

## Assessing biogeographic relationships between North American and Chinese macrofungi

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### Abstract

**Aim** A close biogeographic relationship between the macrofungi of eastern North America and eastern Asia has been documented based on comparisons of species lists. In addition to having a similar species composition, the two regions are reported to share a number of species with putative disjunct distributions. This close biogeographic relationship, however, has rarely been tested within a phylogenetic context. In this paper we examine relationships within three genera, *Armillaria*, *Xerula* and *Suillus* (Fungi, Basidiomycetes, Agaricales) chosen as exemplars of different ecological guilds occupied by macrofungi (plant pathogens, saprobes, mutualists).

**Location** Fieldwork for this project centered in eastern North America, Central America, China and Australasia. Material from additional localities were obtained from cooperating herbaria and additional sequences were downloaded from GenBank.

**Methods** ITS sequence data were used to construct phylogenies for each genus.

**Results** Only one of four tested putative disjunct species, *Xerula hispida* Halling and Mueller, was supported. Material referable to *X. furfuracea* (Peck) Redhead, Ginns and Shoemaker from China and North America do not form a monophyletic group. Disjunct populations of *Suillus spraguei* (Berkeley & Curtis) Kuntze were shown to be paraphyletic. The morphological similarity of the Chinese material to the North American material is likely due to morphological stasis. Finally, morphologically identical material referable to *Suillus americanus* (Peck) Snell in Slipp and Snell from eastern North America and *S. sibiricus* (Singer) Singer from China, along with the morphologically similar western North American *S. sibiricus*, probably represent a single circumboreal taxon.

**Main conclusions** The resulting data, while not refuting the hypothesis that there exists a relatively close biogeographic relationship for macrofungi between eastern North America and eastern Asia, suggest that the relationship may not be as close as indicated by morphological data. These results are similar to emerging data from analyses of flowering plants displaying putative eastern North American/eastern Asian disjunct distribution patterns.

### Keywords

Biogeography, disjunct distributions, Agaricales, *Armillaria*, *Suillus*, *Xerula*.

## INTRODUCTION

Similarities between the biotas of eastern North America and eastern Asia have been noted and discussed for nearly 300 years, and Jonas Halenius, a student of Linnaeus, published on the floristic similarity of the two regions in his dissertation 250 years ago (Halenius, 1750). Asa Gray made detailed comparisons among the north temperate floras and concluded that the floras of eastern North America and eastern Asia were more similar to each other than either were to western North America or Europe (Gray, 1846). Since then, Li (1952), Graham (1972), Raven (1972), Bouffard & Spongberg (1983), Davidse (1983), Tiffney (1985), Wen (1999), and colleagues have provided summaries and updates on our changing understanding of this intriguing biogeographic pattern for flowering plants. While this distribution pattern is best documented for flowering plants, similar biogeographic relationships have been noted for mosses (Iwatsuki, 1958), ferns (Kato & Iwatsuki, 1983; Iwatsuki, 1994), several animal groups (e.g. Suzuki *et al.*, 1977; Patterson, 1981; Enghoff, 1993; Nordlander *et al.*, 1996), lichenized fungi (Culberson, 1972; Dey, 1976), and macrofungi (Hongo & Yokoyama, 1978; Wu & Mueller, 1997; Wu *et al.*, 2000). Further data on distribution patterns among groups other than flowering plants are necessary to see if the emerging pattern is congruent across disparate taxa with very different life histories, as well as to help further elucidate the geological and biological events that gave rise to the observed patterns.

While there have been a few published studies that propose a close eastern North American/eastern Asian geographical relationship for fungi (e.g. Hongo & Yokoyama, 1978), Wu & Mueller (1997) published the first quantitative comparative study to assess the similarity of the macrofungal mycotas (taxonomic composition of mushrooms, bracket fungi, puffballs and other fungi with macroscopic fruiting bodies) between eastern Asia and eastern North America. They undertook a literature and herbarium survey to compare the mycotas of north temperate macrofungi using two methods: (1) direct comparison of taxon lists and (2) calculation of Simpson coefficient of similarity from lists of selected taxa. The study focused principally on the genera *Amanita*, *Lactarius*, *Ramaria* and genera in the family Boletaceae (among the few genera sufficiently well studied to enable a reasonable comparison). The major conclusions of this study were:

- (1) A relatively high similarity exists between the macromycota of eastern North America and temperate eastern Asia based on comparisons of species lists. About 40–52% of the species in these groups reported from eastern Asia also occur in eastern North America. For *Amanita* and *Ramaria* the species overlap is higher between these two regions than between the other regions compared. For *Lactarius* and species of Boletaceae, the species overlap between eastern North America and eastern Asia is only slightly less than reported between eastern and western North America or

between Europe and eastern Asia, respectively. In all cases, the documented species overlap for these taxa between western North America and Europe, and between western North America and Asia is considerably lower.

- (2) Simpson coefficient of similarity values between the two regions ranged from 40 to 59. These values are as high or higher than those observed in comparisons between mycotas of eastern and western North America or between Europe and temperate eastern Asia, and are considerably higher than those calculated between western North America and Europe or between western North America and Asia.
- (3) The number of species displaying putative eastern North American-temperate east Asian disjunctions is relatively high in each of the taxa. These ranged from 5 to 12% of the total number of eastern North American species. These numbers are higher than the numbers reported for North American–European disjuncts or for western North American–eastern Asian disjuncts.
- (4) These distribution patterns were only discernable at the rank of species or lower. Unlike plants that show geographical patterns at the generic level, most genera of macrofungi are widely distributed.

