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Pacific boletes: Implications for biogeographic relationships

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ABSTRACT

The obligate association of boletes with their plant partners is critical to understanding biogeographic distribution of these fungi. Only in rare instances are boletes not obligatory associates of plants; the majority are presumed or proven partners in obligate symbioses with a variety of plants. The array of plant-associated distributions provides a potential handle for evaluating bolete distribution on a global scale. However, migration processes remain unclear and distributions are often disjunct. As an illustration of phylogeographic studies of putatively widespread bolete taxa, we present preliminary analyses for *Tylopilus ballouii* using LSU rDNA and RPB1 sequence data. The LSU data suggest geographic structuring of the tested accessions. However, RPB1 data indicate that long-distance dispersal events (possibly mediated by humans) are possible, or that selection or other factors have obscured geographical patterns. Molecular divergence between samples in RPB1 argues against panmixis, and indicates that populations have been isolated for long periods.

Introduction

The *Boletaceae* as outlined by Singer (1986) has been dissected recently with the realignment of generic affinities in additional families in a single order (*Boletales*), with the recognition of a restricted *Boletaceae* (Binder & Hibbett 2007). The great majority of these taxa are presumed or proven partners in obligate symbioses with a variety of plants. In this role, they are an integral part of all forest systems as they are intimately involved with such basic processes as nutrient cycling, nutrient uptake, and decomposition of organic matter. Extrapolating from previous surveys of fungal diversity (Agerer 1987–1998; Hawksworth *et al.* 1995), roughly 90 % of the species of boletes are potentially ectomycorrhizal, and the boletes may represent 18–25 % of all ectomycorrhizal fungi. The plant families *Betulaceae*, *Caesalpiniaceae*, *Casuarinaceae*, *Dipterocarpaceae*, *Ericaceae*, *Fagaceae*, *Mimosaceae*, *Myrtaceae*, *Pinaceae*, and *Salicaceae* have been proven or implicated to form ectomycorrhizal symbioses with boletes (Halling pers. obs.; Newman & Reddell 1987; Lee *et al.* 1997; Osmundson *et al.* 2007). In forests dominated by trees of these families, boletes are typically a conspicuous element of the mycobiota during the rainy season; e.g. Hongo (1984) stated that the boletes are among the principal components of Japanese evergreen oak forests.

Since the late 1990s, studies by Halling (1997, 2001), Halling & Mueller (2001, 2005), and Mueller & Halling (1995) have substantiated a hypothesis advanced by Moser & Horak (1975) by providing convincing evidence that obligate ectomycorrhizal macromycetes are likely to have migrated with associated plant communities from North America into northern South America (Fig 1). Halling & Ovrebo (1987) postulated a scenario based on plate tectonics and fossil pollen data to account for the disjunct distribution of *Rozites colombiana* in northern

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Fig 1 – Migration route from North America to South America.

South America. Armed with subsequent biogeographic data, Bougher *et al.* (1994) provided a different and more convincing hypothesis for relictual disjunction of *Rozites*. Watling (1988) has discussed similar community migration of plants and associated fungi in the UK. Further support for such a migration scenario is bolstered by Halling (1989) and Mueller & Strack (1992) who described shifts in plant associates of mycorrhizal agaric and bolete fungi. Several biogeographic patterns of distribution of macrofungi in this region have been detailed previously (Halling & Mueller 2001), including one in which there is a North to South clinal trend. *Boletus auriporus*, *Leccinum rugosiceps*, *Pulveroboletus ravenelii*, *Strobilomyces confusus*, and *Xanthoconium separans* are but a few examples in the *Boletaceae*, and there are other examples of ectomycorrhizal agarics (Halling & Mueller 2005).

Field explorations in western Pacific countries over the past several decades have noted amphi-Pacific distributions of bolete morphotaxa (Corner 1972; Halling 2001; Watling 2001) (Fig 2). Examples of such taxa include *Pulveroboletus ravenelii*, *Tylopilus ballouii*, *T. alboater*, *T. eximius*, *Gyroporus cyanescens*, and the *T. chromapes* group. In this latter group, Wolfe & Bougher (1993) described several new species from Asia, Australia, and Central America that were clearly morphologically allied to the well-known *T. chromapes* from eastern North America (Fig 3). In addition, they commented on the biogeography and evolutionary history of the group. Such obvious amphi-Pacific disjunction of sister taxa deserves further study from a molecular perspective.

We suggest that there are three possible hypotheses to account for such apparent disjunction. The first is obvious:

