Cophylogeny and Biogeography of the Fungal Parasite Cyttaria and Its Host Nothofagus, Southern Beech

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Cophylogeny and biogeography of the fungal parasite *Cyttaria* and its host *Nothofagus*, southern beech

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Abstract: The obligate, biotrophic association among species of the fungal genus *Cyttaria* and their hosts in the plant genus *Nothofagus* often is cited as a classic example of cophylogeny and is one of the few cases in which the biogeography of a fungus is commonly mentioned or included in biogeographic analyses. In this study molecular and morphological data are used to examine hypotheses regarding the cophylogeny and biogeography of the 12 species of *Cyttaria* and their hosts, the 11 species of *Nothofagus* subgenera *Lophozonia* and *Nothofagus*. Our results indicate highly significant overall cophylogenetic structure, despite the fact that the associations between species of *Cyttaria* and *Nothofagus* usually do not correspond in a simple one to one relationship. Two major lineages of *Cyttaria* are confined to a single *Nothofagus* subgenus, a specificity that might account for a minimum of two codivergences. We hypothesize other major codivergences. Numerous extinction also are assumed, as are an independent parasite divergence followed by host switching to account for *C. berteroi*. Considering the historical association of *Cyttaria* and *Nothofagus*, our hypothesis may support the vicariance hypothesis for the trans-Antarctic distribution between Australasian and South American species of *Cyttaria* species hosted by subgenus *Lophozonia*. It also supports the hypothesis of transoceanic long distance dispersal to account for the relatively recent relationship between Australian and New Zealand *Cyttaria* species, which we estimate to have occurred 44.6–28.5 mya. Thus the history of these organisms is not only a reflection of the breakup of Gondwana but also of other events that have contributed to the distributions of many other southern hemisphere plants and fungi.

Key words: Australasia, Leotiomycetes, long distance dispersal, South America, southern hemisphere, vicariance

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INTRODUCTION

The obligate, biotrophic relationship between species belonging to the fungal genus *Cyttaria* (Ascomycota, Leotiomycetes) and their hosts in the plant genus *Nothofagus* (Hamamelididae, Nothofagaceae) has captured the attention of evolutionary biologists since Charles Darwin, whose South American collections of these fungi during the *Beagle* voyage became the first two *Cyttaria* species to be described (Berkeley 1842, Darwin 1839). After hearing from Joseph Dalton Hooker that a third *Cyttaria* species had been found in Tasmania, Darwin (1846) commented on the ''singular relationship'' between *Cyttaria* and *Nothofagus* in ''distant parts of the world!''

The history of *Nothofagus* is considered to be important, even key, in understanding southern hemisphere biogeography (Cracraft 1975, Darlington 1965, Steenis 1971). *Nothofagus* pollen is distinctive, produced in copious amounts and is easily fossilized. First appearing by the early Campanian of the Late Cretaceous (~83.5 mya) (Dettmann et al. 1990), its widespread, persistent, and abundant microfossil record, and to a lesser extent its macrofossil record, indicate that *Nothofagus* was widespread throughout much of southern Gondwana before continental breakup. In addition *Nothofagus* includes prominent forest trees that presently exhibit a widespread, disjunct southern hemisphere distribution, with modern representatives in South America (southwestern Argentina and Chile) and Australasia (southeastern Australia, New Zealand, New Guinea and New Caledonia) and with extinct taxa also known from Antarctica (Dettmann et al. 1990, Hill 1991). This disjunct, trans-Antarctic distribution, characterized by areas of endemism, traditionally is hypothesized to be entirely the result of vicariance and extinction because the dispersability of its seeds has been assumed to be insufficient to account for the distribution of *Nothofagus* species. The phylogeny of *Nothofagus* should be a reflection of the geological breakup sequence of southern Gondwana, according to this hypothesis. It is generally accepted that New Zealand was the first to separate ~80 mya and that South America and Australia were connected by Antarctica until ~35 mya (McLoughlin 2001). Thus

the phylogeny of *Nothofagus* should show that species from Australia and South America are more closely related to each other than to species from New Zealand (but see Giribet and Edgecombe 2006). However taxonomic arrangements and phylogenies of *Nothofagus* have inferred a closer relationship between Australian and New Zealand species (Dettmann et al. 1990, Hill and Jordan 1993, Hill and Read 1991, Linder and Crisp 1995, Manos 1997, Martin and Dowd 1993).