Based on the literature and herbarium survey, there appears to be a relatively strong eastern North American/eastern Asian biogeographic pattern at the rank of species for macrofungi (Wu & Mueller, 1997). However, while species lists are important for documenting distribution patterns, they do not provide the historical information needed to test how these patterns came about. Phylogenies provide a rigorous test of relationships and the historical context to investigate biogeographic hypotheses. Additionally, phylogenetic hypotheses, coupled with information on molecular and morphological divergence, are necessary to assess species concepts (i.e. determine if the disjunct populations represent the same taxon), resolve sister species relationships, and investigate evolutionary phenomena such as morphological stasis that have been shown to be common among putative plant disjunctions (e.g. Wen, 1999). The focus of this study therefore is to develop phylogenies of select genera of macrofungi to test the patterns identified in the literature survey of Wu & Mueller (1997).

## MATERIALS AND METHODS

### Taxon sampling

Three genera were chosen for analysis, *Armillaria*, *Xerula* and *Suillus*. Species of *Armillaria* grow on wood and range from saprobes to strong and economically important pathogens of living trees. The genus is widely distributed and contains several morphologically similar, closely related, species groups (Watling *et al.*, 1991; Burdsall & Volk, 1993). A combination of mating studies and molecular analyses (allozymes, RAPDs, sequence data) have been used to identify taxa in this group (e.g. Watling *et al.*, 1991;

Banik & Burdsall, 1998). Species of *Xerula* grow in the soil and are saprobes. Species of the genus have been reported from throughout the world, but individual species appear to have distinct and relatively narrow distribution ranges. The genus has two putative eastern North American/eastern Asian disjuncts: *X. furfuracea* (Peck) Redhead *et al.* and *X. hispida* Halling and Mueller (G.M. Mueller & Q.-X. Wu unpublished). *Suillus* is a genus of fleshy pored fungi (boletes). All species of *Suillus* are obligate mutualists (form ectomycorrhizae) with forest trees (almost exclusively members of the Pinaceae—pines, spruce, firs, etc.) and are restricted to the northern hemisphere in natural communities (some species are commonly found in exotic pine plantations in the southern hemisphere). Two putative eastern North American/eastern Asian disjuncts have been recognized based on morphological characters: *S. spraguei* (Berkeley & Curtis) Kuntze [= *S. pictus* (Peck) Smith & Thiers] (Hongo & Yokoyama, 1978) and *S. americanus* (Peck) Snell in Slipp and Snell (Wu *et al.*, 2000). Their host trees do not display a disjunct distribution pattern. Thus, the three genera, *Armillaria*, *Xerula* and *Suillus*, represent different ecological guilds (pathogens, saprobes and mutualists) and have both putative disjunct and more broadly distributed taxa.

Scientific name, locality, depositary and GenBank accession number of *Xerula* and outgroup *Oudemansiella* specimens used in this study are listed in Table 1. Information on

the employed specimens of *Suillus* is previously published in Wu *et al.* (2000).

### DNA sequencing

Sequences of the ITS region (ITS1–5.8S–ITS2) were obtained directly from specimens or from tissue of fresh basidiomata preserved in a fixative solution (20% DMSO, 250 mM EDTA and saturated NaCl). The DNA extraction, amplification and sequencing methods were previously described (Wu *et al.*, 2000). Briefly, Puregene kit 'DNA isolation from filamentous fungi' (Gentra Systems, Inc., Minneapolis, MN, USA) and CTAB methods (Gardes & Bruns, 1993) were used to isolate DNA. The entire, or a fragment, ITS region of the nuclear ribosomal DNA repeat was amplified using the polymerase chain reaction (PCR) with primers ITS1–ITS4 (White *et al.*, 1990) or primer pairs ITS5–ITS2 and ITS3–ITS4S (Kretzer *et al.*, 1996). The PCR thermal cycling conditions were set as the following: initial denaturation at 94 °C for 1.5 min, 35 cycles of 15 s at 94 °C, 15 s at 50 °C, 30 s at 72 °C, and an extension at 72 °C for 3 min followed by soaking at 4 °C. Cycle-sequencing reactions were performed with the same primers used for the symmetric amplifications with either FS or dRhodamine. Reaction products were then electrophoresed on an ABI 377 automated sequencer (Perkin–Elmer Applied Biosystems, Inc., Boston, MA, USA). The DNA sequences were

**Table 1** List of *Xerula* specimens studied, their geographical origin, and GenBank accession number

Scientific name	Collection No.	Location	Herbarium No.	GenBank No.
<i>Oudemansiella australis</i>	RV95/297	Australia, Queensland	DUKE (2766)	AF321472
<i>O. australis</i>	RV95/416	Australia, Queensland	DUKE (2875)	AF321473
<i>O. australis</i>	RV95/274	Australia, Queensland	DUKE (2742)	AF321474
<i>O. australis</i>	RV95/852	Papua New Guinea	DUKE (3114)	AF321475
<i>O. canarii</i>	JM98/221	China, Yunnan	DUKE (5032)	AF321476
<i>O. canarii</i>	RV96/35	Costa Rica	DUKE	AF321477
<i>O. canarii</i>	RVPR33	Puerto Rico, Luquillo	DUKE (3986)	AF321478
<i>O. canarii</i>	RVPR100	Puerto Rico, Rio Grande	DUKE (4057)	AF321479
<i>Xerula australis</i>	RV95/413	Australia, Queensland	DUKE (2872)	AF321480
<i>X. furfuracea</i> s.l.	QXW2430	China, Yunnan	F (1128660)	AF321481
<i>X. furfuracea</i> s.l.	QXW2446	China, Yunnan	F (1128676)	AF321482
<i>X. furfuracea</i> s.l.	QXW2570	China, Yunnan	F (1128799)	AF321483
<i>X. furfuracea</i> s.l.	JM98/155	China, Yunnan	DUKE (4970)	AF321484
<i>X. hispida</i>	GMM6177	China, Yunnan	F (1129046)	AF321485
<i>X. hispida</i>	GMM4696	Costa Rica, San Jose	F (1136527)	AF321486
<i>X. pudens</i> s.l.	W1983/2424	Austria, Lower Austria	W (1983/2424)	AF321487
<i>X. pudens</i> s.l.	ZT6243	Italy	ZT (6243)	AF321488
<i>X. pudens</i> s.l.	RV95/871	Papua New Guinea	DUKE (3134)	AF321489
<i>X. pudens</i> s.l.	RV95/904	Papua New Guinea	DUKE (3167)	AF321490
<i>X. pudens</i> s.l.	178026	Sweden	UPS (F012635)	AF321491
<i>X. pudens</i> s.l.	179640	Sweden	UPS (F012999)	AF321492
<i>X. pudens</i> s.l.	179651	Sweden	UPS (F013010)	AF321493
<i>X. radicata</i> s.l.	JMCR120	Costa Rica	DUKE	AF321494
<i>X. furfuracea</i>	EK88/27	USA, North Carolina	DUKE (0105)	AF321495
<i>X. furfuracea</i>	DSH4	USA, North Carolina	DUKE (3650)	AF321496
<i>X. furfuracea</i>	RAG 329	USA, Illinois	F (1137555)	AY026918
<i>X. furfuracea</i>	RAG 490	USA, Illinois	F (1137556)	AY026919