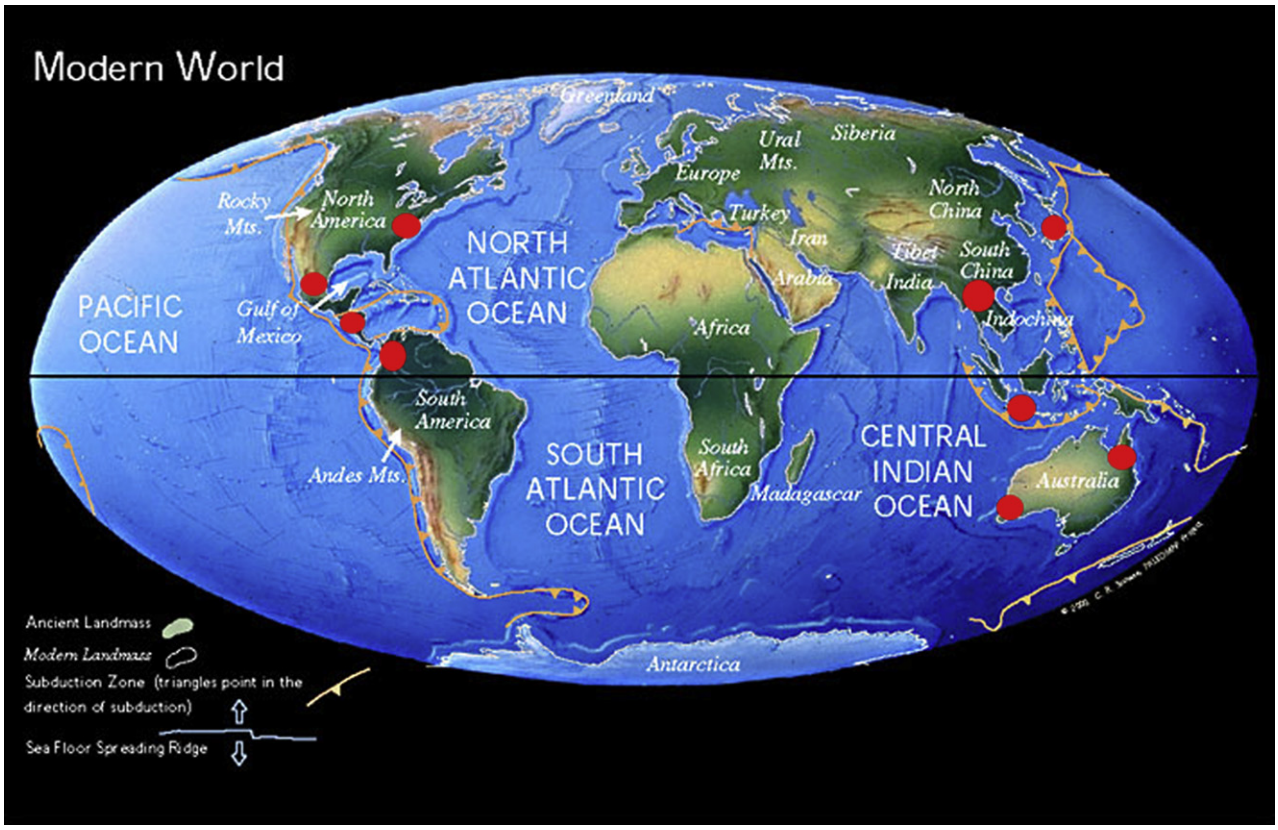

Fig 2 – Amphi-Pacific distributions of some bolete morpho-taxa (base map by Scotese 1997).

long distance dispersal by basidiospores. The second is a post-Cretaceous migration of co-symbionts over land bridges with a change or shift in symbiotic partners. The third hypothesis would embrace an ancient, Pangaean distribution with little, if any, migration and morphological change since the breakup of Pangaea in the Cretaceous.

Biogeographic hypotheses

Long distance dispersal

The best-documented evidence for long-distance dispersal of an ectomycorrhizal species concerns isolates of *Pisolithus* from New Zealand that share an evolutionary relationship to populations originating in Australia, suggesting trans-Tasman long-distance dispersal. This explanation is further supported by purported plant associates (Moyersoen *et al.* 2003).

Evidence supporting a lack of long-distance dispersal in fungi comes primarily from observed patterns of spore deposition, the observation of isolation-by-distance in populations, and the concurrence of phylogenetic trees with vicariance events. Although spores of basidiomycete fungi have been observed in air samples, it is well established that the vast majority of basidiospores are deposited within a few metres of the parent basidiome (Ingold 1971; see reference in Liang *et al.* 2004). For example, a recent study of the ectomycorrhizal basidiomycete *Amanita muscaria* var. *alba* showed that, although spore production occurred at prodigious levels, only 5 % of the spores were dispersed to a location 5.2 m beyond and 2.7 m above the *Amanita* basidiomes, and less than 0.1 % were recovered in a spore trap at a nearby residential location (Li 2005).

Studies of genetic structure between fungal populations reveal evidence of gene flow on small scales, but isolation-by-distance at both moderate and large geographic scales. For example, landscape-level studies of the ectomycorrhizal species *Russula brevipes* by Bergemann and colleagues revealed high levels of gene flow at scales of approximately 1 km, but an isolation-by-distance effect at larger scales (Bergemann *et al.* 2006; Bergemann & Miller 2002). A study of the wood saprotroph *Datronia caperata* in fragmented mangrove forests suggests that high levels of host specificity may exacerbate the effect of isolation-by-distance (Parrent *et al.* 2004).

Migration over land bridges

Since the late 1980s, considerable effort has gone into targeted surveys of agaric and bolete diversity in the montane neotropics, particularly *Quercus* forests in Colombia and Costa Rica (Halling 1989; Halling & Mueller 2001, 2005). As some genera and species were morphologically identical to known genera and species from the Northern hemisphere, particular attention was given to assessing degrees of similarity (Mueller & Halling 1995; Halling 1996). The generic affinities were clearly with Laurasian elements of the Northern hemisphere rather than with the lowland tropics or temperate South America. Conversely, there was a high degree of endemism at the species rank. As the formation of a Panamanian land bridge connecting North America (Laurasian remnant) and