Nothofagus *systematics.*—*Nothofagus* comprises 35 extant species divided into four subgenera that correspond to pollen types (Dettmann et al. 1990, Hill and Jordan 1993, Hill and Read 1991): subgenus *Brassospora* (*brassii* type pollen) with 19 species in New Caledonia and New Guinea, which do not host *Cyttaria* species; subgenus *Fuscospora* (*fusca* type [a] pollen) with five species in southern South America and Australasia, which do not host *Cyttaria* species; subgenus *Lophozonia* (*menziesii* type pollen) with six species in southern South America and Australasia, all which host *Cyttaria* species; and subgenus *Nothofagus* (*fusca* type [b] pollen) with five species in South America, all which host *Cyttaria* species. Pollen types corresponding to the four modern subgenera first appear contemporaneously in the Antarctic fossil record in the late Campanian-early Maastrichtian of the Late Cretaceous (~73 mya) (Dettmann et al. 1990). The monophyly of each of the four extant subgenera is supported by molecular sequence data (Martin and Dowd 1993, Setoguchi et al. 1997) and combined molecular and morphological data (Manos 1997). *Nothofagus* subgenera that host *Cyttaria* species, *Lophozonia* and *Nothofagus* do not together form a single monophyletic group (FIG. 1; Cook and Crisp 2005, Manos 1997, Martin and Dowd 1993, Setoguchi et al. 1997); of the extant subgenera *Nothofagus* is most closely related to *Brassospora*, representing the most recent divergence among subgenera, and subgenus *Lophozonia* is sister of the remaining three subgenera. Subgenus *Lophozonia* exhibits a disjunct trans-Antarctic distribution, with species occurring in Australia (including Tasmania), New Zealand and South America, while subgenus *Nothofagus* occurs only in South America. Thus modern day *Nothofagus* subgenera exhibit three trans-Antarctic relationships: within subgenus *Fuscospora* (with species from Tasmania and New Zealand more closely related to each other than to South American species), within subgenus *Lophozonia* (with species from Australia and New Zealand more closely related to each other than to South American species, and between subgenera *Nothofagus* (with species from South America) and *Brassospora* (with species from New Guinea and New Caledonia species).

Cyttaria *systematics.*—According to phylogenetic analyses of combined morphological and nuclear ribosomal RNA, mitochondrial ribosomal RNA and *TEF1*sequence data, genus *Cyttaria* comprises 12 taxa representing three major clades (FIG. 1; Peterson and Pfister 2010): one clade (A) occurs on subgenus *Nothofagus*, which occurs only in South America; the second (B) occurs only in South America on both subgenus *Nothofagus* and subgenus *Lophozonia*; and the third (C) exhibits a trans-Antarctic distribution, occurring in both South America and Australasia only on subgenus *Lophozonia*. In total seven *Cyttaria* taxa are endemic to southern South America (Argentina and Chile), on subgenera *Lophozonia* and *Nothofagus*, and the other five are endemic to southeastern Australasia and New Zealand on subgenus *Lophozonia*. Of the five Australasian taxa, two are found only in Australia, including Tasmania, and three are found only in New Zealand.

Associations between Cyttaria *and* Nothofagus.—Associations between species of *Cyttaria* and *Nothofagus* usually do not correspond in a simple one to one relationship; several *Cyttaria* species may infect the same *Nothofagus* species and a single *Cyttaria* species may infect several *Nothofagus* species (FIG. 1). Generally an individual *Cyttaria* species is associated with more than one (up to five) *Nothofagus* species, which in turn are associated with more than one (up to four) *Cyttaria* species. However hosts of *Cyttaria* species that are associated with multiple *Nothofagus* species invariably belong to the same subgenus (but see FIG. 1). Furthermore with the exception of one *Cyttaria* species each major *Cyttaria* clade is associated with a single *Nothofagus* subgenus. In no case is there a *Cyttaria* or *Nothofagus* species common to both South America and Australasia or between Australia and New Zealand. Despite extensive searching, *Cyttaria* has not been found on any of the *Nothofagus* species from New Caledonia or New Guinea (Korf 1983). It is unlikely that undiscovered species exist outside the current known range of *Cyttaria*. This is because of the conspicuous nature of the typical *Cyttaria* species, with its spherical, honeycombed fruit bodies (typically up to 4[–8] cm, depending on the species) (FIG. 2), usually obvious perennial cankers (depending on the species and type of canker produced, globose cankers up to 1 m diam on large branches and up to 1.5 m diam on large trunks, as well as longitudinal cankers of up to 1 m long) and often spectacular fruiting (up to 25 densely clustered fruit bodies in a single group and a carpet of fallen

