

SPECIAL
PAPER

Out of the Palaeotropics? Historical biogeography and diversification of the cosmopolitan ectomycorrhizal mushroom family Inocybaceae

P. Brandon Matheny¹*, M. Catherine Aime², Neale L. Bougher³, Bart Buyck⁴, Dennis E. Desjardin⁵, Egon Horak⁶, Bradley R. Kropp⁷, D. Jean Lodge⁸, Kasem Soyong⁹, James M. Trappe¹⁰ and David S. Hibbett¹¹

¹Biology Department, Clark University, Worcester, MA, USA, ²Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA, USA, ³Department of Environment and Conservation, Western Australian Herbarium, Bentley Delivery Centre, Perth, WA, Australia, ⁴Muséum National d'Histoire Naturelle, Département Systématique et Evolution, USM 602 – CP 39, Paris Cedex, France, ⁵Department of Biology, San Francisco State University, San Francisco, CA, USA, ⁶Nikodemweg 5, Innsbruck, Austria, ⁷Department of Biology, Utah State University, Logan, UT, USA, ⁸Center for Forest Mycology Research, US Department of Agriculture, Forest Service, Northern Research Station, Luquillo, Puerto Rico, ⁹Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand and ¹⁰Department of Forest Science, Oregon State University, Corvallis, OR, USA

ABSTRACT

Aim The ectomycorrhizal (ECM) mushroom family Inocybaceae is widespread in north temperate regions, but more than 150 species are encountered in the tropics and the Southern Hemisphere. The relative roles of recent and ancient biogeographical processes, relationships with plant hosts, and the timing of divergences that have shaped the current geographic distribution of the family are investigated.

location Africa, Australia, Neotropics, New Zealand, north temperate zone, Palaeotropics, Southeast Asia, South America, south temperate zone.

Methods We reconstruct a phylogeny of the Inocybaceae with a geological timeline using a relaxed molecular clock. Divergence dates of lineages are estimated statistically to test vicariance-based hypotheses concerning relatedness of disjunct ECM taxa. A series of internal maximum time constraints is used to evaluate two different calibrations. Ancestral state reconstruction is used to infer ancestral areas and ancestral plant partners of the family.

Results The Palaeotropics are unique in containing representatives of all major clades of Inocybaceae. Six of the seven major clades diversified initially during the Cretaceous, with subsequent radiations probably during the early Palaeogene. Vicariance patterns cannot be rejected that involve area relationships for Africa–Australia, Africa–India and southern South America–Australia. Northern and southern South America, Australia and New Zealand are primarily the recipients of immigrant taxa during the Palaeogene or later. Angiosperms were the earliest hosts of Inocybaceae. Transitions to conifers probably occurred no earlier than 65 Ma.

Main conclusions The Inocybaceae initially diversified no later than the Cretaceous in Palaeotropical settings, in association with angiosperms. Diversification within major clades of the family accelerated during the Palaeogene in north and south temperate regions, whereas several relictual lineages persisted in the tropics. Both vicariance and dispersal patterns are detected. Species from Neotropical and south temperate regions are largely derived from immigrant ancestors from north temperate or Palaeotropical regions. Transitions to conifer hosts occurred later, probably during the Palaeogene.

Keywords

Agaricales, Basidiomycota, BEAST, biogeography, dispersal, ectomycorrhizal, fungi, Palaeotropics, relaxed molecular clock, vicariance.

*Correspondence: P. Brandon Matheny, Department of Ecology and Evolutionary Biology, 569 Dabney Hall, University of Tennessee, Knoxville, TN 37996–1610, USA. E-mail: pmatheny@utk.edu

INTRODUCTION

Mushroom-forming fungi, or Agaricomycetes, are poorly represented in historical biogeographical contexts (Sanmartin & Ronquist, 2004) and are virtually absent from texts on biogeography (Cox & Moore, 2000; Lomolino *et al.*, 2006). Basic systematic frameworks for many macro fungal groups are incomplete or require revision (Lodge *et al.*, 2004), morphological species recognition is limited (Taylor *et al.*, 2006), major geographical regions are under-sampled (Mueller *et al.*, 2007), and the fossil record is particularly poor and challenging to interpret (Hibbett & Donoghue, 1997; Taylor & Berbee, 2006) - these conditions have contributed to the under-utilization of fungi as biogeographical markers and to their lack of appeal for studies of historical biogeography (Arnolds, 1997).

Fungi have been presumed to have dispersal strategies similar to those of land plants (Sanmartin & Ronquist, 2004), but this assumption may be overly simplistic in that multiple ecological guilds of mushroom-forming fungi exist - saprotrophic, parasitic, lichenized and mycorrhizal. These varying associations place different constraints on life-history requirements and influence dispersal abilities in different ways (Pirozynski, 1983; Lodge *et al.*, 1995; Mueller *et al.*, 2001).

Biogeographical research on ectomycorrhizal (ECM) fungi is worthwhile for several reasons: (1) patterns of ECM fungal and soil microbial diversity do not necessarily follow those of plant diversity (Allen *et al.*, 1995; Waldrop *et al.*, 2006); (2) several ECM fungal genera are widespread but include species endemic to certain regions (Horak, 1983); and (3) little research has been carried out using recent advances in dating phylogenies (Robinson, 2006) to investigate the biogeographical patterns that underlie evolutionary histories of ECM fungi. Although various studies have investigated fungal distributions in the Northern Hemisphere or broad biogeographical patterns (e.g. Redhead, 1989; Wu & Mueller, 1997; Geml *et al.*, 2006; Petersen & Hughes, 2007), few have specifically evaluated biogeographical patterns of ECM fungi from the tropics or Southern Hemisphere (Horak, 1983; Pirozynski, 1983; Bougher *et al.*, 1994; Mueller & Halling, 1995; Buyck *et al.*, 1996; Watling, 2001a; Martin *et al.*, 2002; Moyersoen *et al.*, 2003; Hosaka *et al.*, 2008) and even fewer have attempted molecular clock dating (Hibbett, 2001; Geml *et al.*, 2004; Matheny & Bougher, 2006a; Jeandroz *et al.*, 2008).

Despite a meager representation in the fossil record (attributed to the ephemeral nature of fruit bodies), two fossils of gilled mushrooms of unknown family affiliation date to the Cretaceous (90–100 Ma) (Hibbett & Donoghue, 1997; Poinar & Buckley, 2007). Molecular clock dating indicates evidence for late Cretaceous origins of the ascolichen genus *Biatora* (Printzen & Lumbsch, 2000) and the mushroom genus *Auritella* (Matheny & Bougher, 2006a). These observations suggest an unanticipated antiquity for some lower-level taxonomic groups of fungi. Mesozoic origins invite hypotheses that attempt to test whether global disjunct patterns are the result of the historical separation of major continental

landmasses (vicariance) or are consistent with models that posit more recent dispersal routes.

The Inocybaceae Julich is a family with a cosmopolitan geographical distribution and ECM association with numerous plant families of angiosperms and conifers (Singer, 1986). It is one of seven major ECM groups that occur throughout the tropics (Buyck *et al.*, 1996). Between 500 (Kirk *et al.*, 2001) and 700 (P.B. Matheny, unpublished data) species are recognized world-wide, including at least 153 species (20–30% of the family diversity) described from the tropics and Southern Hemisphere (see Appendix S1 in Supporting Information). The family is probably primitively ectomycorrhizal (Matheny *et al.*, 2006) and associates with at least 19 families of seed plants. Kuyper (1986) proposed that European species of Inocybaceae form generalist associations with multiple host trees, criss-crossing unrelated clades of angiosperms and conifers.

Based on morphological species recognition criteria alone, almost all Inocybaceae taxa described from the tropics and Southern Hemisphere are regional endemics. This pattern of regional diversity differs sharply from that in the Northern Hemisphere, where continental endemism is suggested to be low (Kuyper, 1986). All seven major clades of Inocybaceae contain species distributed in the Palaeotropics (Fig. 1). In contrast, the Neotropics are represented by species found only in the most derived clade of the family, *Inocybe* s. str. (Fr.) Fr. and its close relative, the *Pseudosperma* clade. Differing species numbers may reflect, in part, collecting efforts in the Northern and Southern hemispheres. Nonetheless, these broad patterns raise four general questions of interest to historical biogeography. (1) When did the major clades of Inocybaceae begin to diversify? (2) Did the Inocybaceae have a temperate or a

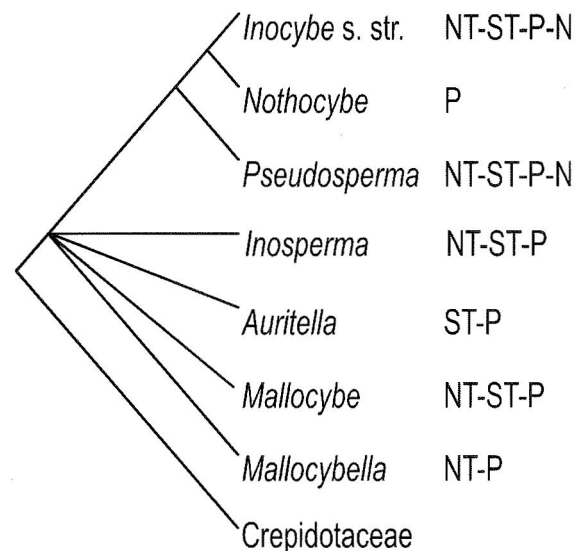


Figure 1 Family cladogram for seven major clades of the Inocybaceae based on Matheny *et al.* (2007) and their general geographical distributions. Abbreviations: NT, north temperate; ST, south temperate; P, Palaeotropical; N, Neotropical.

tropical origin? (3) Are ages of disjunct species patterns consistent with hypotheses predicted by vicariance scenarios? (4) Did the Inocybaceae diversify with angiosperms or with conifers as their plant associates?

MATERIALS AND METHODS

Taxon sampling

We sampled 186 taxa of Inocybaceae, including 74 (40%) from the tropics and Southern Hemisphere, plus three representatives of its sister group, the Crepidotaceae (Matheny *et al.*, 2006) (Appendix S2). All species sampled from Africa, Thailand and India are tropical.