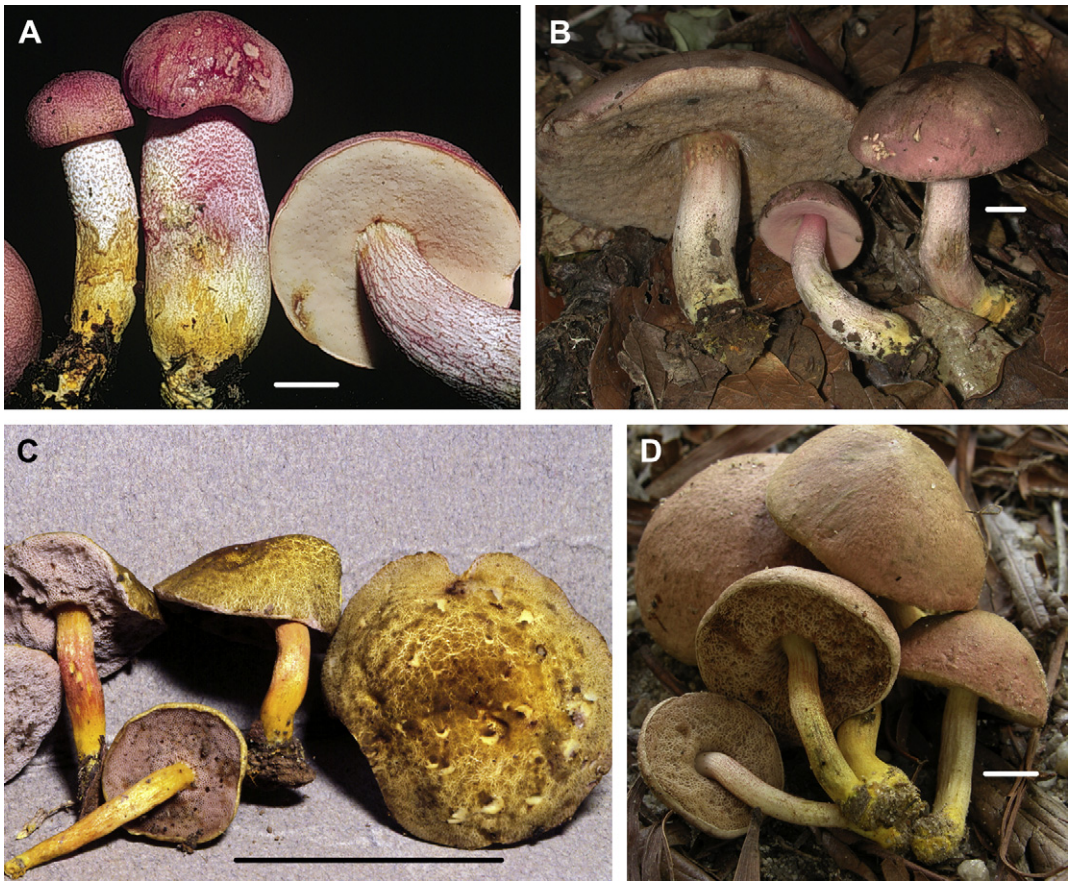

Fig 3 – *Tylopilus chromapes* group. (A) *T. chromapes*. (B) *T. cartagoensis*. (C) *T. pernanus*. (D) *T. queenslandianus*. Bars = 1 cm.

South America (Gondwanan remnant) in the Pliocene, a corridor has developed for the migration of biota across this isthmus (numerous citations in Halling & Mueller 2005: 3) (Fig 4). The efficacy of Beringian or North Atlantic corridors is untenable based on presently known distributions, associations, and paleo-climates.

As the isolated Australian plate has moved northward on a collision course with the Asian plate, the juxtaposition of these two continents since the Pliocene has provided the opportunity for biota to cross the junction known as Wallace's Line (Wallacea; Fig 5). The lowering of sea levels during Pleistocene glaciation provided the requisite land bridge(s). Ectomycorrhizal plant genera, such as *Castanopsis* (*Fagaceae*) and *Anisoptera* (*Dipterocarpaceae*), have migrated from Laurasia to New Guinea, whereas *Leptospermum* (*Myrtaceae*) has moved north from Gondwana. As examples of boletes are documented from either side of Wallacea (e.g. *Pulveroboletus frians*, *P. ravenelii*, *Tylopilus ballouii*, *T. chromapes* group, and *Heimioporus rubropunctus*) (Horak 1980; Wolfe & Bougher 1993; Halling pers. obs.), it is currently difficult to pinpoint an origin and direction of migration.

Relictual Pangaean distribution

The existence of bolete morpho-taxa on continents of both Laurasian and Gondwanan origin could be explained by an original Pangaean distribution and association with then existing mycorrhizal partners (Fig 6). Since the Cretaceous breakup, the bolete–plant partnerships shared subsequent continental habitats with an eventual severing of intercontinental exchange; and during this isolation, little, if any, comparative morphological change transpired (Halling 2001, Halling & Mueller 2001). Each continent then was subjected to local climatic and geologic events that would have had an impact on only those local populations, and the genomes of these populations were thus independently subjected to regional regimes that affected genetic drift, mutation, speciation, and extinction. An example of Gondwanan continental disjunction and speciation was presented for an ectomycorrhizal agaric genus in the *Inocybaceae* (Matheny & Bougher 2006). Our Pangaean hypothesis assumes that the probability of inter-populational gene exchange *via* long-distance dispersal is negligible, and that co-symbiont obligations are necessary for any migration.

Phylogeography of widespread bolete morphotaxa

Molecular genetic data provide powerful tools for assessing the effect of past and current events on the geographic distribution of species. As an example of applying a molecular phylogeographic approach to the question of broad geographic distributions in ectomycorrhizal boletes, a preliminary assessment of phylogeographic pattern is hereby presented for *Tylopilus ballouii*—an example of a widely distributed bolete morphospecies—using specimens collected from a portion of its range, representing both Old World and New World

Fig 4 – Panamanian land bridge connecting Laurasia (North America) and Gondwana (South America).

populations (Table 1). The analyses used a portion of the gene encoding the largest subunit of DNA-dependent RNA polymerase II (RPB1) or a portion of the DNA region encoding nurDNA LSU, or simply LSU. RPB1 consists of eight conserved core regions with amino acid sequences that are alignable between eukaryotes, eubacteria, and archaea, interspersed with intron regions (Jokerst *et al.* 1989), thereby providing the potential for phylogenetic inference at multiple taxonomic scales (Stiller *et al.* 1998; Matheny *et al.* 2002). Even within RPB1 introns, conserved and non-conserved regions have been reported for hymenomycetes (Matheny *et al.* 2002); therefore, the level and location of sequence divergence between samples may allow a qualitative assessment of their degree of historical separation.