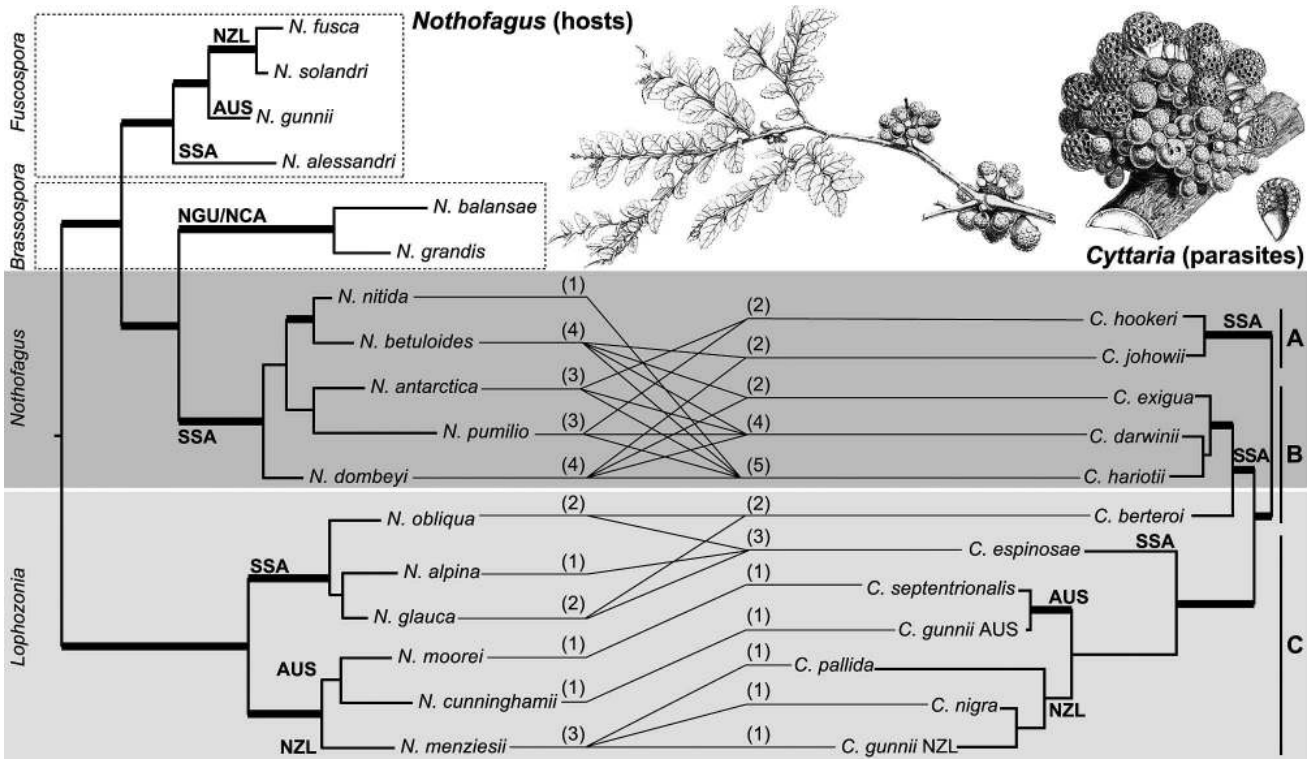

FIG. 1. Relationships between *Nothofagus* and *Cyttaria*. Branches in boldface are well supported (>0.95 posterior probabilities or >70% bootstrap proportion). Lines connecting hosts and parasites represent associations; numbers in parenthesis indicate number of associations per taxon. Shaded boxes indicate *Nothofagus* subgenera that host *Cyttaria* and the *Cyttaria* taxa associated with them. (*Cyttaria* clades A, B and C are discussed in the text and depicted in FIG. 2.) See Gamundí and Minter (2004a, b) and Peterson and Pfister (2010, TABLE I) for reports of possible additional hosts *N. dombeyi* and *N. obliqua* for *C. espinosae* and *C. hookeri* respectively. Illustrations of *C. gunnii* and *N. cunninghamii* from Berkeley (1848) are reproduced courtesy of the library of the Gray Herbarium, Harvard University, Cambridge, Massachusetts. AUS = Australia, NCA = New Caledonia, NGU = New Guinea, NZL = New Zealand, SSA = southern South America.

fruit bodies up to 15 cm deep on the forest floor in one species).