DNA extraction, PCR, sequencing and nucleotide alignments

Protocols for DNA extraction, polymerase chain reaction (PCR), sequencing and nucleotide alignments follow those of Matheny *et al.* (2002) and Matheny (2005). Nuclear gene regions sequenced include coding regions between conserved domains A and C of *rpb1*, domains 6 and 7 of *rpb2*, and the 5' end of the nuclear large subunit ribosomal RNA gene (nLSU). In a few cases, regions of the 5.8S rRNA gene and the second internal transcribed spacer (ITS2) were also sequenced while obtaining the 5' end of nLSU. Because of alignment difficulties and sparse sampling of the 5.8S rRNA gene and ITS2, these regions were not used for phylogenetic analysis, but they are useful for taxonomic identification (Ryberg *et al.*, 2008).

126 sequences of *rpb1*, 122 sequences of *rpb2* and 189 sequences of nLSU were manually aligned using MacClade ver. 4.0 (Maddison & Maddison, 2000) with existing partitions created by Matheny (2005). Alignable regions of *rpb1*-intron2, *rpb1*-intron3 and *rpb1*-intron4 were included. Nucleotide positions too ambiguous to align were removed. New *rpb1*, *rpb2*, nLSU and ITS2-nLSU sequences generated here (156 total) have been deposited at the National Center for Biotechnology Information (NCBI) <http://www.ncbi.nlm.nih.gov/> with accession numbers EU555440-EU555474, EU569834-EU569875, EU600829-EU600904 and EU604546. All sequences are provided in Appendix S2.

Phylogenetic analysis

Phylogenetic analysis was carried out in BEAST ver. 1.4.6 (Drummond & Rambaut, 2006; Drummond *et al.*, 2006, 2007) using a Bayesian Markov chain Monte Carlo (MCMC) tree-sampling procedure. The concatenated data set was imported into BEAUti ver. 1.4.6 to reformat the nexus file into an XML file. In BEAUti we enforced the monophyly of taxon subsets composed of all 183 Inocybaceae taxa and Australian representatives of the genus *Auritella* Matheny & Bougher. These constraints facilitated rooting the final BEAST summary tree and calibrating the divergence of Australian

and African taxa of *Auritella*. Both constraints are consistent with Bayesian and maximum likelihood (ML) estimates of topologies recovered by previous studies (Matheny, 2005; Matheny & Bougher, 2006a). The nucleotide substitution model employed a uniform GTR model of DNA substitution, gamma (1) and invariant (I) site heterogeneity parameters with four rate categories, an uncorrelated lognormal relaxed molecular clock, and the tree prior set to a Yule process. Model selection was based on Matheny (2005), but gene and codon partitions were not modelled separately in this study. All clade names referenced, with the exception of *Auritella* (Matheny & Bougher, 2006b), are informal and have yet to be described or re-circumscribed as per international rules of botanical nomenclature.

Calibration procedure

Molecular clock dating is controversial (Grauer & Martin, 2004; Heads, 2005; Pulquerio & Nichols, 2006), and calibrating a molecular clock for fungi presents a number of challenges (Taylor & Berbee, 2006). However, these techniques may provide insights into the origins and diversification of organisms for which the fossil record is poor (Welch & Bromham, 2005), as is the case for the Inocybaceae. Methodological advances in relaxing rate constancy allow for more realistic assessments of divergence dates and of estimating error around these dates (Sanderson, 1997; Drummond & Rambaut, 2007). Some studies (Berry *et al.*, 2004; Zhou *et al.*, 2006) have employed what Renner (2005) describes as a secondary calibration procedure, whereby a node dated in an initial analysis is used to calibrate the same node in a second more inclusive analysis. We followed this procedure by first establishing a 12-taxon data set of Basidiomycota similar to that of Geml *et al.* (2004). This data set, composed of combined *rpb2* (750 nucleotide positions) and nLSU-rRNA (1282 nucleotide positions) sequences, included exemplars of the Inocybaceae genus *Auritella* in order to date the split between African and Australian species of the genus evaluated previously by Matheny & Bougher (2006a). This date was then used to calibrate the inclusive Inocybaceae tree in a second step. GenBank accession numbers for sequences of taxa used in the primary analysis are provided in Appendix S3.

A prior distribution for tMRCA (time since the most recent common ancestor) between *Ustilago* and the Agaricomycotina was set to a normal distribution with a mean of 430 or 966 Myr and a standard deviation of 50 or 112 Myr, respectively. The later calibration (430 Myr) follows Berbee & Taylor (2001), whereas the earlier one (966 Myr) is derived from Heckman *et al.* (2001). Standard deviations are similar to those employed by Bruns *et al.* (1998). The MCMC was run for 2 million generations, logging parameters every 2000 generations. Before creating a summary tree in TreeAnnotator ver. 1.4.6, a conservative burn-in of 50% was applied. Mixing of trees was assessed in Tracer ver. 1.3 by examination of ESS

(estimated sample size) values. The summary tree was then viewed in FigTree ver. 1.0 available at <http://tree.bio.ed.ac.uk/software/figtree/>.

The 186-taxon data set was run independently seven times between 10 and 50 million generations to ensure sampling of ESS values following the recommendations of Drummond *et al.* (2007). A randomly chosen maximum parsimony (MP) tree reconstructed in PAUP* (Swofford, 2003) was used to construct starting trees for all BEAST runs. A prior distribution for tMRCA between the African *Auritella aureoplumosa* and Australian species of *Auritella* was determined following the initial step of the calibration procedure. Trees were saved every 5000 generations. Log files from each run were imported into Tracer, and trees sampled from the first 1 million generations were discarded. ESS values for every parameter did not reach above 100 until all seven log files were combined and totalled 149,750,000 generations. Tree files from the seven runs were then imported into LogCombiner and combined after burning 1000 trees from each sample. A final summary tree from 23,133 trees was produced in TreeAnnotator and viewed in FigTree.

In order to assess the plausibility of the two alternative calibrations of the *Auritella* crown group for hypothesis testing, we employed a series of internal maximum time constraints on clades optimized with strict angiosperm plant family associations. Host states were scored based on field observations and literature sources (Stuntz, 1954; Horak, 1977; Kuyper, 1986; Stangl, 1989). ECM compatibility and functionality have not been confirmed for most Inocybaceae taxa as these taxa are difficult to grow in pure culture. Each terminal taxon was scored in MacClade with the following host states: Fabaceae = 0, Myrtaceae = 1, Fagaceae = 2, Betulaceae = 3, Phyllanthaceae-Fabaceae = 4, Casuarinaceae = 5, Nothofagaceae = 6, Salicaceae = 7, Dipterocarpaceae = 8 and other = 9. Crown and stem group ages were compared. Ages of plant families were drawn from estimates in Wikstrom *et al.* (2001) and other molecular clock studies (Sytsma *et al.*, 2004) or palynological data (Wurdack *et al.*, 2004). The calibration that violated the series of maximum time constraints the least using 95% confidence intervals was considered the more plausible of the two.

Ancestral area reconstruction analysis

Four major biogeographical areas - the Palaeotropics, Neotropics, north temperate zone and south temperate zone - were scored as potential ancestral areas. This general coding allows us to address whether the Inocybaceae had a temperate or a tropical origin, while taking into account the phylogenetic disparity between Palaeotropical and Neotropical regions evident in Fig. 1. The four areas were scored as presence/absence character states (unordered, symmetric) in the data editor of MacClade, and were optimized under Fitch parsimony. This procedure allows any state to transform to another state and was applied to the summary tree produced by TreeAnnotator. An alternative gross topology from Matheny *et al.* (2007) was also considered.

Host association reconstruction analysis

Every Inocybaceae terminal was scored as an angiosperm associate, conifer associate, generalist (either angiosperm or conifer), or ambiguous in the data editor of MacClade. Host determinations were made based on personal field observations and the literature sources cited above. Southern Hemisphere conifers include the families Araucariaceae, Cupressaceae and Podocarpaceae (Hill & Brodribb, 1999). However, there are no known indigenous ECM conifer hosts of Inocybaceae in temperate regions of the Southern Hemisphere (Horak, 1980), and thus all taxa from these regions were scored as angiosperm only. All tropical taxa sampled to date are exclusively angiosperm-associated except for *Inocybe errata* nom. provo collected in mixed *Dipterocarpus-Pinus* forests in north-west Thailand and under dipterocarps in India.

Data sets

Data sets can be requested from the lead author or obtained online at http://www.clarku.edu/faculty/dhibbett/people_matheny.html. They include the 189-taxon nucleotide alignment and XML formatted file, the 12-taxon nucleotide alignment and XML formatted file, and the MacClade and tree files used for ancestral state reconstruction optimizations.

RESULTS

Secondary calibration

Appendix S3 presents the primary step of the secondary calibration procedure. It illustrates a chronogram of 12 representative Basidiomycota calibrated with two alternative divergence dates at the split between the Agaricomycotina and *Ustilago*. The later of the two calibrations (430 ± 50 Myr) produces a divergence date for *Auritella* at 68 (38-105) Ma (95% confidence intervals in parentheses). The earlier calibration (966 ± 112 Myr) puts the crown origin of *Auritella* at 154 (71-227) Ma. The divergence dates for the crown group of *Auritella* did not differ significantly despite the 500 + million-year difference between the two calibrations.

To assess the plausibility of the two calibrations, we applied a relaxed molecular clock to our entire Inocybaceae sample to compare divergence dates of Inocybaceae symbionts with maximum time constraints imposed by ages of plant family associations. A total of 23,133 trees were sampled after burn-in, combined, and used to produce a summary tree with branch lengths. This tree was imported into MacClade, and plant family host associations were optimized. Twelve clades were observed either to be specific to a given angiosperm family or to possess a shared ancestral state for a given family (Table 1). Divergence dates for Inocybaceae symbionts that significantly pre-date the presumed origin of their angiosperm hosts are given in cells shaded grey. All but one of these significantly older dates include stem lineages. Of 24 comparisons, eight inocyboid divergences pre-date the origin

Table 1 Comparisons of divergence dates in millions of years ago (Ma) for 12 Inocybaceae clades and their ancestrally reconstructed putative angiosperm host families. Two calibrations for the split between *Auritella aureoplumosa* (from Cameroon) and Australian species of *Auritella* (CAL1 vs. CAL2; see Appendix S3) are considered. 95% confidence intervals (CI) are given in parentheses. Angiosperm divergence dates follow Wikström *et al.* (2001) except where indicated. Grey-shaded cells indicate Inocybaceae divergence dates, including 95% CI, that significantly pre-date the age of their ancestrally reconstructed plant host family. 'n.a.' refers to nodes for which confidence intervals were not available.