Materials and methods

DNA was extracted from air-dried or silica-desiccated basidiome tissue using the DNeasy Plant Mini Kit (QIAGEN, Valencia, CA) following the manufacturer's instructions. Table 1 lists the specimens used in this study. LSU sequences were generated for 13 *Tylopilus ballouii* specimens, and RPB1 sequences were generated for 13 specimens (with incomplete overlap with the LSU dataset) and for one specimen of *Pulveroboletus ravenelii* that was used as an outgroup. Additional outgroup sequences were obtained from GenBank. Voucher specimens for the *T. ballouii* accessions have been deposited in the herbarium of the New York Botanical Garden. PCR amplification for LSU used the primers LROR and LR7 (Vilgalys & Hester 1990). Amplification for RPB1 targeted the region between conserved domains A and C using the general forward primer gRPB1-Afor (Stiller & Hall 1997) and fungal-specific reverse primer fRPB1-Crev (Matheny *et al.* 2002). PCR reactions were performed in 25 ml volumes using TaKaRa Ex Taq polymerase (TaKaRa Bio Inc., Otsu, Shiga). PCR for LSU sequences was performed using conditions described by Vilgalys & Hester (1990). PCR for RPB1 sequences was performed using the following thermocycling conditions, modified slightly from Matheny *et al.* (2002): (1) initial denaturation at 95 °C for 4 min; (2) 35 cycles of 94 °C for 1 min, 50 °C for 1.5 min, and 72 °C for 1.5 min; (3) final extension at 72 °C for 10 min. Amplification products were purified using the QIAquick PCR Purification Kit (QIAGEN) following the manufacturer's

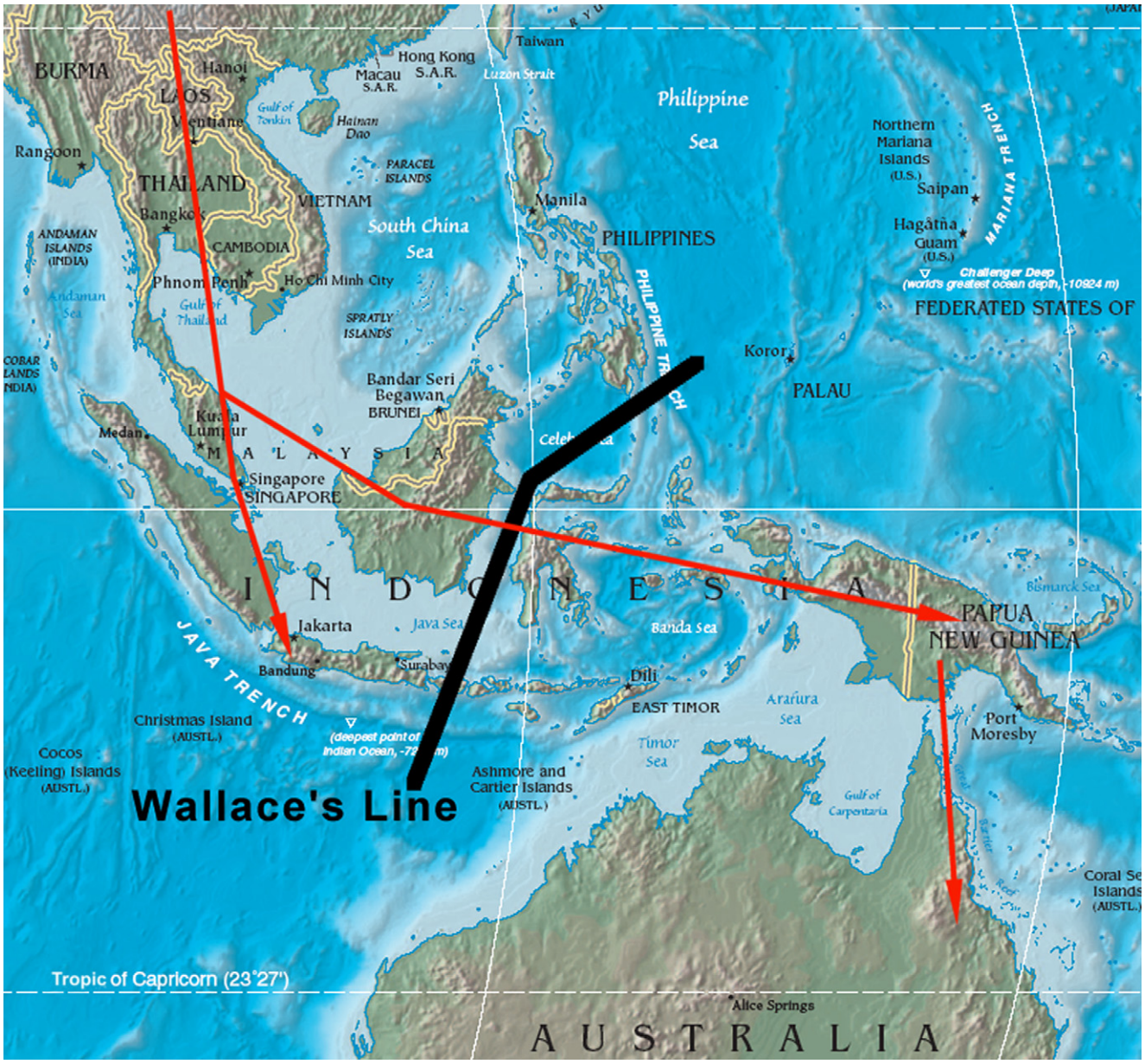

Fig 5 – Migration route(s) across Wallacea.

instructions. DNA sequencing was performed on an ABI 3730XL automated DNA sequencer by Macrogen (Seoul, Korea). Sequencher (Gene Codes, Ann Arbor, MI) was used to edit sequences and assemble contigs. Sequences were aligned using MAFFT version 5.8 (Katoh *et al.* 2005) and adjusted by eye where deemed necessary.