Cyttaria species are presumed to be weak parasites (Gamundí and Lederkremer 1989) that produce trunk and branch cankers on *Nothofagus* trees. These cankers arise due to localized, stimulated cambial activity attributed to the presence of hyphae belonging to *Cyttaria* species, found in the secondary phloem and xylem, cambium and cortex of the hosts (Gutiérrez de Sanguinetti 1988, Wilson 1937).

Many have discussed the idea that parasites, including *Cyttaria* (Humphries et al. 1986, Korf 1983), can serve as taxonomists to elucidate relationships of their hosts. This idea implies that parasite and host phylogenies should be congruent or based on codivergence events. Confounding factors in this association relating to the parasite include host switching, extinction and speciation in the parasite lineage but not the host lineage. Especially problematic with respect to *Cyttaria* and *Nothofagus* is the fact that most *Cyttaria* parasites and *Nothofagus* hosts do not display a simple one to one correspondence. Also of interest is the occurrence of *Cyttaria* species on two relatively unrelated *Nothofagus* subgenera and their absence from the other two.

The relationship between *Cyttaria* and *Nothofagus* often is cited as a classic example of cophylogeny (e.g. Korf 1983) and is one of the few cases in which the biogeography of a fungus is commonly mentioned. Crisci et al. (1991), Seberg (1991), Linder and Crisp (1995) and Sanmartín and Ronquist (2004) included *Cyttaria* in their biogeographical analyses. Humphries et al. (1986) reconstructed five codivergence events, including the concurrent origins of *Cyttaria* and *Nothofagus*, which they inferred was significant cophylogeny in the associations between *Cyttaria* species and their *Nothofagus* hosts.

In this study we used molecular and morphological datasets derived from our work for *Cyttaria* (Peterson and Pfister 2010) and from Martin and Dowd (1993), Manos (1997) and Setoguchi (1997) for *Nothofagus* to test assertions of cophylogeny between the fungal