Major clade or genus	Crown group node		Putative angiosperm family host*	Plant host divergence	Symbiont divergence (CAL1)	Symbiont divergence (CAL2)
	Stem group node	Species				
<i>Auritella</i>	8	<i>chamaecephala</i>	Myrtaceae	70–86†	49 (32–65)	111 (72–147)
	7	<i>dolichocystis</i>			68 (38–105)	154 (86–238)
		<i>geoaustalis</i>				
		<i>serpentinocystis</i>				
<i>Inosperma</i>	16	<i>submaculata</i>	Phyllanthaceae	34–55‡	31 (15–49)	70 (34–111)
	15	sp. PC 96013	Fabaceae	56–68	64 (40–93)	145 (91–211)
		sp. BB3233				
<i>Mallocybe</i>	38	<i>subflavospora</i>	Myrtaceae	70–86	31 (16–48)	70 (36–109)
	11	<i>subtilisior</i>			57 (38–78)	129 (86–177)
		<i>jarrahae</i>				
<i>Mallocybe</i>	56	<i>agardhii</i>	Salicaceae	20–25	5.5 (n.a.)	12 (n.a.)
	54	<i>dulcamara</i>			9 (4–14)	20 (9–32)
<i>Inocybe</i>	85	<i>velatorimosa</i>	Phyllanthaceae	34–55‡	44 (23–67)	100 (52–152)
	84	<i>conspicuospora</i>	Fabaceae	56–68	64 (41–89)	145 (93–202)
<i>Inocybe</i>	86	<i>epidendron</i>	Fabaceae	56–68	47 (28–68)	109 (65–157)
	84	<i>pulchella</i>			64 (41–89)	145 (93–202)
		<i>lasseri</i>				
<i>Inocybe</i>	92	<i>gilvida</i> DED8043	Fagaceae	34–37	12 (5–19)	27 (11–43)
	91	<i>alabamensis</i>			16 (8–26)	36 (18–59)
		<i>gilvida</i> DED8050				
<i>Inocybe</i>	97	<i>densifolia</i>	Phyllanthaceae	34–55‡	29 (15–43)	66 (40–93)
	96	<i>glaucodisca</i>	Fabaceae	56–68	46 (28–65)	104 (63–147)
		<i>leucophaea</i>				
<i>Inocybe</i>	102	<i>ayangannae</i>	Fabaceae	56–68	57 (35–82)	129 (79–186)
	101	<i>lilacinosquamosa</i>			69 (n.a.)	156 (n.a.)
		sp. MCA 1490				
<i>Inocybe</i>	142	cf. <i>serrata</i>	Myrtaceae	70–86	55 (37–75)	125 (84–170)
	136	<i>serrata</i>			61 (41–83)	138 (93–188)
		<i>eriodcaulis</i>				
		cf. <i>murrayana</i>				
		sp. PBM 2181				
		<i>cazaresii</i>				
		<i>bicornis</i>				
		<i>fibrillosibrunnea</i>				
		cf. <i>umbrosa</i> PDD				
		<i>albovestita</i>				
		cf. <i>umbrosa</i> PBM				
		sp. BK02991				
		<i>persicinipes</i>				
		aff. <i>serrata</i>				
		<i>spadicea</i>				
	166	<i>thailandica</i>			21 (10–32)	48 (23–72)
	164	<i>actinospora</i>			46 (30–63)	104 (68–143)
		<i>calospora</i>				
	183	<i>nothopedes</i>			39 (24–53)	88 (54–120)
	182	<i>pseudocystis</i>			42 (n.a.)	95 (n.a.)
		<i>fulvilubrica</i>				
		<i>calopedes</i>				
		<i>scissa</i>				
		<i>emergens</i>				
		<i>sinuospora</i>				

*Ancestral plant family host as reconstructed in MacClade.

†See Sytsma *et al.* (2004).

‡Palynological evidence supports diversification of Phyllanthaceae by the Eocene. See Wurdack *et al.* (2004).

of their angiosperm hosts when calibrated by the earlier calibration (154 Myr). In contrast, the later calibration (68 Myr) produced no dates that were significantly older than their associated angiosperm families. For this reason we find the later calibration (68 Myr) to be the more plausible of the two and evaluate subsequent hypotheses in this context (Figs 2 & 3). All nodes are numbered and listed with their divergence dates (mean node heights) and 95% confidence intervals in Appendix 54.

Major lineages of Inocybaceae diversified by the Cretaceous followed by temperate radiations during the Palaeogene

The Inocybaceae diversified between 99 and 191 Ma with a mean divergence date of 143 Ma, which lies near the Cretaceous–Jurassic boundary (Fig. 2; Appendix 54). Divergence dates for the seven major lineages of the family occur between mean values of 68 and 80 Ma and are of Cretaceous age, except for *Mallochybe*, whose crown group dates to 57 (38–78) Ma during the Palaeocene (95% confidence intervals are in parentheses). *Auritella* diversified 68 (38–105) Ma, *Mallochybella* 75 (33–120) Ma, *Inosperma* 80 (51–110) Ma, *Pseudosperma* 70 (40–105) Ma and *Inocybe* s. str. 79 (53–106) Ma. *Nothocybe* is a single-stem lineage that split from *Inocybe* s. str. 103 (70–138) Ma. All six temperate radiations (Figs 2 & 3) have mean divergence dates between 66 and 39 Ma, five of these dating to the Palaeogene and pre-dating the Oligocene.

The Palaeotropics are the ancestral area of the Inocybaceae

Ancestral area (AA) reconstructions are illustrated on the left-hand sides of Figs 2 and 3. In total, 18 unambiguous changes between area states are observed with 11 transitions from north temperate (NT) to other regions, and seven from the Palaeotropics (P) to other regions. No unambiguous transitions from the Neotropics (N) and the south temperate (ST) zone to other regions are observed. Frequencies are as follows: NT → N: 1, NT → P: 4, NT → ST: 6; P → N: 2, P → NT: 4, P → ST: 1; N → NT: 0, N → P: 0, N → ST: 0; ST → N: 0, ST → NT: 0, ST → P: 0. The earliest unambiguous transition from a Palaeotropical area to any other area, in this case the north temperate zone, does not occur until the late Cretaceous (node 65). Earlier transitions are possible, but are ambiguous (nodes 5, 6, 12–14). Despite an alternative placement for *Mallochybe* and *Auritella* (Fig. 4), a Palaeotropical origin is still inferred.

South temperate taxa primarily diversified from north temperate progenitors by means of dispersal

Five nodes, 76 (Fig. 2) and 127, 136, 160, 176 (Fig. 3), demonstrate unambiguous origins of Australian taxa from north temperate clades. Of these, four (127, 136, 160, 176) are too old to be explained by recent migratory events that would

have been possible between Australia and Southeast Asia up to 15 Ma (Raven, 1979). Node 136 is dated as Palaeocene, 160 and 176 as Eocene and 127 as Oligocene, suggesting old colonization of Australia by long-distance dispersal. Two south temperate radiations (nodes 142, 183) are nested within a single large north temperate radiation (node 104) (Fig. 3). Node 142 dates at least to the early Eocene and gave rise to a clade of at least 15 smooth-spored species now distributed in temperate Australia, southern South America and New Zealand. Node 183 dates to the late Eocene and gave rise to a sample of seven nodulose-spored species from temperate Australia and New Zealand.

Four species from New Zealand stem from nodes 33, 148, 149 and 186. All exhibit mean divergence dates less than 35 Ma and appear to represent Eocene to Miocene immigrants from Australia (nodes 148, 149, 186) or the north temperate zone (node 33). Because none of these taxa exhibits ages older than 80 Myr, vicariance between Australia and New Zealand is rejected (McLoughlin, 2001). An alternative hypothesis of post-Oligocene arrival to New Zealand (Campbell & Landis, 2001; Waters & Craw, 2006) cannot be rejected for taxa that stem from nodes 33 (*I. calamistratoides*; Fig. 2) and 186 (*J. scissa*; Fig. 3) with mean divergence dates in the Miocene.

Neotropical taxa are immigrant taxa from Africa and the north temperate zone

Two weakly supported nodes (84, 96) in *Inocybe* s. str. (Fig. 3) suggest reciprocal monophyly between northern South American and African taxa. Neither node is old enough (> 95 Myr) to support vicariance (Raven & Axelrod, 1974; Sanmartin, 2002). Three other lineages of Neotropical taxa in the *Inocybe* s. str. clade diverged during the Palaeogene (nodes 102, 179, 182) with mean divergences dating back to the mid Palaeocene and mid Eocene. The Neotropical member of the *Pseudosperma* clade (node 67) appears to have evolved during the late Eocene. In total, Neotropical taxa of Inocybaceae exhibit up to six independent origins during or just before the Cenozoic. None is closely related to species from temperate southern South America.

Two species from southern South America associate with indigenous species of *Alnus* (*I. actinospora* nom. prov., node 167) and *Salix* (*Inocybe* sp. GDa, node 119) (Fig. 3). Both fungi are considerably older than the earliest palynological evidence that places *Alnus* (2 Ma) and *Salix* (5 Ma) in South America (Graham, 1999), but neither is significantly older than the divergences of Betulaceae and Salicaceae, respectively (Wikström *et al.*, 2001).