assembled and manually aligned with Sequencher 3.0 (Gene Codes Co., Ann Arbor, MI, USA).

### Phylogenetic analyses

Maximum parsimony and subsequent bootstrapping analyses were conducted with PAUP\* 4.0b1a (Swofford, 1998). Sequence regions in which nucleotides could be aligned in different ways as a result of the presence of indels were defined as ambiguously aligned regions and subsequently excluded from all analyses. Constant sites were also excluded. Gaps were treated as the fifth character state. Changes among character states were unequally weighted using a symmetric step matrix incorporating evolution costs. The costs were estimated by counting the number of each type of change, computing their relative frequencies and converting them to costs by negative natural logarithm (Felsenstein, 1981; Wheeler, 1990).

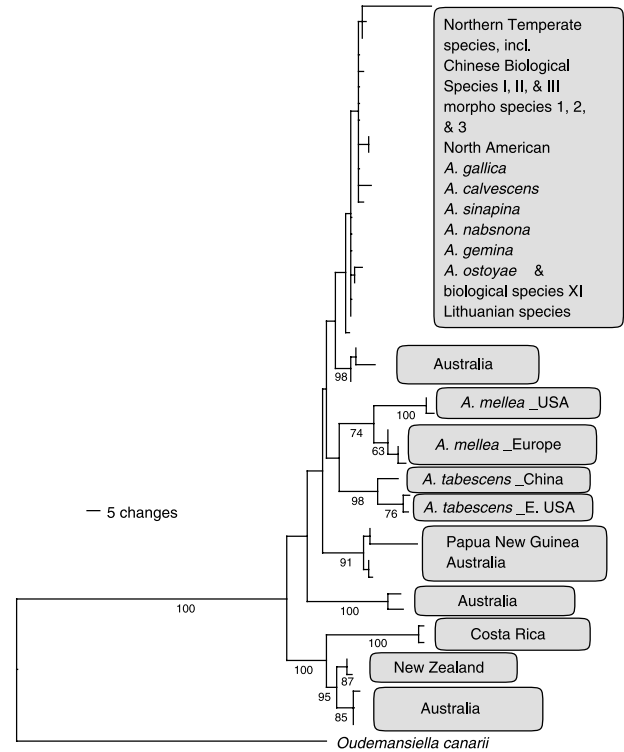
Most parsimonious trees were obtained by running the heuristic search with tree-bisection-reconnection (TBR) branch swapping and up to 1000 random-addition sequence replications. Clade stability of most parsimonious trees was estimated by 1000 bootstrap replicates (Felsenstein, 1985) using the same parameters set up in the initial heuristic search.

Maximum likelihood analysis was performed by using the Hasegawa–Kishino–Yano model (Hasegawa *et al.*, 1985). Ambiguously aligned regions were excluded from the maximum likelihood analysis. Base frequencies, transition/transversion ratio, and the number of rate categories to account for site to site variation were estimated using the most parsimonious tree. Parameters were optimized using a likelihood ratio test (Huelsenbeck & Crandall, 1997). The heuristic search of up to 1000 replicates was performed with random-addition sequences, TBR, and branches collapsed if maximum branch length was zero. Clade stability was estimated by up to 1000 bootstrap replicates after setting base frequencies, transition/transversion ratio, and number of rate categories identical to those in the initial maximum-likelihood analysis.

## RESULTS

### Armillaria

Sequences of the ITS region of the nuclear ribosomal RNA tandem repeats were obtained for a total of 46 collections from both the northern and southern hemispheres. Many gaps were created during alignment for both ITS1 and ITS2 regions because of insertions and deletions that occurred separately in the northern hemisphere and southern hemisphere species. *Oudemansiella canarii* (Jungh.) Höhn. served as an outgroup. The final alignment consisted of 981 sites. 13 ambiguously aligned regions and constant characters were excluded from the analysis, covering 805 sites. Of the remaining 176 included sites, 83 characters were parsimony-informative. The step matrix implemented to weight character state changes consisted of  $A \leftrightarrow C = 2.39$ ;  $A \leftrightarrow G = 1.96$ ;  $A \leftrightarrow T = 2.18$ ;  $A \leftrightarrow \text{gap} = 2.81$ ;  $C \leftrightarrow G = 2.18$ ;



**Figure 1** The single most parsimonious tree generated for *Armillaria* based on ITS sequence data. Geographic origin, rather than taxon name, is provided to clearly depict the observed biogeographical pattern. Branch lengths were short, and neither taxonomic nor geographical structure was evident within the large north temperate clade. Numbers below each internode are the percentage of 1000 bootstrap replicas supporting that binary partition (value  $\leq 50$  not shown). Shaded areas encompass northern hemisphere species of *Armillaria*.