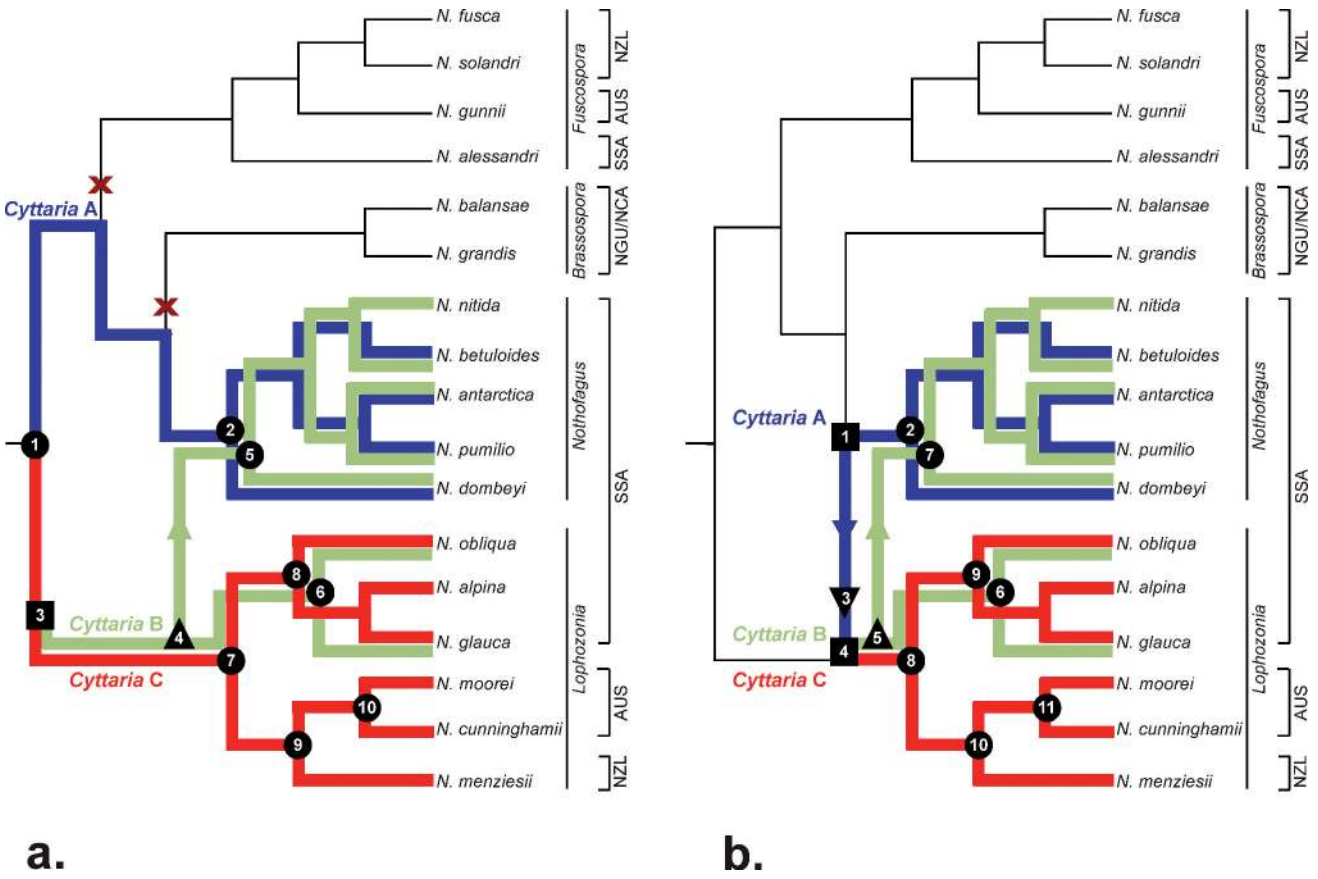

FIG. 2. Hypothesized cophylogenetic reconstructions of *Cyttaria* taxa and *Nothofagus* hosts depicting major events. a. Represents the concurrent origin of *Cyttaria* and *Nothofagus*. b. Represents the colonization by *Cyttaria* after the origin of *Nothofagus* to explain the absence of *Cyttaria* on *Nothofagus* subgenera *Brassospora* and *Fuscospora*, which do not host *Cyttaria*. 5s represent extinction or failure to track the host by *Cyttaria*. Events, labeled 1–10, are: circles, codivergence events; squares, duplication; and triangles, host switching. *Cyttaria* clades A, B and C represent the three major monophyletic lineages. AUS = Australia, NCA = New Caledonia, NGU = New Guinea, NZL = New Zealand, SSA = southern South America.

parasite *Cyttaria* and its host plant *Nothofagus*. These results were used to examine the biogeographic history of *Cyttaria*.

MATERIALS AND METHODS

Parasite and host phylogenies.—Character alignments and GenBank sequences were obtained from studies on *Cyttaria* and *Nothofagus*. For *Cyttaria* an alignment of nuclear small subunit (nucSSU), nuclear large subunit (nucLSU), mitochondrial small subunit (mitSSU) ribosomal RNA (rRNA) and *TEF1* sequence data as well as morphological data were obtained from our work (Peterson and Pfister 2010; GenBank EU107178–203, -205–232, -234–249). For *Nothofagus* alignments of chloroplast DNA (cpDNA) *rbc*L sequences (Martin and Dowd 1993; GenBank L13341–345, -348, -350–358, -360, -362), nuclear internal transcribed spacer (nucITS) rRNA sequences (Manos 1997; GenBank U96849–857, -859, -863, -865–870), cpDNA *atp*B-*rbc*L intergenic spacer sequences (Setoguchi et al. 1997; GenBank AF015687–690, -692, -696, -698–708) and morphological data (Manos 1997) were obtained from the original publications; the dataset of Knapp et al. (2005) was not used because it did not include all *Nothofagus* species that host *Cyttaria*. Seventeen *Nothofagus* representatives for which data were available for all four partitions were included. This included all *Nothofagus* species that host *Cyttaria* taxa, as well as select representatives from the other two *Nothofagus* subgenera. Methods for reconstructing phylogenies follow Peterson and Pfister (2010). *Cyttaria* phylogenies were taken from Peterson and Pfister (2010), where phylogenies resulting from the exclusion of morphological data also were considered.