Intermittent filter routes occurred between Eurasia and tropical Africa

Three instances of sister group relationships are observed between taxa from Eurasia and tropical Africa, at nodes 13, 47 (Fig. 2) and 175 (Fig. 3). The oldest of these dates to 75 (33–120) Ma, the late Cretaceous, and entails the only two

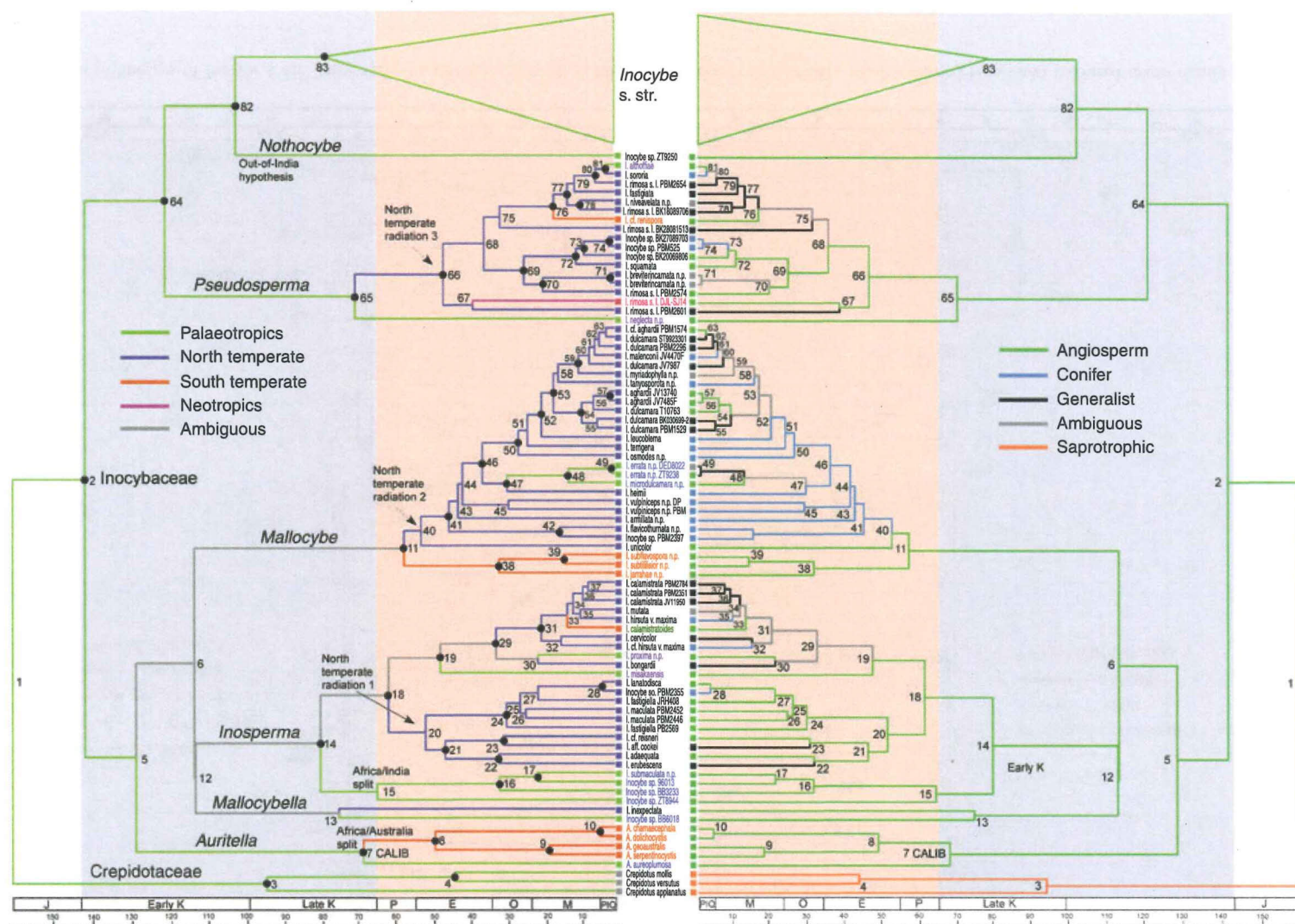


Figure 2 Chronogram of basal lineages of Inocybaceae. The time-scale is set to match the 50% majority-rule summary tree and mean divergence dates (node heights) produced in BEAST. Numbered nodes refer to mean divergence dates and their 95% confidence intervals provided in Appendix S4. Black filled circles indicate posterior probabilities greater than 0.95. The left-side topology indicates an ancestral area reconstruction with branches coloured according to area. The right-side topology indicates an ancestral plant partner reconstruction with branches coloured by general plant association. Small coloured boxes indicate coded character states. Coloured taxon names indicate geographic origin: black, north temperate; purple, India or Thailand; green, New Zealand; orange, Australia; light blue, southern South America; dark blue, tropical Africa; magenta, northern South America and the Caribbean. Abbreviations in the geological time scale are as follows: J, Jurassic; K, Cretaceous; P, Palaeocene; E, Eocene; O, Oligocene; M, Miocene; Pl, Pliocene; Q, Quaternary. The abbreviation 'n.p.' after a species name indicates *nomen provisorium* (provisional name).

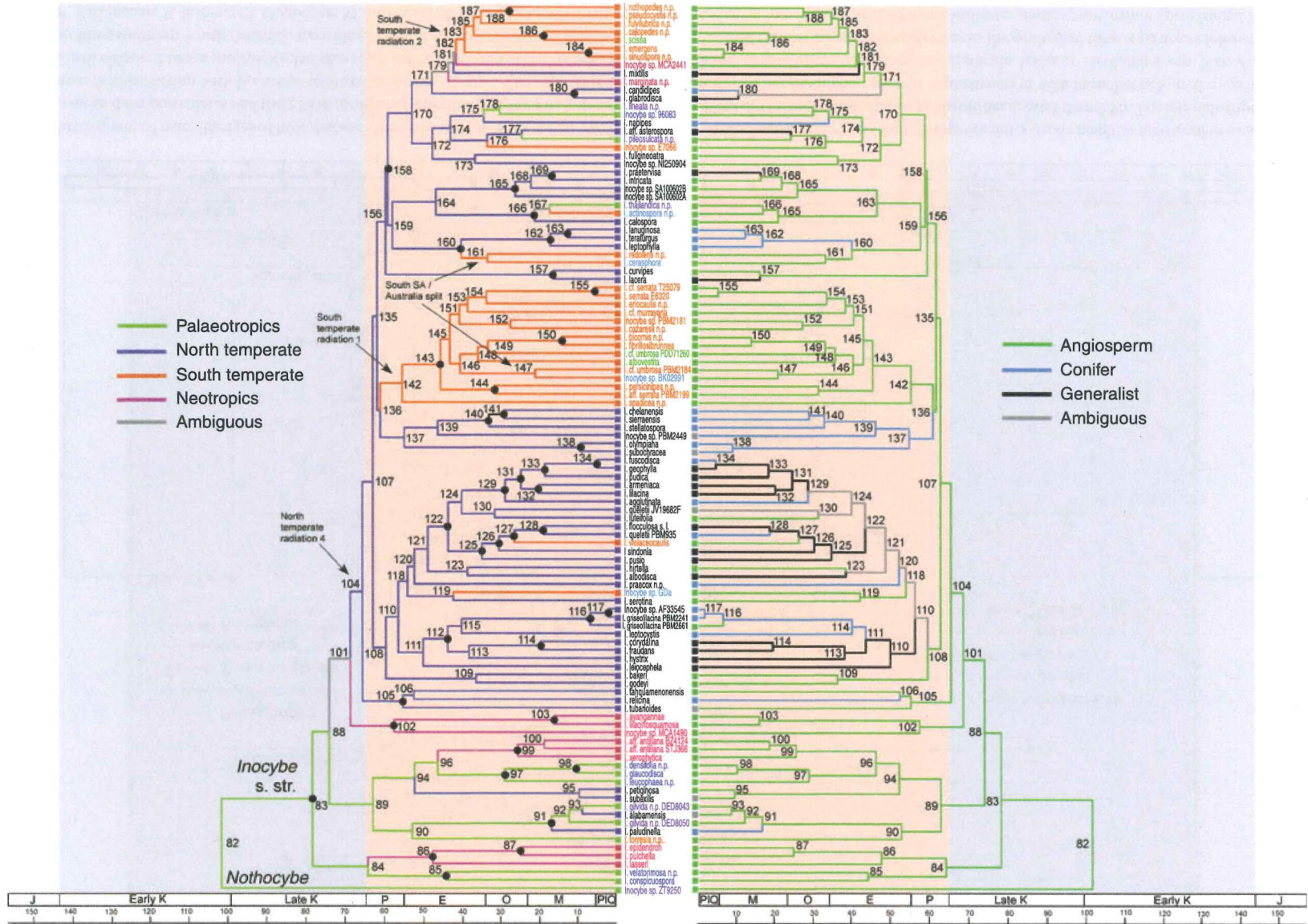


Figure 3 Chronogram of lineages of *Inocybe* s. str. Time-scale, numbered nodes, black filled circles, coloured branches, small coloured boxes and coloured taxon names are as in Fig. 2.

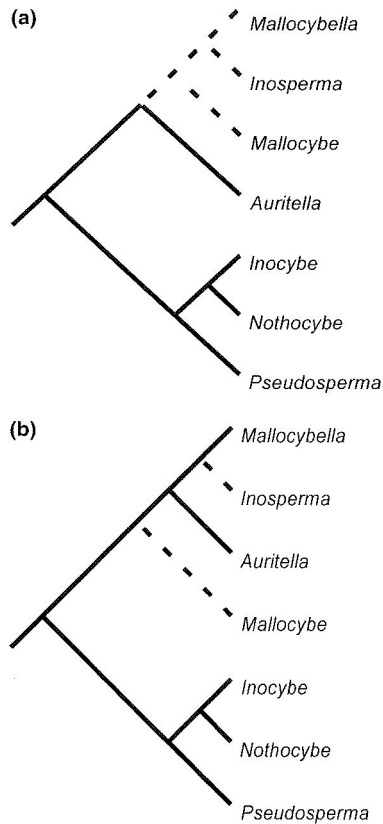


Figure 4 Summary cladograms of optimal and alternative gross topologies of major clades of Inocybaceae, and reconstruction of the Palaeotropics as an ancestral area. Solid lines are optimized as Palaeotropical in ancestry. Dashed lines are equivocal. (a) Summary of topology presented in Fig. 2. (b) Summary Bayesian topology in Fig. 2 after Matheny *et al.* (2007).

members of the *Mallocybella* clade, *I. inexpectata* from Europe (Villarreal *et al.*, 1998) and an undescribed species from tropical Africa (Fig. 2). Node 47 demonstrates support for dispersal 29 (18–41) Ma, during the Oligocene, from Europe into tropical Africa, India and Thailand. Node 175 (Fig. 3) suggests another filter route from Eurasia into tropical Africa and Southeast Asia 34 (20–49) Ma.