To assess the monophyly of the *T. ballouii* accessions prior to phylogeographic analyses, a preliminary analysis was conducted using LSU sequences from additional bolete species, including the broadest sampling of *Tylopilus* species available from GenBank. Although a monophyletic *T. ballouii* was not obtained in the analysis [MP analysis conducted using PAUP 4.0 b10 (Swofford 2002), heuristic search with 1 K random addition sequences and tree bisection–reconnection (TBR) branch swapping; data not shown], a nonparametric Templeton test failed to reject a hypothesis of non-monophyly for *T. ballouii* when most-parsimonious trees from a constrained analysis (with *T. ballouii* accessions constrained as monophyletic) were compared with those from the unconstrained analysis (constrained analysis: 36 trees of 732 steps; unconstrained analysis: 24 trees of 719 steps; $P = 0.1183–0.3815$).

Assessments of phylogeographic pattern were conducted using analyses of LSU and RPB1 sequences. Because the results of a partition homogeneity test in PAUP indicated highly significant non-homogeneity ($P = 0.01$), the loci were analysed separately. An analysis of LSU sequences was conducted using PAUP with MP as the optimality criterion. All characters were specified as unordered and equally weighted, with gaps treated as missing data. A branch-and-bound search was performed using 1 K random-addition sequences with Multrees option enabled, branches collapsed when maximum branch length equalled zero, and keeping minimal trees only.

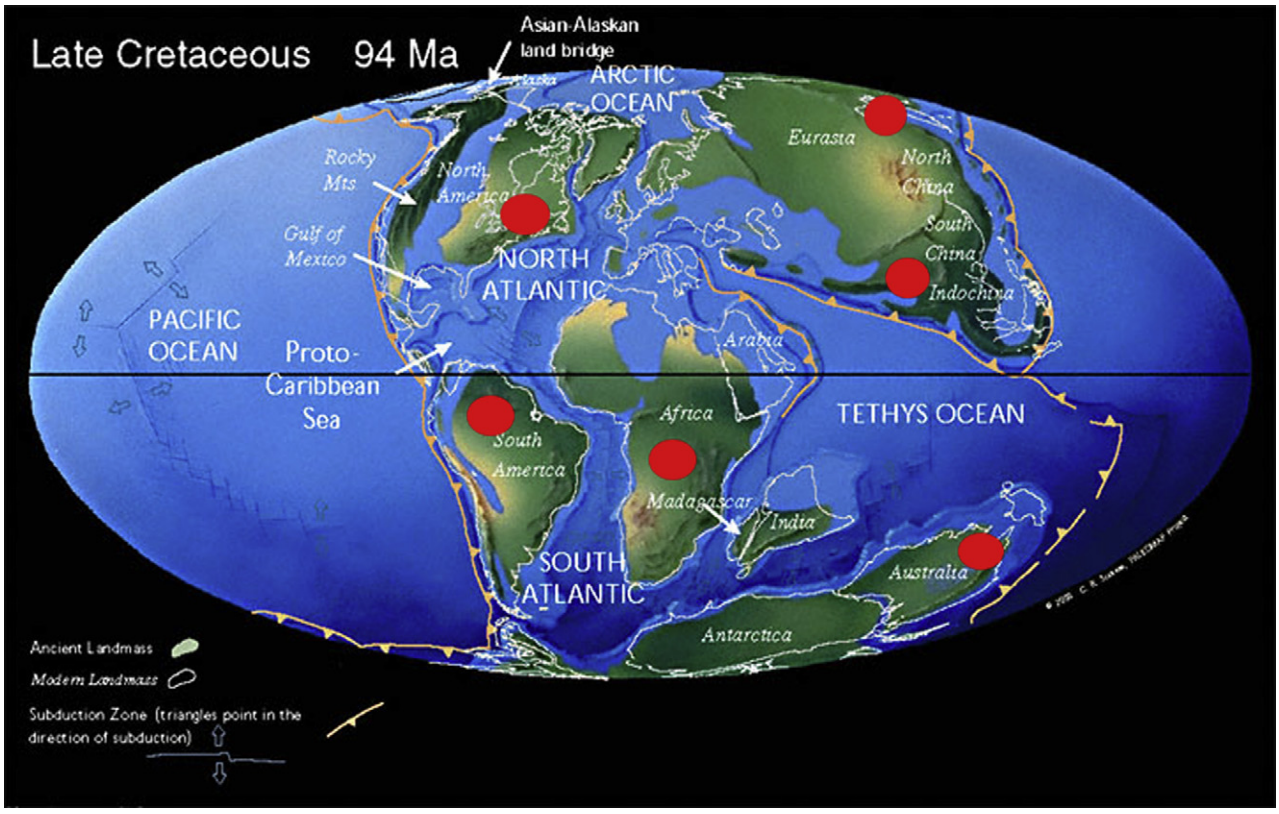

Fig 6 – Hypothetical Pangaean distribution of bolete morpho-taxa (base map by Scotese 1997).

Parsimony-uninformative characters were excluded from the analysis. Branch confidence was assessed using 1 K BS replicates with a branch-and-bound search, ten random addition sequences per BS replicate, and Multrees option enabled. In order to incorporate modelling of nucleotide substitution within RPB1 coding regions by codon position, Bayesian phylogenetic analyses were conducted using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The combined dataset was partitioned into introns and first, second, and third codon positions. The best-fit model of nucleotide substitution for the analysis was selected using a hierarchical likelihood ratio test, implemented in MrModeltest 2.2 (Nylander 2004). The model selected was GTR + G. The analysis was conducted with four chains run over 3×10^6 MCMC generations, temperature parameter set to 0.1, and the first 300 K trees specified as the burn-in phase and discarded.