$C \leftrightarrow T = 1.55$ ;  $C \leftrightarrow \text{gap} = 3.16$ ;  $G \leftrightarrow T = 2.15$ ;  $G \leftrightarrow \text{gap} = 2.81$ ;  $T \leftrightarrow \text{gap} = 3.01$ .

The maximum parsimony analysis generated a single most parsimonious trees (tree length = 572.90 steps, consistency index [CI] = 0.828, retention index [RI] = 0.881, rescaled consistency index [RC] = 0.729) (Fig. 1). While the tree was resolved, little support was detected for the backbone of the tree by bootstrap analysis. Our analyses indicate that the genus rapidly radiated into the northern hemisphere from the south. The region of the tree encompassing most of the northern hemisphere specimens was characterized by uniform short branch lengths with limited biogeographic structure. Material identified as *A. mellea* (Vahl. : Fries) Kummer and *A. tabescens* (Scop. : Fries) Enel. formed well-supported clades separate from the large northern hemisphere clade.

### Xerula

The ITS region was sequenced for a total of 27 collections, including 19 in the genus *Xerula* and 8 collections in

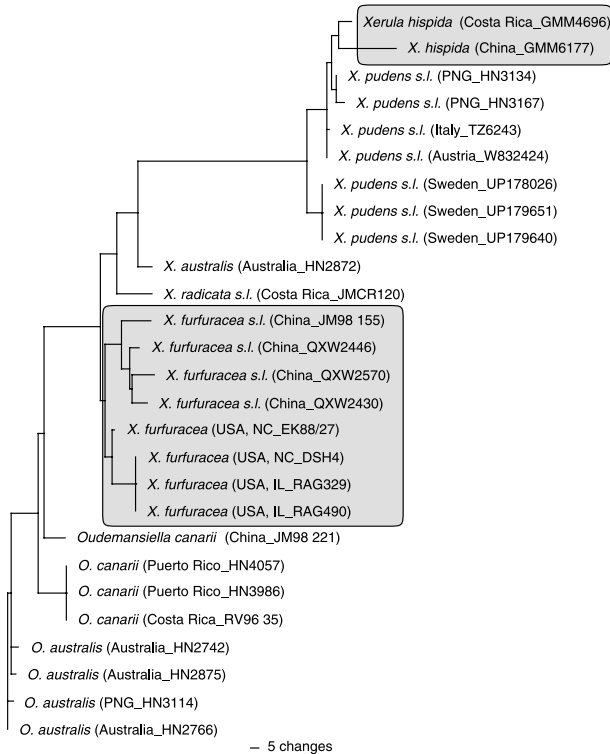
*Oudemansiella* as outgroups. The final alignment consisted of 775 sites. 12 ambiguously aligned regions were excluded from the analysis, covering 288 sites. Of the remaining 487 nonambiguously aligned sites, 119 were variable, 97 characters were parsimony-informative. The step matrix implemented to weight character state changes consisted of  $A \leftrightarrow C = 2.66$ ;  $A \leftrightarrow G = 1.87$ ;  $A \leftrightarrow T = 2.17$ ;  $A \leftrightarrow \text{gap} = 2.91$ ;  $C \leftrightarrow G = 2.74$ ;  $C \leftrightarrow T = 1.33$ ;  $C \leftrightarrow \text{gap} = 2.60$ ;  $G \leftrightarrow T = 2.41$ ;  $G \leftrightarrow \text{gap} = 3.00$ ;  $T \leftrightarrow \text{gap} = 2.74$ .

The maximum parsimony analyses generated 10 most parsimonious trees (tree length = 406.02 steps, CI = 0.762, RI = 0.933, RC = 0.710) (Figs 2 & 3). The genus has not undergone a modern revision, and several of the specimens included in this study vary from published taxa in some micromorphological characters. These specimens are listed as *X. furfuracea* s.l., *X. radicata* s.l. (Relhan; Fries) Dörfelt or *X. pudens* s.l. (Persoon) Singer.

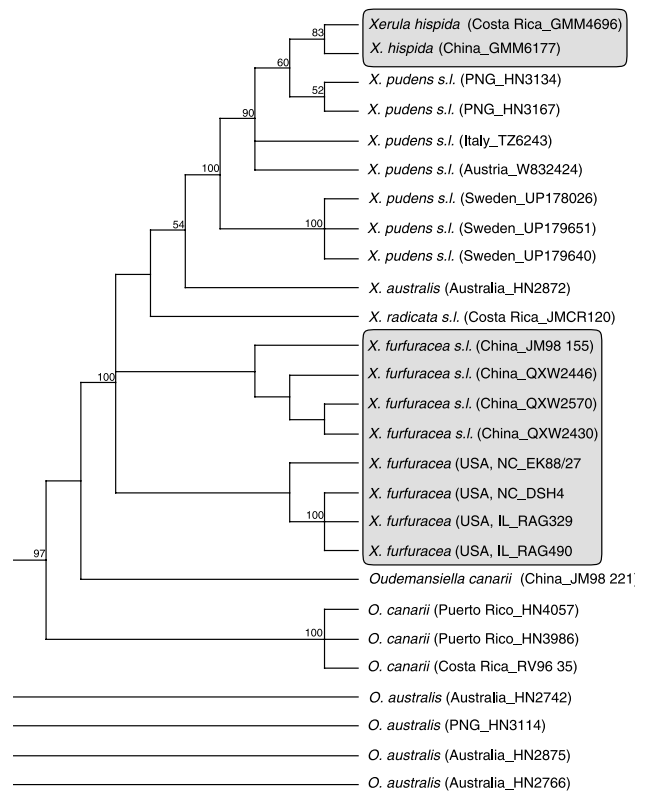
As depicted on the strict consensus tree (Fig. 3), the 10 most parsimonious trees varied primarily in the relationships among material identified as *O. australis* Stevenson & G. Taylor (taxon restricted to Australasia) and the relationship of the Chinese to the North American *X. furfuracea* clades. The two *X. furfuracea* clades were sister taxa in five of the 10 trees. Species characterized by having long, dense hairs covering the pileus and stipe, i.e. *X. hispida* and *X. pudens* s.l., formed a well supported clade

(100%). Within this clade, the two disjunct specimens of *X. hispida* formed a distinct, well supported clade (82%) as did *X. pudens* from Sweden (100%). *Xerula pudens* s.l. from Papua New Guinea is the sister group to *X. hispida*. *Xerula australis* (Dörfelt) Petersen is basal to the *X. pudens*–*X. hispida* clade. *Oudemansiella canarii* has a pantropical distribution pattern but material from China showed molecular divergence from the well-supported Costa Rican and Puerto Rican clade (100%).