Vicariance scenarios cannot be rejected for species with disjunct distributions in Africa–Australia, Africa–India and southern South America–Australia

Node 7 (Fig. 2) supports an area relationship between Africa and Australia. This node is dated by the primary calibration analysis to 68 (38–105) Ma (Appendix S3). Raven (1979) postulated that migratory events between the two regions were possible up to 70 Ma, whereas Ali & Aitchison (2008) suggest 90–95 Ma or possibly later via the Kerguelen platform, which would have served as a migratory stepping stone between India–Seychelles–Madagascar and Australia–Antarctica. In any case, a vicariance episode between Africa and Australia cannot be rejected.

Node 82 (Fig. 3) subtends an isolated branch occupied by a species with affinities to *I. cutedructa* from India (*Inocybe* sp. ZT9250) and dates to the early Cretaceous 130 (70–138) Ma. This result is consistent with an 'out-of-India' Gondwanan origin and is older than a dispersal model would predict (Karanth, 2006) (Fig. 2). Node 15 supports divergence between an Indian species (*Inocybe* sp. ZT8944) and three African taxa 64 (40–93) Ma, a result consistent with vicariance between India and Africa per Raven (1979), but not entirely inconsistent with the hypothesis that exchanges between India and Africa (and Madagascar) occurred during the late Cretaceous (Briggs, 2003). Node 48 demonstrates support for a more recent mid-Miocene split between taxa from Africa and Southeast Asia.

Vicariance between southern South America and Australia cannot be rejected if migration was still possible between the two regions up to 28–32 Ma (McLoughlin, 2001). Argentine *Inocybe* sp. BK02991 (node 147) may have diverged as early as 35 Ma from Australian *Inocybe* cf. *umbrosa* PBM2184; and Australian *I. redolens* nom. prov. diverged from the Chilean *I. cerasphora* about 33 Ma.

The Inocybaceae initially diversified with angiosperm hosts

Twenty-one unambiguous transitions are inferred under Fitch parsimony between angiosperm (A), conifer (C) and generalist (G) hosts. Fifteen of these favour switches from angiosperms to conifers and generalists, five from generalists to angiosperms and conifers, and one from conifers to angiosperms: A → C: 8, A → G: 7; C → A: 1, C → G: 0; G → A: 2, G → C: 3. Ancestral host reconstruction is illustrated on the right-hand side of Figs 2 and 3. Switches to conifer or generalist associations do not occur at the earliest until the Palaeogene (nodes 40, 136).

DISCUSSION

Early members of Inocybaceae are Palaeotropical in origin and were associated with angiosperms most likely during the Cretaceous

ECM fungi have long been thought of as features of boreal and temperate forest communities. Indeed, Kuyper (1986) hypothesized a temperate ancestral area for *Inocybe* and allies based on the relative paucity of species described from the tropics up to that point and their presumably derived morphological traits. Pirozynski (1983), and more recently Alexander (2006), however, have hypothesized a Palaeotropical origin for the ECM habit. Recent research into the biodiversity of ECM fungi from the tropics indicates that they are routinely underestimated regarding their occurrence and importance (Taylor & Alexander, 2005). Samples of ECM genetic diversity from the tropics have been suggested to be on a par with those from boreal and temperate forests (Buyck *et al.*, 1996; Alexander & Lee, 2005; Riviere *et al.*, 2007). Our results suggest that the

Palaeotropical zone is the 'centre of origin' or most likely ancestral area for the Inocybaceae. Ancestral state reconstruction analysis of plant associates is not inconsistent with the hypothesis of a Palaeotropical ancestral area, in that early members of Inocybaceae were associated with angiosperms, which today form extensive ECM communities in the Palaeotropics (Alexander, 1989; Lee, 1990; Thoen, 1993; Watling, 1994; Buyck *et al.*, 1996). These results are significant because tropical species of *Inocybe* and allies were suggested to possess rather advanced morphological features that could support their derived status (Kuyper, 1986). However, four of the seven major lineages of Inocybaceae are optimally reconstructed as Palaeotropical in origin (*Auritella*, *Pseudosperma*, *Nothocybe* and *Inocybe* s. str. clades). An alternative topology from Matheny *et al.* (2007) is also consistent with a Palaeotropical origin for the family (Fig. 4).

Species such as *Auritella aureoplumosa* from Cameroon, *Inocybe neglecta* nom. provo from Thailand, *Inocybe* sp. ZT 9250 from India, and others that have yet to be formally described from Africa or Southeast Asia are old relictual species with restricted areas of geographic distribution, in some cases sporting unusual gross morphologies (Watling, 2001b). Additional relictual lineages may be found elsewhere in the Palaeotropics as taxon sampling intensifies (Horak, 1979, 1980; Morris, 1990; Turnbull, 1995; Sims *et al.*, 1997; Bougher & Syme, 1998; Turnbull & Watling, 1999; Natarajan *et al.*, 2005; Wilme *et al.*, 2006). A few old isolated lineages have persisted also in temperate zones: in Europe (*I. inexpectata*), North America (*I. unicolor*) and Australia (*I. spadicea* nom. prov.).

Thoen (1993) and Buyck *et al.* (1996) suggested the Palaeotropics as the centre of origin for another ECM group, *Russula*, based on the rich diversity of species encountered in African rain forests. Species of Inocybaceae are rather poorly represented in African rain forests (Watling, 1993) but are considerably more conspicuous in dry tropical forests such as those in Zambia (Buyck & Eyssartier, 1999). Other ECM groups that are richly represented in tropical Africa include Amanitaceae, Boletales and Cantharellales (Thoen, 1993). Whether these taxa have Palaeotropical origins has not been explicitly evaluated.

Almost all Cretaceous lineages are optimized as angiosperm associates with plant families Casuarinaceae, Fabaceae, Fagaceae, Myrtaceae or Phyllanthaceae. In contrast, *Nothofagus* and Dipterocarpaceae associates are quite young. *Nothofagus*-associated species of Inocybaceae from South America and New Zealand do not appear until 13–33 Ma (early Oligocene to mid Miocene). However, we cannot exclude the possibility that unsampled *Nothofagus* associates from Papua New Guinea (Horak, 1980), New Caledonia, Australia, or elsewhere might represent relictual lineages, as appears to be the case in the ascomycete genus *Torreliella* (Johnston, 2006). Although Moyersoen (2006) suggested an ancient tropical Gondwana (c. 135 Myr old) ECM habit in Dipterocarpaceae, our three dipterocarp-associated species diversified no earlier than 49 Ma (nodes 48, 175, 177; Appendix S4) based on 95% confidence intervals.

Which angiosperm families could have been possible ECM partners of the Inocybaceae as it initially diversified between 99 and 191 Ma? Molecular clock studies tend to support the origin of angiosperms between 140 and 190 Ma (Sanderson & Doyle, 2001; Wikstrom *et al.*, 2001; Bell *et al.*, 2005), with diversification of basal eudicots about 120 Ma (Anderson *et al.*, 2005). Earlier origins for angiosperms are possible (up to 275–281 Ma), depending on the methods and data used. Regardless, an early to late Jurassic origin of angiosperms does not reject the view that the Inocybaceae could have been primitively ECM with angiosperms. Numerous ECM fungal groups have evolved independently (Bruns *et al.*, 1998; Hibbett *et al.*, 2000; Bruns & Shefferson, 2004; Matheny *et al.*, 2006; Wang & Qiu, 2006) and probably followed the radiation of their angiosperm partners as suggested by Pirozynski (1983). Taking our results and associated error into account, two alternative hypotheses are possible: either clades of Inocybaceae acquired the ECM symbiosis independently at later dates (a rather unparsimonious solution); or extant ECM angiosperm families are older than minimal dating based on fossil evidence suggests (Wikstrom *et al.*, 2001).

Evolution of conifer symbioses did not emerge until the Palaeogene

Members of Pinaceae are primitively ECM and have a fossil and molecular clock record that dates back to about 200 Ma, in the Jurassic (Malloch *et al.*, 1980; Cairney, 2000; Wang *et al.*, 2000). Our findings, however, do not support the evolution of symbioses with ECM conifers until about 50–60 Ma, during the late Palaeocene and early Eocene (nodes 40, 136) (Figs 2 & 3). Ancestral area analyses do not support evolution of Inocybaceae in northern latitudes, regions where contact with ECM conifers would have been most probable, until about 70–75 Ma, a period of diversification for many genera of Pinaceae such as *Abies*, *Keteleeria*, *Larix*, *Pseudotsuga* and *Tsuga* (Wang *et al.*, 2000).

This period of potential co-divergence between ECM mushrooms and Pinaceae is consistent with a Palaeogene diversification of the 'suilloid group' (Suillineae), a monophyletic group of Boletales with a strict ECM association with Pinaceae (Bruns *et al.*, 1998). Examination of a global phylogeographical study of *Pisolithus* (Martin *et al.*, 2002), another ECM mushroom group of Boletales, indicates that transition to conifer hosts is a derived trait (data not shown). Similar observations are observed in ECM Russulaceae (B. Buyck, unpublished data) and Hysterangiales (Hosaka *et al.*, 2008). Our results suggest that of four major north temperate radiations in the Inocybaceae (Figs 2 & 3), only one (in *Mallochybe*) is unambiguously associated with conifers (node 41). Thus, it seems unlikely that switches from angiosperms to conifers promoted cladogenesis in Inocybaceae exclusively, as angiosperm-associated radiations were taking place concurrently in the Southern Hemisphere. Future research should consider the phylogenetic breadth

of ancestral associations between ECM Agaricomycetes and angiosperms.

Neotropical Inocybaceae are derived lineages composed of immigrant taxa

The Neotropics include some early lineages but also have been the recipient of immigrant lineages from north temperate and African progenitors on six distinct occasions, primarily during the Palaeocene and Eocene. Despite the high number of dispersal events, the diversification of lowland Neotropical species has been limited to two clades only: *Pseudosperma* and *Inocybe* s. str.