Results

The LSU data matrix consisted of 15 taxa and 1500 characters, of which 1230 characters were constant, 104 variable but parsimony-uninformative, and 166 parsimony-informative. The analysis resulted in two most-parsimonious trees of 434 steps (Fig 7). The gene tree based on LSU sequences shows two major clades (though one receives only moderate BS support), each containing an Australasian and an American subclade.

The RPB1 data matrix consisted of 18 taxa and 1362 characters. The RPB1 gene tree suggests a much different history than that indicated by the LSU tree. Like the LSU tree, the RPB1 tree supports a possible Australasian origin for *Tylopilus ballouii* (though with low Bayesian PP), but relationships between accessions exhibit few easily discernable geographical patterns. Well-supported clades (Bayesian PP = 1) include groupings of an accession from Thailand within a Costa Rica–Mexico clade, accessions from Belize with those from Australia, and USA with Australian accessions (Fig 8).

Both RPB1 intron and exon sequences were alignable with minimal difficulty within the *T. ballouii* accessions; however, difficulty in unambiguously aligning the ingroup and outgroup sequences required omitting five short regions within introns 1–3 from the analysis. For the ingroup sequences, within exon A, synonymous nucleotide substitutions occurred in six of 23 (26 %) of the codons. A non-synonymous substitution occurs at amino acid position 18, resulting in a lysine in members of clade 1 and the Australian accessions TWO 1121 and 1117, and an arginine in members of clade 2. Within exon B, synonymous nucleotide substitutions occur in 27 of 82 (33 %) of the codons. Six non-synonymous substitutions occur, in codon 27 (aspartic acid in clade 2A, serine in TWO 1117, and asparagine in the remaining accessions), codon 47 (alanine in clade 2B, clade 1A, and TWO 1117; threonine in the remaining accessions), codon 54 (asparagine in clade 1B, isoleucine in clade 1A, and valine in the remaining accessions), codon 56 (lysine in clade 2A, glutamic acid in the remaining accessions), codon 69 (valine in the Australian accessions TWO 1121 and TWO 1117, deletion in clades 1A and 1B, and threonine in clade 2), and codon 93 (methionine in clade 2B, leucine in the

Table 1 – Specimens used in the phylogeographic analyses

Taxon	Country	Locality	Collector and number	LSU	RPB1	Presumed Photobiont
Tylopilus ballouii	Australia	Queensland: Paluma	*T.W. Osmundson* 1087	N/A	EU434337	*Eucalyptus*
T. ballouii	Australia	Queensland: Julatten	*T.W. Osmundson* 1105	EU430738	EU434331	*Acacia* and/ or *Eucalyptus*
T. ballouii	Australia	Queensland: Daintree National Park	*T.W. Osmundson* 1111	N/A	EU434330	*Myrtaceae*, *Acacia*
T. ballouii	Australia	Queensland: Atherton	*T.W. Osmundson* 1117	EU430741	EU434334	*Allocasuarina* and/ or *Eucalyptus*
T. ballouii	Australia	Queensland: Mareeba	*T.W. Osmundson* 1121	EU430743	EU434333	*Allocasuarina* and/ or *Eucalyptus*
T. ballouii	Australia	Queensland: Mareeba	*T.W. Osmundson* 1122	EU430742	N/A	*Allocasuarina* and/ or *Eucalyptus*
T. ballouii	Australia	Queensland: Cooloola	*T.W. Osmundson* 1132	EU430739	EU434332	*Eucalyptus*
T. ballouii	Belize	Cayo District: Mountain Pine Ridge	*R.E. Halling* 8521	EU430735	EU434339	*Quercus* sp.
T. ballouii	Belize	Cayo District: Mountain Pine Ridge	*R.E. Halling* 8526	EU430736	EU434336	*Pinus* sp.
T. ballouii	Costa Rica	Cartago: Guarco, Estrella	*R.E. Halling* 8087	EU430731	EU434329	*Quercus* sp.
T. ballouii	Costa Rica	Cartago: Guarco, Palo Verde	*R.E. Halling* 8187	EU430732	EU434342	*Quercus* sp.
T. ballouii	USA	New York: Bronx, NY Bot. Garden	*R.E. Halling* 8292	EU430734	N/A	*Quercus* sp.
T. ballouii	Mexico	Tamaulipas	*FMNH* 1073250	EU430733	EU434341	Unknown
T. ballouii	Thailand	Chiang Mai	*T.W. Osmundson* 1198	EU430740	EU434340	Dipterocarpaceae
T. ballouii	USA	New York: Bronx, NY Bot. Garden	*T.W. Osmundson* 1030	EU430737	EU434338	*Fagus* sp.
Additional and outgroup taxa						
Pulveroboletus ravenelii	Costa Rica	San José: Dota	*R.E. Halling* 8636	N/A	EU434335	
Boletinellus merulioides	USA	Massachusetts: Rockhouse	*M. Binder* 02-199	N/A	DQ435803	
Suillus pictus	USA	Massachusetts: Rutland St Park	*M. Binder* 03-002	N/A	AY858965	
Boletus edulis	GenBank direct submission		*F. Oberwinkler* 46874	N/A	DQ067991	
Strobilomyces floccopus	USA	Massachusetts: Rutland St Park	*M. Binder* 03-102	N/A	AY858963	
Boletellus projectellus			*M. Binder* 03-118	N/A	AY684158	
T. felleus			*K.H. Larsson* 8542	N/A	AY586723	

GenBank accession numbers for LSU and RNA polymerase II largest subunit (RPB1) sequences are provided, as well as the presumed ectomycorrhizal photobiont for the *Tylopilus ballouii* accessions based on field associations.

remaining accessions). Within exon C, synonymous nucleotide substitutions occur in 39 of 108 (36 %) of the codons. Three non-synonymous substitutions occur, in codon 130 (serine in all accessions except a proline in TWO 1121), codon 156 (isoleucine in clade 1, valine in the remaining accessions), and codon 180 (tyrosine in all accessions except histidine in TWO 1121).