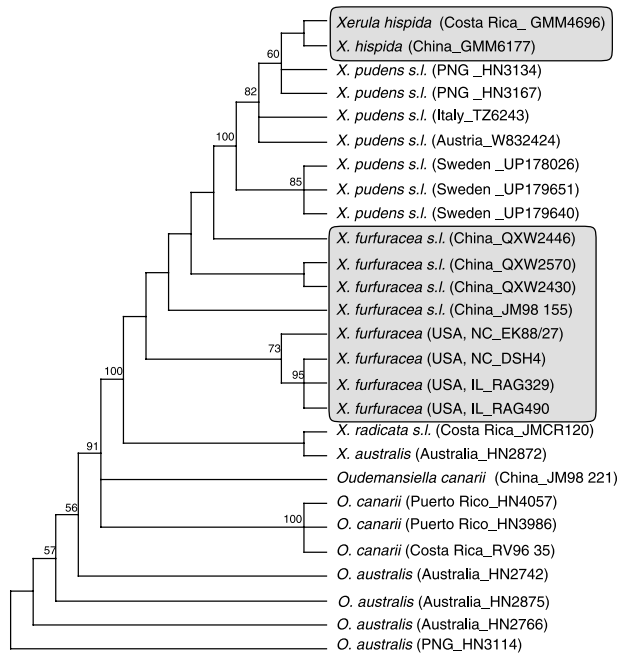
Maximum likelihood analysis was performed on the *Xerula* data set. Estimated base frequencies were A: 0.21662, C: 0.24323, G: 0.29912, T: 0.34003. Estimated transition/transversion ratio was 2.77862. All sites were assumed to evolve at the same rate because rate heterogeneity among sites was not detected. Heuristic search using these parameters generated three best trees [–Ln = 880.99564] (Fig. 4). The most likely trees are similar to the most parsimonious trees in topology, differing only in that material referable to *X. furfuracea* s.l. from China formed a grade rather than a distinct clade and that this grade is basal to the *X. hispida*–*X. pudens* s.l. clade, rather than the grade of *X. australis* plus *X. radicata* s.l. from Costa Rica as seen in the most parsimonious trees. Unlike the maximum parsimony analysis, there was no



**Figure 2** One of the 10 most parsimonious MPUEW trees generated for *Xerula* based on ITS sequence data depicted as a phylogram to illustrate branch lengths. Shaded area indicates a putative disjunct species.



**Figure 3** Strict consensus MPUEW tree generated for *Xerula* based on ITS sequence data. Numbers below each internode are the percentage of 1000 bootstrap replicas supporting that binary partition (value  $\leq 50$  not shown). Shaded area indicates a putative disjunct species.



**Figure 4** Strict consensus ML tree generated for *Xerula* based on ITS sequence data. Numbers below each internode are the percentage of 100 bootstrap replicas supporting that binary partition (value  $\leq 50$  not shown). Shaded area indicates a putative disjunct species.

bootstrap support for the observed monophyly of the two representatives of *X. hispida* in the maximum likelihood analysis.

This study was most interested in the two putative disjunct taxa, *X. furfuracea* and *X. hispida*. Subsequent macro and micromorphological study of the included North American and Chinese material initially identified as *X. furfuracea* uncovered greater morphological variation than has been reported in the literature (unpublished). However, the material formed distinct clades in the maximum parsimony analysis (Fig. 3), although the Chinese clade was not supported. As mentioned above, Chinese and North American material of *X. furfuracea* were sister taxa in five of the 10 most parsimonious trees. A clade of Chinese *X. furfuracea* was not discovered through maximum likelihood analysis (Fig. 4). The two specimens of *X. hispida*, one from China and the other from Costa Rica, are monophyletic and share a most recent common ancestor with *X. pudens* s.l. from Papua New Guinea (Figs 2–4). These relationships are well supported in the parsimony analysis (Fig. 3) but not in the maximum likelihood trees (Fig. 4). Morphological characters are concordant with the hypothesis that *X. hispida* is a distinct taxon (Halling & Mueller, 1999) with a disjunct distribution between eastern Asia and southern-Central America/northern South America (in addition to oak dominated forests of southern Costa Rica, the species is reported from oak [*Quercus humboldtii* Bonpland] dominated forests in Colombia).