Two Neotropical clades are unambiguously sister to Old World clades in Africa (nodes 84, 96), but the timing of New World/Old World divergences is not old enough (<5 Myr) to support vicariance between northern South America and Africa, both northern Gondwanan remnants. Neither are these divergences entirely consistent with a model predicted by the boreotropics hypothesis, which invokes the evolution of Neotropical taxa from a grade of North American ancestors, the earliest representative of which is sister to Old World taxa (Lavin & Luckow, 1993; Pennington & Dick, 2004). Three lowland Neotropical species (*Inocybe* sp. DJL-SJI4, 1. *marginata* nom. provo and *Inocybe* sp. MCA2441; nodes 67, 179, 182, respectively) are reconstructed as having north temperate ancestry and appearing during the Eocene (elements consistent with the boreotropics hypothesis), but necessary sister relationships to Old World taxa are currently lacking. Moyersoen (2006) recently confirmed two samples of *Inocybe* from ECM roots of South American Dipterocarpaceae that share DNA sequence affinities to nodulose-spored taxa of *Inocybe* s. str. based on BLAST comparisons. Thus, there is potentially a third New World/Old World connection that merits further scrutiny.

Whereas 53 currently described species are from the Palaeotropics, only 24 species are acknowledged from the Neotropics (Matheny *et al.*, 2003; Appendix S1). The reason for this discrepancy (apart from sampling effort) could be a combination of factors - extinction, paucity of ECM host diversity, or competition. Buyck & Ovrebo (2002) observed a similar discrepancy in the diversity of ECM Russulales in the Neotropics, as did Lodge *et al.* (1995) for ECM Boletales.

South temperate lineages of Inocybaceae are derived primarily from north temperate ancestry

The evolution of Australian ECM fungi has not been explicitly evaluated, but hypotheses that posit the origins of myrtaceous-associated ECM taxa from *Nothofagus*-associated ancestors have been raised (Horak, 1983; Bougher *et al.*, 1994; Bougher, 1995). The underlying premise is that former *Nothofagus* ECM associates switched hosts to Myrtaceae (e.g. *Eucalyptus*), Fabaceae, or Casuarinaceae upon extinction of their *Nothofagus* hosts. All six myrtaceous-associated nodes (8, 38, 119,

127, 142, 183) have mean divergence dates between 26 and 55 Ma, dates consistent with the diversification of eucalypts in Australia (Crisp *et al.*, 2004). None of the austral lineages is unambiguously derived from conifer-only ancestral lineages, although *I. violaceocaulis* (node 127) appears to be derived from generalist ancestors.

Our results suggest that south temperate species of Inocybaceae associated with Myrtaceae or *Nothofagus* are mostly derived from north temperate ancestry. No models exist that propose vicariance between the north temperate zone and Australia exclusively. Dispersal during the Cenozoic may have been facilitated by taxa with the ability to switch hosts from other angiosperm groups to Myrtaceae and/or *Nothofagus* (Tedesco *et al.*, 2007).

Despite a north temperate influence on the Inocybaceae component of Australia's mycoflora, at least three instances of vicariance that involve lineages from Australia cannot be rejected - one ancient, two more recent. The first entails support for a late Cretaceous split between African and Australian lineages of *Auritella* (Appendix S3). This result is consistent with a strict molecular clock analysis of *Auritella* calibrated by geological evidence (Matheny & Bougher, 2006a). Two other instances date more or less to the Oligocene and fail to reject vicariance between South America and Australia, regions that were last in biotic contact 28–32 Ma. None of the four New Zealand taxa (nodes 33, 146, 149, 186) demonstrates support for vicariance, which is consistent with conclusions drawn from studies of *Pisolithus* (ECM Boletales, Moyersoen *et al.*, 2003) and *Lentinula* (Hibbett, 2001), a genus of saprotrophic Agaricales.

In temperate regions of South America, Singer (1953) observed no connections between species of fungi associated with *Alnus* and *Salix*, on the one hand, and with *Nothofagus*, on the other. Our results reinforce this view. Members of Inocybaceae associated with *Alnus* and *Salix* descended from north temperate ancestry but are too old to have migrated with their hosts into South America. There is also no biogeographical relationship, and no history of ECM exchange, between tropical South American taxa and temperate South American taxa, results consistent with observations made by Singer (1969). Sanmartin & Ronquist (2004) reported evidence that supports a high degree of non-relatedness between these regions based on biogeographical surveys of animals and plants. Macrofungi may adhere to this pattern as well.

A mosaic of vicariance and dispersal events has contributed to the historical biogeography of the Inocybaceae

Our current view of Inocybaceae historical biogeography is a mixture of several ancient vicariance events involving Africa, India and Australia, followed by late Cretaceous to Palaeogene movement into the north temperate zone, and then later into southern temperate areas. Radiations within major clades of the family did not take place until the Palaeogene,

primarily in north and south temperate regions. Based on current taxon sampling, tree topology, and parsimony reconstruction analyses of major geographic areas, vicariance hypotheses can be rejected that involve northern South America-Africa and Australia-New Zealand. Contrary to an expectation that ECM elements of the south temperate zone inherited their biogeographical history from ancient *Nothofagus* associations, the south temperate zone has been the recipient of long-distance dispersal events from lineages primarily associated with angiosperm lineages in the Northern Hemisphere. A view that early *Inocybaceae* history was associated with conifers (by virtue of their older age than angiosperms) is rejected. On the contrary, ancestral *Inocybaceae* from the early and late Cretaceous were involved in ECM associations with angiosperms from Palaeotropical regions, a result that revives Pirozynski's (1983) hypothesis that ECM fungi originated from a 'Cretaceous tropical source'.

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REFERENCES

Alexander, I. (1989) Systematics and ecology of ectomycorrhizal legumes. *Advances in legume biology* (ed. by CH. Stirton and J.L. Zarucchi), *Monographs in Systematic Botany from the Missouri Botanical Garden*, 29, 607-624.

Alexander, I. (2006) Ectomycorrhizas - out of Africa? *New Phytologist*, 172, 589-591.

Alexander, I. & Lee, S.S. (2005) Mycorrhizas and ecosystem processes in tropical rain forest: implications for diversity. *Biotic interactions in the tropics: their role in the maintenance of species diversity* (ed. by D.F.R.P. Burslem, M.A. Pinar and S.E. Hartley), pp. 165-203. Cambridge University Press, Cambridge.

Ali, J.R. & Aitchison, J.C. (2008) Gondwana to Asia: Plate tectonics, paleogeography and the biological connectivity of the India sub-continent from the Middle Jurassic through latest Eocene (166-35 Ma). *Earth-Science Reviews*, 88, 145-166.

Allen, E.B., Allen, M.F., Helm, D.J., Trappe, J.M., Molina, R. & Rincon, E. (1995) Patterns and regulation of mycorrhizal plant and fungal diversity. *Plant and Soil*, 170, 47-62.

Anderson, C.L., Bremer, K. & Friis, E.M. (2005) Dating phylogenetically basal eudicots using *rbcL* sequences and multiple fossil reference points. *American journal of Botany*, 92, 1737-1748.

Arnolds, E.J.M. (1997) Biogeography and conservation. *The Mycota IV. Environmental and microbial relationships* (ed. by D.T. Wicklow and B.E. Soderstrom), pp. 115-131. Springer-Verlag, Berlin.

Bell, C.D., Soltis, D.E. & Soltis, P.S. (2005) The age of the angiosperms: a molecular timescale without a clock. *Evolution*, 59, 1245-1258.

Berbee, M.L. & Taylor, J.W. (2001) Fungal molecular evolution: gene trees and geologic time. *The Mycota VII Part B: systematics and evolution* (ed. by D. McLaughlin, E. McLaughlin and P. Lemke), pp. 229-245. Springer-Verlag, Berlin.

Berry, P.E., Hahn, W.J., Sytsma, K.J., Hall, J.C. & Mast, A. (2004) Phylogenetic relationships and biogeography of *Fuchsia* (Onagraceae) based on noncoding nuclear and chloroplast DNA data. *American journal of Botany*, 91, 601-614.

Bougher, N.L. (1995) Diversity of ectomycorrhizal fungi associated with eucalypts in Australia. *Mycorrhizas for plantation forestry in Asia* (ed. by M. Brundrett, B. Dell, N. Malajczuk and G. Mingqin), pp. 8-15. ACIAR, Canberra.

Bougher, N.L. & Syme, K. (1998) *Fungi of southern Australia*. University of Western Australia Press, Nedlands, Australia.

Bougher, N.L., Fuhrer, B.A. & Horak, E. (1994) Taxonomy and biogeography of Australian *Rozites* species mycorrhizal with *Nothofagus* and Myrtaceae. *Australian Systematic Botany*, 7, 353-375.

Briggs, J.C. (2003) The biogeographic and tectonic history of India. *Journal of Biogeography*, 30, 381-388.

Bruns, T.D. & Shefferson, R.P. (2004) Evolutionary studies of ectomycorrhizal fungi: recent advances and future directions. *Canadian journal of Botany*, 82, 1122-1132.

Bruns, T.D., Szaro, T.M., Gardes, M., Cullings, K.W., Pan, J.J., Taylor, D.L., Horton, T.R., Kretzer, A., Garbelotto, M. & Li, Y. (1998) A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. *Molecular Ecology*, 7, 257-272.

Buyck, B. & Eyssartier, G. (1999) Two new species of *Inocybe* (Cortinariaceae) from African woodland. *Kew Bulletin*, 54, 675-681.

Buyck, B. & Ovrebo, C.L. (2002) New and interesting *Russula* species from Panama. *Mycologic*, 94, 888-901.

Buyck, B., Thoen, D. & Watling, R. (1996) Ectomycorrhizal fungi of the Guinea-Congo region. *Proceedings of the Royal Society of Edinburgh*, 104 (B), 313-333.

Cairney, J.W.G. (2000) Evolution of mycorrhiza systems. *Naturwissenschaften*, 87, 467-475.

Campbell, H.J. & Landis, C.A. (2001) New Zealand awash. *New Zealand Geographic*, 51, 6-7.

Cox, C.B. & Moore, P.D. (2000) *Biogeography: an ecological and evolutionary approach*, 6th edn. Blackwell Science Ltd, Oxford.