Divergence time estimation.—Because the molecular data could be rejected as evolving in clock-like ($P < 0.001$), based on a likelihood ratio test, we used two relaxed clock dating methods: (i) penalized likelihood and (ii) an uncorrelated method implemented in BEAST 1.4.8 (Drummond and Rambaut 2007). An uncorrelated lognormal (UCLN) model implemented in BEAST was used to infer divergence times. Convergence of each chain to the target distribution was assessed with Tracer 1.4 (Rambaut and Drummond 2007) and by plotting time series of the log

posterior probability of sampled parameter values. After convergence each chain was sampled every 1000 steps until 50 000 samples were obtained. Model fit of the UCLN relaxed clock models was assessed with Bayes factors as implemented in Tracer 1.4. In addition divergence times were estimated with penalized likelihood (PL, Sanderson 2002) as implemented in the program r8s (Sanderson 2003). The optimal smoothing parameter (λ) was determined by cross validation. We constructed confidence intervals for our PL based estimates with a bootstrap resampling technique (Baldwin and Sanderson 1998). First, the original dataset was resampled 1000 times with SEQBOOT 3.6 (Felsenstein 2005). Each replicate was used to re-estimate edge lengths on the optimal topology with ML in PAUP* 4.0b10 (Swofford 2002). Replicate trees were transformed in r8s and divergence time estimates summarized across trees with the PROFILE command.

Peterson and Pfister (2010, FIG. 1) was used to estimate divergence times relating to events pertaining to the *Cyttaria* clade. *Paleopyrenomycites*, widely regarded as the most important fungal fossil (e.g. Lücking et al. 2009, Taylor and Berbee 2006), was used to calibrate divergence among groups. It is uncertain to which fungal group this 400 myo fossil from the Rhynie Chert belongs. Taylor and Berbee (2006) regard it as an ancestral member of the Ascomycota, whereas Lücking et al. (2009) proposed it as an ancestral member of the Pezizomycotina. We therefore tested both hypotheses.

Host-parasite associations.—Data were taken from Calvelo and Gamundí (1999), Gamundí (1971) and Rawlings (1956).

Tree-based analyses of cophylogeny.—These methods take into account four basic types of cophylogenetic events: codivergence (cospeciation), duplication (independent speciation of the parasite), host switching and extinction; the latter is a type of loss, which also includes two other phenomena, missing the boat (failure to track all host lineages following a speciation) and sampling failure (failure of the researcher to observe parasites on their hosts); also confounding is the scenario in which parasites fail to diverge with their hosts (see review by Charleston and Perkins 2006). Based on the one host-one parasite assumption, these tree-based methods are unable to accommodate widespread parasites (parasites associated with more than one host); thus none were used in this study to calculate significance values. TreeMap (Charleston and Page 2002) however was used to provide a graphical depiction of hypothesized cophylogenetic events.

Distance-based analysis of cophylogeny.—COPYCAT (Meier-Kolthoff et al. 2007), a wrapper or interface for ParaFit (Legendre et al. 2002), was used to assess the null hypothesis of random association between parasites and hosts as performed in ParaFit. Host and parasite trees including branch lengths plus an association file representing all 25 parasite-host combinations (because most *Cyttaria* taxa are associated with more than one host) were input into COPYCAT, and tests of random association were performed with 9999 permutations globally across both phylogenies for each host-parasite association. Unlike earlier methods, ParaFit is able to accommodate any type of host-parasite association (Legendre et al. 2002), including widespread parasites (as well as trees with polytomies).

Data-based analysis of cophylogeny.—The incongruence length difference (ILD) test (Farris et al. 1995) was used to seek evidence that the *Cyttaria* and *Nothofagus* datasets were not samples of the same phylogenetic history. Complete parasite and host datasets were treated as partitions, such that each parasite-host pair consisted of a concatenation of all aligned data from a *Cyttaria* species and an associated *Nothofagus* host species. All 25 parasite-host taxa combinations were tested with 10 000 replicates of the partition-homogeneity test in PAUP* 4.0b10 (Swofford 2002) with parsimony uninformative characters excluded.

RESULTS

Parasite and host phylogenies.—*Cyttaria* phylogeny (FIG. 1) recovered these notable clades are one composed of the South American species *C. hookeri* and *C. johowii* (A), which forms a monophyletic sister group with the remaining species, one composed of the South American species *C. berteroi*, *C. darwinii*, *C. exigua* and *C. hariotii* (B), which forms a monophyletic sister group with the remaining species, one composed of the South American species *C. espinosae* plus the Australasian species (C), a monophyletic Australian lineage and a monophyletic New Zealand lineage.