## Suillus

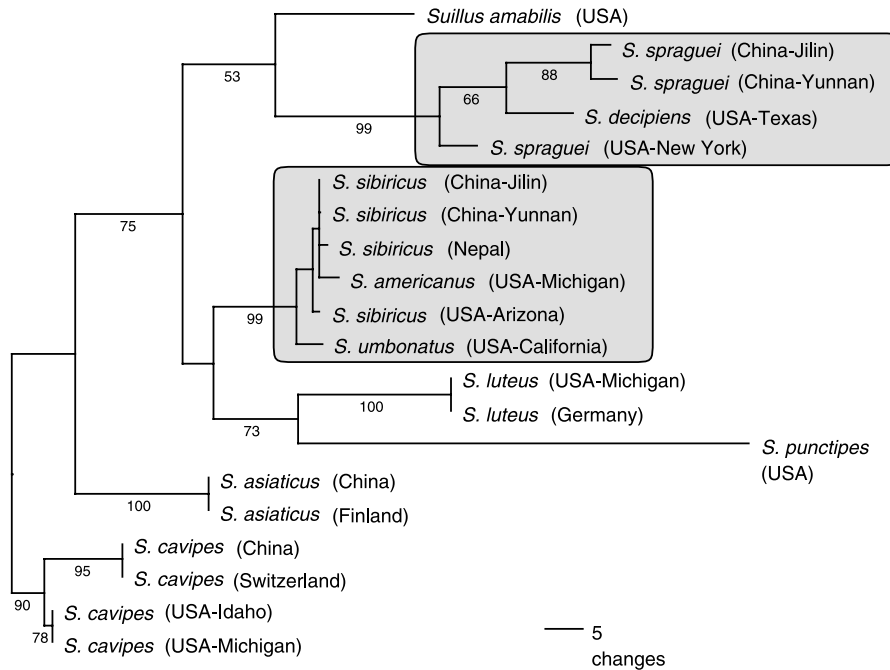
Sequences of the ITS region were obtained for a total of 20 collections, each representing a different geographical region. This is a subset of the data included in the larger study of Wu *et al.* (2000). The final alignment included 608 sites. Nine ambiguously aligned regions of a total of 119 sites were delimited and excluded from the analysis. Ninety-two out of the 489 nonambiguously aligned sites were variable sites, among which 64 characters were parsimony-informative. The step matrix implemented to weight character state changes consisted of  $A \leftrightarrow C = 2.04$ ;  $A \leftrightarrow G = 2.29$ ;  $A \leftrightarrow T = 2.09$ ;  $A \leftrightarrow \text{gap} = 4.93$ ;  $C \leftrightarrow G = 2.36$ ;  $C \leftrightarrow T = 0.92$ ;  $C \leftrightarrow \text{gap} = 3.32$ ;  $G \leftrightarrow T = 2.36$ ;  $G \leftrightarrow \text{gap} = 4.93$ ;  $T \leftrightarrow \text{gap} = 4.93$ . The maximum parsimony analyses generated a single most-parsimonious tree [tree length = 258.17 steps, CI = 0.729, RI = 0.833, RC = 0.607] (Fig. 5).

This analysis identified relationships among these taxa congruent to those identified in the more inclusive analyses of Wu *et al.* (2000). Morphologically identical specimens identified as *S. spraguei* from North America and China are paraphyletic based on ITS sequence data, with North American *S. spraguei* being basal to a monophyletic group (66%) consisting of *S. spraguei* from China and the morphologically distinct *S. decipiens* (Berkeley & Curtis) Kuntze from south-eastern United States. Material identified as *S. sibiricus* (Singer) Singer, *S. americanus* and *S. umbonatus* Dick and Snell form a well supported (99%) monophyletic group. Branch lengths are short in this clade, and while possible biogeographic structure is evident, i.e. *S. sibiricus* from North America is basal to a group consisting of Asian *S. sibiricus* and North American *S. americanus*, there is no bootstrap support for these relationships. *Suillus cavipes* (Opat.) Smith & Thiers displays a circumboreal distribution. In our analyses, and those of Wu *et al.* (2000), European and Chinese populations of the circumboreal *S. cavipes* are more closely related to each other than either is to North American populations of this species.

## DISCUSSION

The biogeography of macrofungi has been receiving increasing attention (reviewed in Wu & Mueller, 1997). Most of the biogeographic hypotheses proposed in these papers, however, are based on studies of morphological characters, comparisons of species lists and/or untested assumptions regarding the phylogenetic relationships of the taxa. Thus, while there have been a few recent historical biogeographic studies published (e.g. Vilgalys & Sun, 1994; Wu *et al.*, 2000, Hibbett, in press, this volume), there is a need to rigorously test most of the putative biogeographic relationships proposed for macrofungi.

In this paper we focus on testing one set of these hypotheses, the putative close relationship between the macrofungi of eastern North America and eastern Asia. Unfortunately, as discussed by Redhead (1989) and Wu & Mueller (1997), the lack of modern taxonomic revisions for most genera of macrofungi coupled with the high number of



**Figure 5** The single most parsimonious tree generated for *Suillus* based on ITS sequence data. Numbers below each internode are the percentage of 1000 bootstrap replicas supporting that binary partition (value  $\leq 50$  not shown). Shaded area indicates a putative disjunct species.

species in many broadly distributed genera greatly limits the genera or other taxa currently amenable to historical biogeographic analyses. Our discussion is limited to the biogeographic relationships among species in three different genera: *Armillaria*, *Xerula* and *Suillus* as data on other taxa are not available. However, these three genera are reasonable exemplars for an initial assessment of this biogeographic pattern in macrofungi as the three genera are relatively large, broadly distributed and represent the three major ecological guilds that macrofungi occupy, i.e. parasite saprobe, and mutualist (ectomycorrhizal), respectively. While interpretation of the results reported in this paper may be limited by the lack of thorough, modern taxonomic revisions of these genera, especially for *Xerula* and *Armillaria*, there is sufficient systematic information available for these genera to enable reasonable taxon sampling and to assess broad biogeographic patterns. Several general observations were evident from these analyses.

#### Biogeographic pattern is evident in at least part of each data set

Although the apparent rapid radiation into the northern hemisphere renders ITS sequence data uninformative for assessing relationships among northern hemisphere taxa of *Armillaria*, there is a southern hemisphere/northern hemisphere pattern seen in the data with Australasian taxa forming the base of each clade or grade (< 50% bootstrap support for most of these clades) (Fig. 1). This hypothesis needs to be tested with a faster evolving gene(s). Unlike most of the northern hemisphere taxa, all of the Australasian

*Armillaria* taxa formed well supported clades based on the ITS data, possibly indicating that the Australasian taxa are older. The tree topologies obtained for both *Xerula* and *Suillus* are congruent with the geographical distributions of the included material of the same species (Figs 2–5). Vilgalys & Sun (1994), Hibbett *et al.* (1998), Hibbett (in press; this volume), and colleagues also have documented discrete biogeographic patterns for the taxa that they studied, as did Wu *et al.* (2000) in their more inclusive study of *Suillus*. Thus, phylogenetic analyses have provided data consistent with the current hypothesis that species of macrofungi generally display discrete distribution patterns that mirror those observed for species of plants and animals, although these fungi have spores that may be dispersed great distances.

