Dolabra nepheliae is characterized by bitunicate asci and abundant, unbranched, thin-walled, septate pseudoparaphyses that suggest placement in the Pleosporales, Dothideomycetes. Booth and Ting (1964) stated that "the structure of the locules in the upper half of the stroma is typical of the Pleosporales…" and "the ascocarps suggest a member of the Nitschkiaceae…". However, as discussed below, neither of these placements is correct. The anamorph known to be associated with the canker on rambutan trees as well as in culture was first described and illustrated by Zalasky et al. (1971) (see Figs. 8–11). Although they referred to this anamorph as *Rhabdospora nepheliae*, this scientific name was not validly published because of the lack of a Latin description, nor was a type specimen designated.

Instead of confirming our initial assessment as a member of the Pleosporales, Dothideomycetes, the multigene sequence data suggest that *Dolabra nepheliae* represents a new lineage, placed as *incertae sedis* in the subclass Chaetothyriomycetidae of the Eurotiomycetes (see Figs. 1, 2). The Eurotiomycetes includes diverse orders ranging from the so-called "black yeasts" of the Chaetothyriales and lichenized Pyrenulales and Verrucariales to the "common moulds" of the Eurotiales and host-specialized Coryneliales and Onygenales (Geiser et al. 2006). The majority of the "black yeasts," animal and human opportunistic pathogens, were previously thought to be related to other loculoascomycetes in the Dothideomycetes because of similarities in morphology and development of the ascomata (Luttrell 1951; Lumbsch and Huhndorf 2007). Since the advent of phylogenies derived from molecular data, these two groups of fungi having bitunicate asci have proven to be distinct and separate (Winka et al. 1998; Spatafora et al. 2006).

Morphological characters such as the bitunicate asci are common in the subclass Chaetothyriomycetidae. The phylogenetic placement of *Dolabra nepheliae* in the Chaetothyriomycetidae, Eurotiomycetes, is surprising given that this species has pseudoparaphyses. Most members of the Eurotiomycetes including the Chaetothyriales lack interthecial elements, although pseudoparaphyses are present in the Verrucariales (Gueidan et al. 2009). Their presence in *Dolabra* suggests convergence between the Dothideomycetes and Eurotiomycetes. The characteristic feature of *Dolabra nepheliae* most indicative of the Eurotiomycetes is the schizogenous ascomatal apex that splits to form an irregularly shaped pore and the stalked base of the ascomata. The other two isolates in this clade represent



Figs. 8–11 Anamorph of *Dolabra nepheliae*. **8** Pycnidia developing in culture. **9** Transverse section of pycnidia produced in culture. **10** Conidiogenous cells with developing conidia. **11** Conidia. *Bars* **8** 100 µm; **9–11** 10 µm

fungi with little morphology. *Phaeomoniella chlamydospora* lacks a fruiting body, producing only a hyphomycetous state.

The Eurotiomycetes are an ecologically diverse class with well-defined clades focused on specific life strategies including lichens (most of the Pyrenulales, Verrucariales), animal pathogens (Onygenales), and plant-associated fungi (*Dolabra*, *Phaeomoniella*, Coryneliales), as highlighted in Fig. 2. Within the class, new lineages continue to emerge, such as a clade of marine fungi associated with the breakdown of wood on mangroves *Dactylospora mangrovei* and *D. manglicola*, previously placed in the lichenized order Lecanoromycetes on the basis of morphology (Kohlmeyer and Kohlmeyer 1979; Jones et al. 1999; Schoch et al. 2009). Plant-associated fungi are included in the Chaetothyriales and Coryneliales. The latter are parasites of the Podocarpaceae, have fissitunicate asci and ascolocular ascomata, and, until recently, were considered an order of uncertain position within the Pezizomycotina (Eriksson 2006). In many ways fungi in the Coryneliales are unique, producing thin-walled asci that were shown to have multiple layers (Johnston and Minter 1989). The *Dolabra* lineage is only distantly related to Coryneliales but within the context of the Eurotiomycetes presents another example of a plant-associated fungus. Given the relationship of the two plant pathogens, *Dolabra nepheliae* with *Phaeomoniella chlamydospora*, it seems realistic to expect to discover additional related fungi involved in plant disease in this novel lineage. Our study clearly suggests an underestimation of morphological and ecological diversity within the Eurotiomycetes.

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