Nothofagus phylogeny (FIG. 1) recovered four monophyletic groups corresponding to subgenera in agreement with Manos (1997), Martin and Dowd (1993) and Setoguchi et al. (1997) and for the reduced set of taxa included here identical in topology to the combined consensus tree presented by Manos (1997). As indicated by earlier studies, the two subgenera that host *Cyttaria*, *Lophozonia* and *Nothofagus*, did not form a clade. Instead subgenus *Lophozonia* (Australia including Tasmania, New Zealand and South America), the designated outgroup in this study, was sister of the remaining subgenera. In this and previous studies the next to diverge, subgenus *Fuscospora* (Australia and Tasmania, New Zealand, and South America), was sister of the final two, subgenera *Brassospora* (New Caledonia and New Guinea) and *Nothofagus* (South America). Thus two subgenera, *Fuscospora* and *Lophozonia*, exhibit trans-Antarctic distributions. Furthermore subgenera *Brassospora* and *Nothofagus* together exhibit a third trans-Antarctic distribution.

Divergence time estimation.—Notable dates estimated for the *Cyttaria* lineage (TABLE I) include the origin of modern *Cyttaria*, estimated at 148.4–112.4 mya, the divergence between Australian and New Zealand

TABLE I. Divergence estimates (mya) for key splits. BEAST ages represent the mean of the 95% highest posterior density (HPD). AUS = Australia, NZL = New Zealand

	PL[a]	BEAST[a]	PL[b]	BEAST[b]
Origin of *Cyttaria*	112.2 (101–123)	112.4 (64–157)	148.4 (133–162)	146.3 (84–178)
AUS/NZL split	28.5 (24–33)	33.9 (14–58)	37.8 (33–42)	44.6 (18–72)
C. espinosae split	61.5 (55–67)	62.1 (29–96)	81.4 (73–88)	81.7 (38–102)
C. berteroi split	31.7 (26–38)	47.5 (13–87)	41.9 (38–47)	60.6 (17–98)

[a] Fossil placed at the divergence of the Ascomycota.
[b] Fossil placed at the divergence of the Pezizomycotina.

species estimated at 44.6–28.5 mya, the divergence of the clade that includes *C. espinosae* at 81.7–61.5 mya and the divergence of the clade that includes *C. berteroi* at 60.6–31.7 mya. With the exception of the divergence time estimate between the Australian and New Zealand species of *Cyttaria*, the two placements of the fossil *Paleopyrenomycites* produced times that were considerably older than geological and other events that would explain the divergences between these groups of *Cyttaria*. One example of this phenomenon may be that our estimates for the origin of *Cyttaria* predate the origin of *Nothofagus*, which has been estimated at 93–83.5 mya (Cook and Crisp 2005), and the origin of extant *Nothofagus* at 55–40 mya (Cook and Crisp 2005). These discrepancies at least in part likely are due to the problem of using a fossil far removed from our group of interest.

Distance-based analysis of cophylogeny.—The distance-based method ParaFit found highly significant overall cophylogenetic structure between the *Nothofagus* and *Cyttaria* datasets ($P < 0.0001$), regardless of correction method used in the DistPCoA setting (Lingoes, Cailliez, or no correction). Tests of individual links between host-parasite pairs found 23 (out of 25) links to be significant (significant P values = 0.0001–0.03). The two nonsignificant links were those between *C. berteroi* and its two hosts.

Data-based analysis of cophylogeny.—The ILD test detected no significant difference between *Cyttaria* and *Nothofagus* datasets ($P = 0.97$). That is there was no evidence to indicate that they were not samples of the same phylogenetic history.

DISCUSSION

Cophylogeny between Cyttaria *and* Nothofagus.—Analyses of cophylogeny (distance-based ParaFit and data-based ILD) indicate that *Cyttaria* taxa exhibit highly significant cophylogeny with *Nothofagus* hosts, even though associations between species of *Cyttaria* and *Nothofagus* usually do not correspond in a simple one to one relationship.

Parafit and ILD do not provide graphical depictions of probable cophylogenetic events. Given that both tests detected highly significant cophylogeny between *Cyttaria* and *Nothofagus*, we attempted to reconstruct their cophylogenetic history with TreeMap and present two possible, general scenarios (FIG. 2).