#### Putative North American/east Asian disjunct taxa recognized in *Suillus* and *Xerula*, but not yet in *Armillaria*

As predicted, material of *X. hispida* from China and Costa Rica formed a monophyletic group on the most parsimonious tree (83% bootstrap) (Figs 2 & 3) and the most likely tree (< 50% bootstrap) (Fig. 4). Specimens from these two populations are morphologically indistinguishable. Thus, the disjunct distribution of *X. hispida* is not refuted in this study. *Xerula hispida* shares a most recent common ancestor with material referable to *X. pudens* s.l. from Papua New Guinea, suggesting a southern hemisphere origin, although the continental European material of *X. pudens* s.l. is basal to the *X. hispida*/PNG *X. pudens* clade. Unexpectedly, material

of *X. pudens* s.l. from Sweden formed a well-supported clade separate from other material referable to *X. pudens* s.l. Micromorphological examination of all of these specimens revealed more morphological variation than has been reported in the literature, and a detailed examination of additional specimens, especially of type material for *X. pudens* and its synonyms, will be necessary to resolve the nomenclatural issues presented by our findings. While we can not assign names to the taxa, based on both morphological and molecular data, several taxa occur in the *X. pudens* group.

The disjunct distribution of *X. furfuracea* was not supported by these analyses. The relationship between Chinese and North American *X. furfuracea* was unresolved in the maximum parsimony trees (Figs 2 & 3) and maximum likelihood trees (Fig. 4). North American material referable to *X. furfuracea* formed a distinct, well-supported clade in both analyses. The Chinese material formed a distinct clade (< 50%) in the maximum parsimony trees but not in the maximum likelihood trees. As was the case with the *X. pudens* group, micromorphological examination of material referable to *X. furfuracea* from North and Central America and China uncovered more morphological variation than has been reported in the literature (unpublished data). Thus, increased sampling within this group, as well as the inclusion of additional characters, could help resolve these relationships.

Morphologically similar material of *S. spraguei* from China and eastern North America are paraphyletic based on the ITS data, with the morphologically distinct *S. decipiens* being the sister taxon to Chinese *S. spraguei* (Fig. 5). These two taxa plus North American *S. spraguei* share a most recent common ancestor. Thus, while there is a close biogeographical relationship among these taxa, the group consists of a disjunct sister species pair rather than disjunct populations of one species and predicted relationships based on morphology were not supported by the molecular data. Material of *S. spraguei* from China and North America are morphologically indistinguishable. They also show strong host association and have only been reported associated with species of *Pinus* subgenus *Strobus* (five-needle pines). Conversely, *S. decipiens*, which is restricted to the south-eastern United States, differs significantly in a number of morphological characters and forms ectomycorrhizae with different species of pines, those in *Pinus* subgenus *Pinus* (Wu *et al.* 2000). Thus, divergence following geographical isolation from a common ancestor followed different paths, with *S. decipiens* changing in morphology, ITS sequence, and host switching to another subgenus while *S. spraguei* only showing sequence divergence.

This lack of observable morphological differences between the two isolated, disjunct, paraphyletic populations of *S. spraguei* is an example of morphological stasis in the Chinese population. Morphological stasis is the lack of significant morphological change in a species over a long time period. Morphological stasis has been proposed as a common phenomenon in the evolution of eastern North American and eastern Asian disjunct plants (see Wen, 1999).

Two general mechanisms have been proposed for morphological stasis (Wen, 1999): (1) stasis by constraints is the lack of significant changes through genetic or developmental architecture and (2) stabilizing selection comes about through the elimination of all phenotypes that deviate significantly from the population mean. Both the Chinese and eastern North American populations occur in similar forest types, moist temperate mixed forests with a canopy dominated by Fagaceae and *Pinus* subgenus *Strobus*. Thus, stabilizing selection, rather than stasis by constraints, may be operating in this case. Parks and his collaborators (e.g. Parks & Wendel, 1990; Qiu *et al.*, 1995) have suggested that stabilizing selection plays a larger role than stasis by constraints in flowering plants, but as Wen (1999) states, this needs to be tested. More phylogeny based biogeographic studies of macrofungi need to be undertaken before we can estimate the potential frequency of morphological and/or molecular stasis.

The disjunct populations of *S. americanus* and *S. sibiricus* show little ITS sequence divergence (Fig. 3). While there appears to be slight morphological differences between *S. sibiricus* from western North America and the other material in this clade, specimens of *S. americanus* from eastern North America and the material that we called *S. sibiricus* from eastern Asia are morphologically indistinguishable. As discussed by Wu *et al.* (2000), the traditional key diagnostic characters delimiting North American *S. americanus* (restricted to eastern North America) from *S. sibiricus* (previously reported only from western North America and western and central Asia–Siberia and Nepal) is the presence or absence of a partial veil (annulus), a slight difference in colour change upon bruising, and the species of five-needle pine with which it is associated (*S. americanus* restricted to *P. strobus*). Upon examination of material labelled as both taxa, including the type specimen of *S. sibiricus*, we found that the morphological characters formed a mosaic and were not informative to circumscribe separate species. The lack of molecular divergence coupled with the lack of good diagnostic morphological characters suggest that *S. americanus* and *S. sibiricus* could be synonymized into one circumboreal taxon. Eastern North American and Chinese *S. americanus*/*S. sibiricus* formed a weakly supported clade separate from western North America *S. sibiricus* in the more inclusive study of Wu *et al.* (2000) so more information would be helpful to resolve the circumscription of this taxon.