- Crisp, M., Cook, L. & Steane, D. (2004) Radiation of the Australian flora: what can comparisons of molecular phylogenies across multiple taxa tell us about the evolution of diversity in present-day communities? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 359, 1551-1571.
- Drummond, A.J. & Rambaut, A. (2006) *BEAST* v1.4. Available at: <http://beast.bio.ed.ac.uk/> (last accessed 30 September 2008).
- Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, 7, 214.
- Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology*, 4, e88.
- Drummond, A.J., Ho, S.Y.W., Rawlence, N. & Rambaut, A. (2007) A rough guide to BEAST 1.4. Available at: http://beast.bio.ed.ac.uk/#A_Rough_Guide_to_BEAST_1.4 (last accessed 30 September 2008).
- Geml, J., Geiser, D.M. & Royse, D.J. (2004) Molecular evolution of *Agaricus* species based on ITS and LSU rDNA sequences. *Mycological Progress*, 3, 157-176.
- Geml, J., Laursen, G.A., O'Neill, K.O., Nusbaum, H.C. & Taylor, D.L. (2006) Beringian origins and cryptic speciation events in the fly agaric (*Amanita muscaria*). *Molecular Ecology*, 15, 225-239.
- Graham, A. (1999) The Tertiary history of the northern temperate element in the northern Latin American biota. *American Journal of Botany*, 86, 32-38.
- Grauer, D. & Martin, W. (2004) Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends in Genetics*, 20, 80-86.
- Heads, M. (2005) Dating nodes on molecular phylogenies: a critique of molecular biogeography. *Cladistics*, 21, 62-78.
- Heckman, D.S., Geiser, D.M., Eidell, B.R., Stauffer, R.L., Kardos, N.L. & Hedges, S.B. (2001) Molecular evidence for the early colonization of land by fungi and plants. *Science*, 293, 1129-1133.
- Hibbett, D.S. (2001) Shiitake mushrooms and molecular clocks: historical biogeography of *Lentinula*. *Journal of Biogeography*, 28, 231-241.
- Hibbett, D.S. & Donoghue, M.J. (1997) Fossil mushrooms from Miocene and Cretaceous ambers and the evolution of homobasidiomycetes. *American Journal of Botany*, 84, 981-991.
- Hibbett, D.S., Gilbert, L.-B. & Donoghue, M.J. (2000) Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. *Nature*, 407, 506-508.
- Hill, R.S. & Brodribb, T.J. (1999) Southern conifers in time and space. *Australian Journal of Botany*, 47, 639-696.
- Horak, E. (1977) Fungi Agaricini Novaezelandiae VI. *Inocybe* (Fr.) Fr. and *Astrosporina* Schroeter. *New Zealand Journal of Botany*, 15, 713-747.
- Horak, E. (1979) *Astrosporina* (Agaricales) in Indomalaya and Australasia. *Persoonia*, 10, 157-205.
- Horak, E. (1980) *Inocybe* (Agaricales) in Indomalaya and Australasia. *Persoonia*, 11, 1-37.
- Horak, E. (1983) Mycogeography in the South Pacific region: Agaricales, Boletales. *Australian Journal of Botany Supplemental Series*, 10, 1-41.
- Hosaka, K., Castellano, M.A. & Spatafora, J.W. (2008) Biogeography of Hysterangiales (Phallomycetidae, Basidiomycota). *Mycological Research*, 112, 448-462.
- Jeandroz, S., Murat, C., Wang, Y., Bonfante, P. & Le Tacon, F. (2008) Molecular phylogeny and historical biogeography of the genus *Tuber*, the 'true truffles'. *Journal of Biogeography*, 35, 815-829.
- Johnston, P.R. (2006) New Zealand's nonlichenised fungi - where they came from, who collected them, where they are now. *Proceedings of the 7th and 8th Symposia on Collection Building and Natural History Studies in Asia and the Pacific Rim* (ed. by Y. Tomida). *National Science Museum Monographs*, 34, 37-49.
- Karant, E.P. (2006) Out-of-India Gondwanan origin of some tropical Asian biota. *Current Science*, 90, 789-792.
- Kirk, P.M., Cannon, P.F., David, J.C. & Stalpers, J.A. (2001) *Ainsworth & Bisby's dictionary of the fungi*, 9th edn. CABI Publishing, Wallingford.
- Kuyper, T.W. (1986) A revision of the genus *Inocybe* in Europe I. Subgenus *Inosperma* and the smooth-spored species of subgenus *Inocybe*. *Persoonia (Suppl.)*, 3, 1-247.
- Lavin, M. & Luckow, M. (1993) Origins and relationships of tropical North America in the context of the boreotropics hypothesis. *American Journal of Botany*, 80, 1-14.
- Lee, S.S. (1990) The mycorrhizal association of the Dipterocarpaceae in the tropical rain forests of Malaysia. *Ambia*, 19, 383-385.
- Lodge, D.J., Chapela, I., Samuels, G., Uecker, F.A., Desjardin, D., Horak, E., Miller, O.K., Jr, Hennebert, G.L., Decock, C.A., Ammirati, J., Burdsall, H.H., Jr, Kirk, P.M., Minter, D.W., Halling, R., Laessle, T., Mueller, G., Huhndorf, S., Oberwinkler, F., Pegler, D.N., Spooner, B., Petersen, R.H., Rogers, J.D., Ryvarden, L., Watling, R., Turnbull, E. & Whalley, A.J.S. (1995) A survey of patterns of diversity in non-lichenized fungi. *Mitteilungen der Eidgenössischen Forschungsanstalt für Wald, Schnee und Landschaft*, 70, 157-173.
- Lodge, D.J., Ammirati, J.F., O'Dell, T.E., Mueller, G.M., Huhndorf, S.M., Wang, C.J., Stokland, J.N., Schmit, J.P., Ryvarden, L., Leacock, P.R., Mata, M., Umana, L., Wu, Q. & Czederpiltz, D.L. (2004) Terrestrial and lignicolous macrofungi. *Biodiversity of fungi. Inventory and monitoring methods* (ed. by G.M. Mueller and G. Bills), pp. 127-172. Academic Press, Amsterdam.
- Lomolino, M.V., Riddle, B.R. & Brown, J.H. (2006) *Biogeography*, 3rd edn. Sinauer Associates, Sunderland, MA.
- Maddison, D.R. & Maddison, W.P. (2000) *MacClade 4: analysis of phylogeny and character evolution*. Sinauer Associates, Sunderland, MA.
- Malloch, D.W., Pirozynski, K.A. & Raven, P.H. (1980) Ecological and evolutionary significance of mycorrhizal symbi-

- oses in vascular plants (a review). *Proceedings of the National Academy of Sciences USA*, 77, 2113-2118.
- Martin, F., Diez, J., Dell, B. & Delaruelle, C. (2002) Phylogeography of the ectomycorrhizal *Pisolithus* species as inferred from nuclear ribosomal DNA ITS sequences. *New Phytologist*, 153, 345-357.
- Matheny, P.B. (2005) Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; Agaricales). *Molecular Phylogenetics and Evolution*, 35, 1-20.
- Matheny, P.B. & Bougher, N.L. (2006a) The new genus *Auritella* from Africa and Australia (Inocybaceae, Agaricales): molecular systematics, taxonomy and historical biogeography. *Mycological Progress*, 5, 2-17.
- Matheny, P.B. & Bougher, N.L. (2006b) Validation of the genus *Auritella*. *Mycotaxon*, 97, 231-233.
- Matheny, P.B., Liu, Y.J., Ammirati, J.F. & Hall, B.D. (2002) Using RPB1 sequences to improve phylogenetic inference among mushrooms (*Inocybe*, Agaricales). *American Journal of Botany*, 89, 688-698.
- Matheny, P.B., Aime, M.C. & Henkel, T.W. (2003) New species of *Inocybe* from *Dicymbe* forests of Guyana. *Mycological Research*, 107, 495-505.
- Matheny, P.B., Curtis, J.M., Hofstetter, V., Aime, M.C., Moncalvo, J.M., Ge, Z.W., Yang, Z.L., Slot, J.C., Ammirati, J.F., Baroni, T.J., Bougher, N.L., Hughes, K.W., Lodge, D.J., Kerrigan, R.W., Seidl, M.T., Aanen, D.K., DeNitis, M., Daniele, G.M., Desjardin, D.E., Kropp, B.R., Norvell, L.L., Parker, A., Vellinga, E.C., Vilgalys, R. & Hibbett, D.S. (2006) Major clades of Agaricales: a multilocus phylogenetic overview. *Mycologia*, 98, 982-995.
- Matheny, P.B., Aime, M.C., Buyck, B., Desjardin, D., Horak, E. & Lodge, D.J. (2007) The evolution of tropical species of Inocybaceae (Agaricales). *Abstracts of the Mycological Society of America Annual Meeting, Louisiana State University, Baton Rouge, USA, 5-9 August 2007*. Mycological Society of America, Baton Rouge, LA.
- McLoughlin, S. (2001) The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany*, 49, 271-300.
- Morris, B. (1990) An annotated check-list of the macrofungi of Malawi. *Kirkia*, 13, 323-364.
- Moyersoen, B. (2006) *Pakaraimaea dipterocarpacea* is ectomycorrhizal, indicating an ancient Gondwanaland origin for the ectomycorrhizal habit in Dipterocarpaceae. *New Phytologist*, 172, 753-762.
- Moyersoen, B., Beever, R.E. & Martin, F. (2003) Genetic diversity of *Pisolithus* in New Zealand indicates multiple long-distance dispersal from Australia. *New Phytologist*, 160, 569-579.
- Mueller, G.M. & Halling, R.E. (1995) Evidence for high biodiversity of Agaricales (Fungi) in neotropical montane *Quercus* forests. *Biodiversity and conservation of neotropical montane forests* (ed. by S.P. Churchill, H. Balslev, E. Forero and J.L. Luteyn), pp. 303-312. The New York Botanical Garden, New York.
- Mueller, G.M., Wu, Q.X., Huang, Y.Q., Guo, S.Y., Aldana-Gomez, R. & Vilgalys, R. (2001) Assessing biogeographic relationships between North American and Chinese macrofungi. *Journal of Biogeography*, 28, 271-281.
- Mueller, G.M., Schmit, J.P., Leacock, P.R., Buyck, B., Cifuentes, J., Desjardin, D.E., Halling, R.E., Hjortstam, K., Iturriga, T., Larsson, K.H., Lodge, D.J., May, T.W., Minter, D., Rajchenberg, M., Redhead, S.A., Ryvarden, L., Trappe, J.M., Watling, R. & WU, Q. (2007) Global diversity and distribution of macrofungi. *Biodiversity and Conservation*, 16, 37-48.
- Natarajan, K., Narayanan, K., Ravindran, C. & Kumaresan, V. (2005) Biodiversity of agarics from Nilgiri Biosphere Reserve, Western Ghats, India. *Current Science*, 88, 1890-1893.
- Pennington, R.T. & Dick, C.W. (2004) The role of immigrants in the assembly of the South American rainforest tree flora. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 359, 1611-1622.
- Petersen, R.H. & Hughes, K.W. (2007) Some agaric distribution patterns involving Pacific landmasses and Pacific Rim. *Mycoscience*, 48, 1-14.
- Pirozynski, K.A. (1983) Pacific mycogeography: an appraisal. *Australian Journal of Botany Supplemental Series*, 10, 137-159.
- Poinar, G.O. & Buckley, R. (2007) Evidence of mycoparasitism and hypermycoparasitism in Early Cretaceous amber. *Mycological Research*, 111, 503-506.
- Printzen, C. & Lumbsch, H.T. (2000) Molecular evidence for the diversification of extant lichens in the Late Cretaceous and Early Tertiary. *Molecular Phylogenetics and Evolution*, 17, 379-387.
- Pulquerio, M.J.F. & Nichols, R.A. (2006) Dates from the molecular clock: how wrong can we be? *Trends in Ecology and Evolution*, 22, 180-184.
- Raven, P.H. (1979) Plate tectonics and Southern Hemisphere biogeography. *Tropical botany* (ed. by K. Laursen and L.B. Holm-Nielsen), pp. 3-24. Academic Press, London.
- Raven, P.H. & Axelrod, D.L. (1974) Angiosperm biogeography and past continental movements. *Annals of the Missouri Botanical Garden*, 61, 539-673.
- Redhead, S.A. (1989) A biogeographical overview of the Canadian mushroom flora. *Canadian Journal of Botany*, 67, 3003-3062.
- Renner, S.S. (2005) Relaxed molecular clocks for dating historical plant dispersal events. *Trends in Plant Science*, 10, 550-558.
- Riviere, T., Diedhiou, A.G., Diabate, M., Senthilarasu, G., Natarajan, K., Verbeken, A., Buyck, B., Dreyfus, B., Bena, G. & Ba, A.M. (2007) Genetic diversity of ectomycorrhizal Basidiomycetes from African and Indian tropical rain forests. *Mycorrhiza*, 17, 415-428.
- Robinson, R. (2006) Relaxing the clock brings time back into phylogenetics. *PLoS Biology*, 4, e106.
- Ryberg, M., Nilsson, R.H., Kristiansson, E., Topel, M., Jacobsson, S. & Larsson, E. (2008) Mining meta data from