An attempt to construct a dataset of nu-rDNA ITS sequences was abandoned due to the discovery of multiple, possibly paralogous (based on preliminary phylogenetic analyses) sequences within accessions. Additional research is currently underway to more precisely determine the nature of ITS haplotype distributions within and between populations.

Discussion

The occurrence of widespread fungal taxa presents something of a biogeographical enigma, raising questions of biogeographic history and the potential for long-distance dispersal by spores or other means. Widespread distributions for ectomycorrhizal taxa—obligate symbionts that require a suitable plant symbiont for survival—are particularly intriguing. This paper describes examples of widespread morphotaxa among the boletes, a speciose group of ectomycorrhizal fungi, and presents several biogeographic hypotheses that could explain these distributions; it then presents an example of bringing molecular data to bear on this question for a widespread morphospecies, *Tylopilus ballouii*. Such data can foster a better understanding of evolutionary mechanisms that generate biodiversity at historic timescales and, when combined with population-level genetic markers, can enable identification of unique genetic lineages and inference of dispersal capacity within morphospecies, two types of information valuable to biodiversity conservation assessment and planning.

The analysis of separate gene trees, as presented in the *T. ballouii* example, can provide some insight into forces — some of them opposing — that may have shaped the genetic

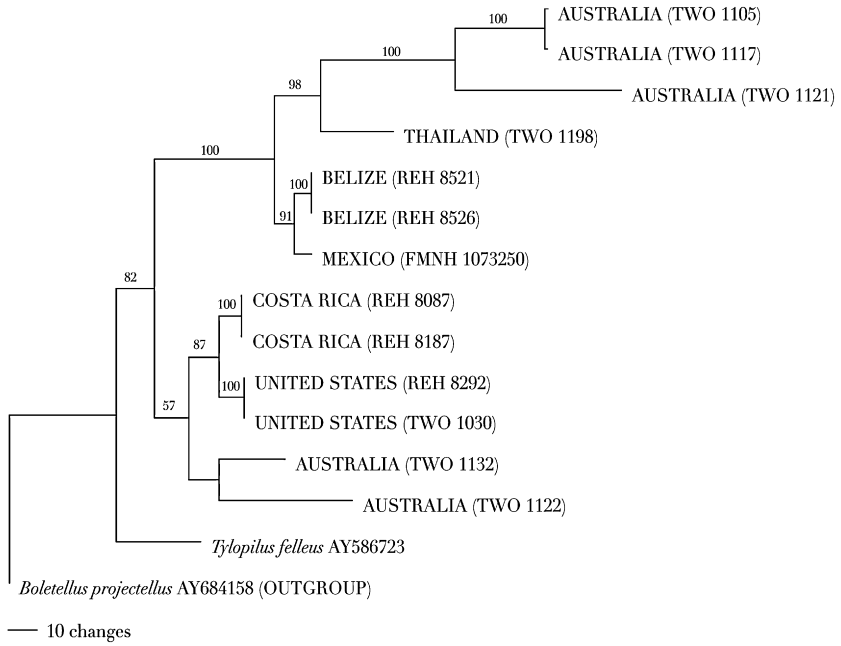

Fig 7 – Phylogram of one of the two most-parsimonious trees generated from an analysis of nu-rDNA LSU sequences of *Tylopilus ballouii* specimens. Nonparametric BS values > 50 appear above branches. *Boletellus projectellus* (GenBank accession AY684158) was used as an outgroup. *T. felleus* (GenBank accession AY586723) was included in the analysis but not specified as an outgroup. (Tree length = 434, CI = 0.8041, RI = 0.8262, RC = 0.6644.)

make-up within taxa. In *T. ballouii*, LSU data strongly suggest the geographic structuring of populations and the possibility of cryptic speciation. Conversely, RPB1, a protein-coding gene, indicates that selection or other processes have resulted in geographic patterns being obscured. Ectomycorrhizal photobiont associations (*Myrtaceae* and *Casuarinaceae* for the Australian collections, *Dipterocarpaceae* for the Thai collection, and *Fagaceae* for the collections from the Americas) are more consistent with the results of the LSU tree. Examination of additional coding and non-coding loci should provide interesting insights into the historical and contemporary forces that shape the geographical distributions of these obligately symbiotic organisms.

The recognition of several well-supported clades and the degree of sequence divergence in protein coding, as well as non-coding regions, observed between accessions suggest that *T. ballouii* as currently circumscribed represents a species complex rather than a single species, echoing the observations of Watling (2001) and Watling & Gregory (1988). Micromorphological examinations of collections and a review of macromorphological characters from field notes are currently underway in an effort to better understand the circumscription, evolution, and distribution of taxonomic entities in this group.