A faster evolving gene, coupled with more extensive taxon sampling from Asia and South America, is necessary to examine fine scale distribution patterns in *Armillaria*. Our data are similar to those of Anderson & Stasovski (1992) who found that ITS sequence data were insufficient to resolve relationships among closely related species of *Armillaria*, but too variable to easily align for more distantly related taxa such as *A. mellea* and *A. tabescens*. They reported that sequence data from the highly variable intergenic region of the nuclear RNA tandem repeat (IGR) was informative for closely related taxa (Anderson & Stasovski, 1992). Our ITS data place the eastern Asian

*Armillaria* material nested within the large clade of northern hemisphere taxa that excluded *A. mellea* and *A. tabescens*. Thus, IGR data, or other rapidly diverging genes, may prove useful for uncovering eastern North American/eastern Asian disjunct populations.

#### Data support a relatively close biogeographic relationship between the macrofungus mycota of eastern North America and eastern Asia

Several different biogeographic patterns were uncovered in this test of eastern North American/eastern Asian disjunct species of macrofungi. *Xerula hispidia* represents a species with disjunct populations in Central America/northern South America and eastern Asia (Figs 2–4). Chinese material referable to *S. spraguei* is the sister taxon to *S. decipiens* from south-eastern United States and is paraphyletic to the morphologically similar *S. spraguei* from eastern North America (Fig. 5). The more inclusive *Suillus* study of Wu *et al.* (2000) uncovered sequence divergence between populations of *S. spraguei* in north-eastern and south-western China. If one recognized the two Chinese populations as separate species (we do not), they would be sister taxa, and there would be no intercontinental disjunct sister taxon relationship. Our study does not support treating material of *X. furfuracea* from China and North America as disjunct populations of one species. Rather, morphological and ITS sequence data suggest that they represent distinct, but closely related taxa. Finally, our data suggest that material of *S. americanus* and *S. sibiricus* from China, while related, can be collapsed into one species that includes material referable to *S. sibiricus* from western North America. In *Armillaria* (Fig. 1) eastern Asian exemplars representing different species were nested within the unresolved northern hemisphere clade. Chinese and North American material identified as *A. tabescens* formed a monophyletic group, but European material attributable to this taxon was not included in the analysis. Thus, while there is an evident close relationship among north temperate taxa, a distinct pattern among these taxa was not identifiable with this data set. In summary, the data presented in this paper document a relatively close relationship between the macrofungi of eastern North America and eastern Asia. However, the relationship may not be as close as had been hypothesized solely on morphology. Data on additional taxa are necessary to assess the nature of other putative disjunct taxa and to determine if the eastern North America/eastern Asia relationship is generally closer than that seen between eastern and western North America.

Similar patterns have been identified for putative disjunct plant taxa. While numerous disjunct species have been reported for flowering plants, examination of these hypotheses within a phylogenetic framework has shown that few plant species have eastern North American/eastern Asian disjunct populations (Wen, 1999). Rather, most represent discrete species that are most closely related to other species within the same continent (Wen, 1999). Thus, while there are many instances of closely related taxa occurring in

eastern North America and eastern Asia, there are few intercontinental sister taxa now recognized among the flowering plants. More putative macrofungi disjuncts need to be tested before one can predict if the same pattern will emerge for these fungi.

The low level of ITS sequence divergence observed among the putative disjunct macrofungal taxa is consistent with a hypothesis that these disjunct populations represent formerly broadly distributed taxa that were relatively recently isolated through vicariance-promoted extinction. Gene flow between these widely separated disjunct populations is another possible explanation for the observed low level of sequence divergence. Unfortunately, without a good fossil record or a way to calibrate a molecular clock, these hypothesis remain untestable for macrofungi. Most studies of fungal biogeography, however, emphasize that as fungi are closely associated with plants as mutualists, parasites, or substrate, current fungal distributions are greatly influenced by the same factors controlling plant distributions (see citations in Wu & Mueller, 1997). Several possible migration routes for, and a wide range of divergence times among, eastern North American and eastern Asian plant disjuncts have been established (Wen, 1999). Thus, we expect that the observed relatively close relationship between the macrofungi of eastern North America and eastern Asia is not the result of a single event, but that each of the putative disjuncts have individual histories, each affected by climatic and geological events.

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## BIOSKETCHES

**Greg Mueller's** research focuses on the systematics, biogeography, biodiversity and ecology of macrofungi. Field work in the last 10 years has been concentrated in China, Costa Rica and eastern North America with a goal of documenting biogeographic and biodiversity patterns and investigating various processes that may have shaped these patterns (e.g., geological history, plant migrations and human caused stressors).

**Qiuxin Wu** has focused her research on the systematics of macrofungi and on documenting Chinese macrofungal diversity and biogeography, factors influencing these patterns, and how these data can be used for conservation.

**Yongqing Huang** is interested in the biodiversity and ecology of macrofungi. He has undertaken several long-term, plot-based inventories that compare the species composition and diversity of different forest communities in China.

**Shouyu Guo** works on the systematics of lichenized fungi with a focus on taxa that show putative disjunct distributions.

**Ruth Aldana-Gómez** is a PhD student with interests in morphological and molecular systematics of macrofungi, fungal biodiversity and biogeography.

**Rytas Vilgaly's** research program used the techniques of molecular biology as well as more conventional approaches (culturing, mating studies, etc.) to answer questions about the natural history of fungi. Current research is focused on three areas: (1) phylogenetic biology and systematics, especially of the Agaricales (mushrooms); (2) genetics of speciation, including patterns of morphological vs. genetic divergence, and analysis of the genetic factors underlying development of intersterility between related species; and (3) population biology of fungi, estimation of breeding systems and measurement of gene flow in natural populations.