- unidentified ITS sequences in GenBank: a case study in *Inocybe* (Basidiomycota). *BMC Evolutionary Biology*, 8, 50.
- Sanderson, M.J. (1997) A nonparametric approach to estimating divergence times in the absence of rate constancy. *Molecular Biology and Evolution*, 14, 1218-1231.
- Sanderson, M.J. & Doyle, J.A. (2001) Sources of error and confidence intervals in estimating the age of angiosperms from *rbcL* and 18S rDNA data. *American Journal of Botany*, 88, 1499-1516.
- Sanmartin, I. (2002) *A paleogeographic history of the Southern Hemisphere*. Available at: <http://www.ebc.uu.se/systzoo/staff/sanmartin/PaleoHistSH.pdf> (last accessed 30 September 2008).
- Sanmartin, I. & Ronquist, F. (2004) Southern Hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic Biology*, 53, 216-243.
- Sims, K., Watling, R., De la Cruz, R. & Jeffries, P. (1997) Ectomycorrhizal fungi of the Philippines: a preliminary survey and notes on the geographic biodiversity of the Sclerodermatales. *Biodiversity and Conservation*, 6, 45-58.
- Singer, R. (1953) Four years of mycological work in southern South America. *Mycologia*, 45, 865-891.
- Singer, R. (1969) Mycoflora Australis. *Beihefte zur Nova Hedwigia*, 29, 1-405.
- Singer, R. (1986) *The Agaricales in modern taxonomy*, 4th edn. Koeltz Scientific Books, Koenigstein, Germany.
- Stangl, J. (1989) Die Gattung *Inocybe* in Bayern. *Hoppea*, 46, 1-409.
- Stuntz, D.E. (1954) Studies on the genus *Inocybe* II. New and noteworthy species from Michigan. *Papers of the Michigan Academy of Science, Arts, and Letters*, 39, 53-84.
- Swofford, D.L. (2003) *PAUP*. Phylogenetic analysis using parsimony (*and other methods)*. Version 4. Sinauer Associates, Sunderland, MA.
- Sytsma, K.J., Litt, A., Zjhra, M.L., Pires, J.C., Nepokroeff, M., Conti, E., Walker, J. & Wilson, P.G. (2004) Clades, clocks, and continents: historical and biogeographical analysis of Myrtaceae, Vochysiaceae, and relatives in the Southern Hemisphere. *International Journal of Plant Sciences*, 165(Suppl. 4), 585-5105.
- Taylor, A.F.S. & Alexander, I. (2005) The ectomycorrhizal symbiosis: life in the real world. *Mycologist*, 19, 102-112.
- Taylor, J.W. & Berbee, M.L. (2006) Dating divergences in the Fungal Tree of Life: review and new analyses. *Mycologia*, 98, 838-849.
- Taylor, J.W., Turner, E., Townsend, J.P., Dettman, J.R. & Jacobson, D. (2006) Eukaryotic microbes, species recognition and the geographic limits of species: examples from the kingdom Fungi. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 361, 1947-1963.
- Tedersoo, L., Beaver, K. & Koljalg, U. (2007) Ectomycorrhizal fungi of the Seychelles: diversity patterns and host shifts from the native *Vateriopsis seychellarum* (Dipterocarpaceae) and *Intsia bijuga* (Caesalpinaceae) to the introduced *Eucalyptus robusta* (Myrtaceae), but not *Pinus caribea* (Pinaceae). *New Phytologist*, 175, 321-333.
- Thoen, D. (1993) Looking for ectomycorrhizal trees and ectomycorrhizal fungi in tropical Africa. *Aspects of tropical mycology* (ed. by S. Isaac, J.C. Frankland, R. Watling and A.I.S. Whalley), pp. 192-205. Cambridge University Press, Cambridge.
- Turnbull, E. (1995) *Inocybe* in peninsular Malaysia. *Edinburgh Journal of Botany*, 52, 351-359.
- Turnbull, E. & Watling, R. (1999) Taxonomic and floristic notes on Malaysian larger fungi III. *Malayan Nature Journal*, 53, 189-200.
- Villarreal, M., Esteve-Raventos, F., Heykoop, M. & Horak, E. (1998) *Inocybe inexpectata*, a new and unusual species of subgenus *Mallochybe*. *Mycological Research*, 102, 472-482.
- Waldrop, M.P., Zak, D.R., Blackwood, C.B., Curtis, C.D. & Tilman, D. (2006) Resource availability controls fungal diversity across a plant diversity gradient. *Ecology Letters*, 9, 1127-1135.
- Wang, B. & Qiu, Y.L. (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*, 16, 299-363.
- Wang, X.Q., Tank, D.C. & Sang, T. (2000) Phylogeny and divergence times in Pinaceae: evidence from three genomes. *Molecular Biology and Evolution*, 17, 773-781.
- Waters, J.M. & Craw, D. (2006) Goodbye Gondwana? New Zealand biogeography, geology, and the problem of circularity. *Systematic Biology*, 55, 351-356.
- Watling, R. (1993) Comparison of the macromycete biotas in selected tropical areas of Africa and Australia. *Aspects of tropical mycology* (ed. by S. Isaac, J.C. Frankland, R. Watling and A.I.S. Whalley), pp. 171-191. Cambridge University Press, Cambridge.
- Watling, R. (1994) Ectomycorrhizal fungi in the Palaeotropics. *Mycologia Helvetica*, 2, 129-138.
- Watling, R. (2001a) The relationships and possible distributional patterns of boletes in south-east Asia. *Mycological Research*, 105, 1440-1448.
- Watling, R. (2001b) An unusual *Inocybe* sp. from West Africa. *Czech Mycology*, 52, 329-334.
- Welch, I.J. & Bromham, L. (2005) Molecular dating when rates vary. *Trends in Ecology and Evolution*, 20, 320-327.
- Wikstrom, N., Savolainen, V. & Chase, M.W. (2001) Evolution of the angiosperms: calibrating the family tree. *Proceedings of the Royal Society B: Biological Sciences*, 268, 2211-2220.
- Wilme, L., Goodman, S.M. & Ganzhorn, J.U. (2006) Biogeographic evolution of Madagascar's microendemic biota. *Science*, 312, 1063-1065.
- WU, Q.X. & Mueller, G.M. (1997) Biogeographic relationships between the macro fungi of temperate eastern Asia and eastern North America. *Canadian Journal of Botany*, 75, 2108-2116.
- Wurdack, K.J., Hoffmann, P., Samuel, R., de Bruijn, A., van der Bank, M. & Chase, M.W. (2004) Molecular phylogenetic

analysis of Phyllanthaceae (Phyllanthoideae pro parte, Euphorbiaceae sensu lato) using plastid *RBCL* DNA sequences. *American Journal of Botany*, 91, 1882-1900.

Zhou, S., Renner, S.S. & Wen, J. (2006) Molecular phylogeny and inter- and intracontinental biogeography of Calycanthaceae. *Molecular Phylogenetics and Evolution*, 39, 1-15.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Number of Inocybaceae species described from the tropics and Southern Hemisphere.

Appendix S2 List of accessions, collection numbers, geographic origins, and GenBank accession numbers of sequences used in this study.

Appendix S3 Chronogram of 12 selected Basidiomycota used in the initial step of the secondary calibration procedure.

Appendix S4 Estimated ages and 95% confidence intervals (CI) for numbered nodes on the Inocybaceae chronogram (Figs 2 & 3).

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BIOSKETCH

P. Brandon Matheny is an assistant professor at the University of Tennessee with research interests in the systematics, evolution and biogeography of Agaricales.

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