While the results of the RPB1 analysis cannot discount the possibility that infrequent long-distance dispersal events have occurred, the significant degree of sequence divergence observed between accessions and the occurrence of several non-synonymous nucleotide substitutions do indicate that *T. ballouii* is not panmictic across its geographic range. These results, when considered in combination with other lines of evidence (e.g. limited numbers of basidiospores released beyond the vicinity of the parent basidiome, evidence for isolation-by-distance at the landscape scale), and considering the specific requirement for symbiosis with a compatible plant symbiont, suggest that hypotheses (2) and (3) (see Introduction) may provide a more reasonable explanation than hypothesis (1) for the occurrence of amphi-Pacific disjunct distributions in ectomycorrhizal basidiomycetes and that selection may obscure geographic patterns for some loci. The possibility of a relict Pangaean distribution cannot be refuted by estimated divergence dates from existing molecular clock analyses. Although these analyses lack the taxonomic sampling density necessary to date divergences within the *Boletaceae* and within bolete genera, a Pangaean origin for these taxa is well within estimated dates of the hymenomycete–ustilaginomycete divergence (966 ± 86 M years ago; Heckman *et al.* 2001) and *Basidiomycota* crown divergence (299–1361 M years ago, depending upon choice of calibration point; Taylor & Berbee 2007). Further support for this hypothesis is provided by an estimated divergence date of 86 M years ago for African and Australian lineages within the Inocyboid genus *Auritella* (Matheny & Bougher 2006).

The relatively scant sampling presented in the *T. ballouii* example requires that these biogeographic conclusions be considered preliminary at this point. Currently, additional loci are being sampled for an expanded dataset that includes improved geographical representation, as well as additional samples from within geographical areas. The addition of neutral genetic markers may help to illuminate the patterns observed for coding regions.

To date, most biogeographic studies of the hymenomycetes have focused on saprobic species (e.g. Vilgalys & Sun 1994; Hughes *et al.* 1998; Hughes *et al.* 1999; James *et al.* 1999; Methven *et al.* 2000; Jin *et al.* 2001; Lickey *et al.* 2002); however,

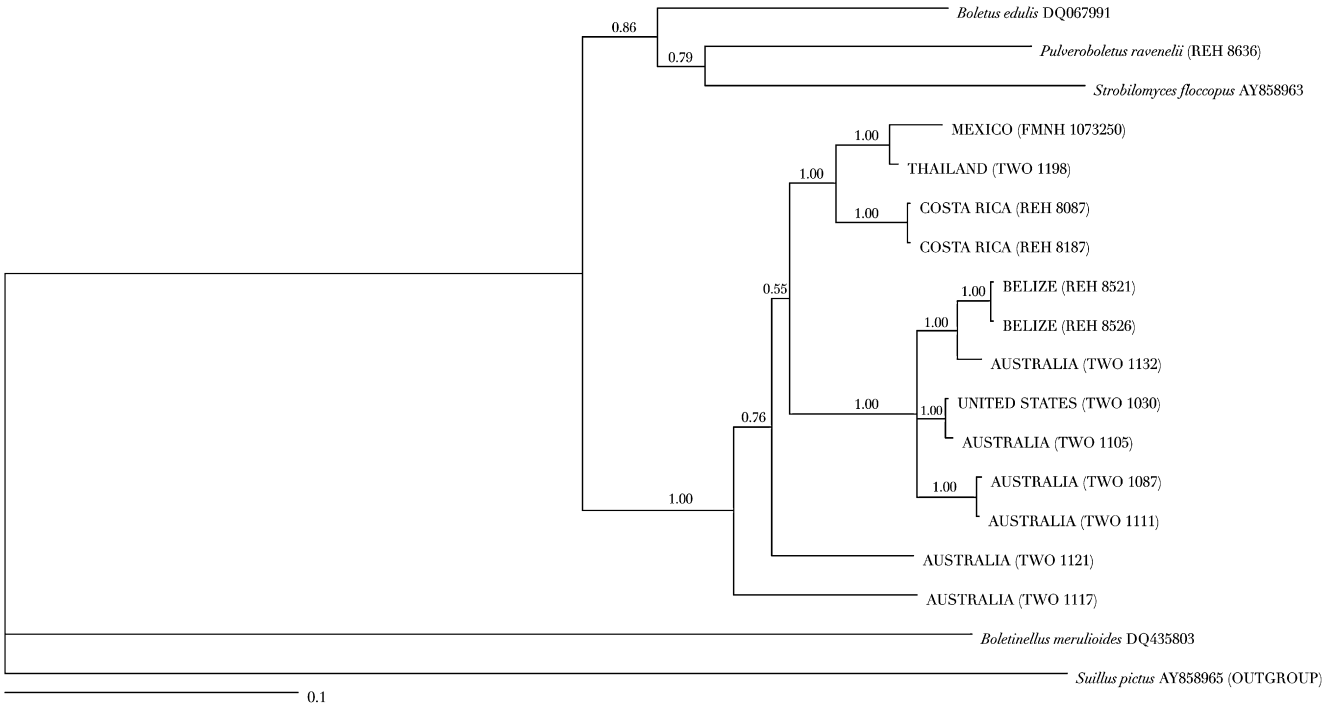

Fig 8 – Phylogram of the best-fit tree generated from a Bayesian analysis of *Tylopilus ballouii* partial RPB1 (from conserved domains A to C) sequences. Bayesian PP values > 0.5 appear above branches. *Suillus pictus* (GenBank accession AY858965) was used as an outgroup. *Boletinellus merulioides* (GenBank DQ435803), *Strobilomyces floccopus* (GenBank AY858963), *Boletus edulis* (GenBank DQ067991), and *Pulveroboletus ravenelii* (*R.E. Halling* 8636, generated during this study) were included as additional taxa in the analysis but not specified as outgroups.

ectomycorrhizal taxa are likely to exhibit different biogeographic patterns than saprobic taxa due to the obligately symbiotic life style of the former. As a widely-distributed and easily recognized morphospecies, *T. ballouii* is a promising model species for examining the biogeography and population genetics of ectomycorrhizal basidiomycetes over multiple spatial and temporal scales. We are currently studying additional *T. ballouii* accessions, as well as other ectomycorrhizal bolete taxa, in an effort to draw more generalized conclusions.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.mycres.2007.11.021.

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