Cophylogeny scenario a.—Two *Cyttaria* lineages each are confined to a single *Nothofagus* subgenus (but see FIG. 1), a specificity that accounts for a minimum of two incidents of codivergence. Major incidents in the cophylogeny (events 1–10, FIG. 2a) are summarized: (i) an early codivergence at the concurrent origin of *Cyttaria* and extant *Nothofagus* leading to the South American subgenus *Nothofagus* hosting *C. hookeri* and *C. johowii* (*Cyttaria* clade A) and subgenus *Lophozonia* hosting the lineage that gave rise to all other *Cyttaria* taxa (*Cyttaria* clades B and C); (ii) a codivergence between the early diverging South American species *C. hookeri* and *C. johowii* (clade A) and their hosts in subgenus *Nothofagus*; (iii) a duplication (independent divergence) in the *Cyttaria* lineage on subgenus *Lophozonia*, which gave rise to (1) the South American *C. espinosae* plus all of the Australasian species (clade C) and (2) to the remaining *Cyttaria* species (clade B); (iv) host switching producing a dichotomy between the South American *C. berteroi* (clade B), which parasitizes subgenus *Lophozonia*, and the South American species *C. darwinii*, *C. exigua* and *C. hariotii* (clade B), which parasitize subgenus *Nothofagus*; (v) codivergence between *C. darwinii*, *C. exigua*, and *C. hariotii* (clade B) and their hosts, subgenus *Nothofagus*; (vi) codivergence between *C. berteroi* (clade B) and the South American lineage of subgenus *Lophozonia*; (vii) codivergence leading to the South American subgenus *Lophozonia* hosting *C. espinosae* (clade C) and the Australasian subgenus *Lophozonia* hosting all Australasian members of *Cyttaria* (clade C); (viii) codivergence between *C. espinosae* (clade C) and the South American subgenus *Lophozonia*; (ix) codivergence within the Australasian members of *Cyttaria* (clade C) and subgenus *Lophozonia*, giving rise to the Australian species and

the New Zealand species; and (x) codivergence within Australian members of *Cyttaria* (clade C) and subgenus *Lophozonia* giving rise to *C. gunnii* sensu stricto and *C. septentrionalis* and respective hosts, *N. cunninghamii* and *N. moorei*. The absence of *Cyttaria* species on subgenera *Brassospora* and *Fuscospora* in this scenario is explained by extinction or missing the boat. If *C. espinosae* and *C. hookeri* also occur respectively on *N. dombeyi* and *N. obliqua* (see FIG. 1) two additional host-switching events also are inferred.

Cophylogeny scenario b.—This one (FIG. 2b) explains the absence of *Cyttaria* species on subgenera *Brassospora* and *Fuscospora* by inferring the infection of one clade of *Nothofagus* with a subsequent host jump to the other clade.

Biogeography of Cyttaria *and* Nothofagus.—Given fossil evidence that the four extant subgenera were widespread, occurring in Antarctica, Australia and South America before continental drift (Dettmann et al. 1990), vicariance resulting from the breakup of southern Gondwana comprise a plausible hypothesis to explain the three trans-Antarctic distributions exhibited by *Nothofagus* (FIGS. 1, 2). In support of this hypothesis a biogeographic reconstruction for *Nothofagus* by Sanmartín et al. (2007) inferred that these three Australasian-South American relationships (within subgenus *Fuscospora*, within subgenus *Lophozonia* and between subgenera *Brassospora* and *Nothofagus*) arose from vicariance. Knapp et al. (2005) estimated divergence at 65–36 mya between the Australasian and South American species of subgenus *Fuscospora* that were consistent with vicariance, but their results for the divergence between Australasian and South American species of subgenus *Lophozonia* were equivocal (38–21 mya, the lower point being too recent to be indicative of vicariance). In a similar study Cook and Crisp (2005) estimated divergence consistent with vicariance between the Australasian and South American species of subgenera *Fuscospora* at 45–30 mya and *Lophozonia* at 30 mya, as well as between Australasian subgenus *Brassospora* and South American subgenus *Nothofagus* at 45–30 mya. Considering the historical association of *Cyttaria* and *Nothofagus*, the cophylogenetic reconstructions (FIG. 2) may support the vicariance hypothesis for the trans-Antarctic distribution between Australasian and South American *Cyttaria* species hosted by subgenus *Lophozonia*. The codivergence of one *Lophozonia* lineage with Australia and the other *Lophozonia* lineage with South America followed the divergence of their associated *Cyttaria* parasites. Our divergence estimates however are too early to support the vicariance hypothesis to explain the disjunction of *Cyttaria* species in clade C present on subgenus *Lophozonia*, at 81.7–61.5 mya. The hypothesis of the presence of *C. berteroi* from clade B on subgenus *Lophozonia* being the result of host switching also is supported by our divergence estimates, at 60.6–31.7 mya, later than the divergence among *C. espinosae* and its Australasian relatives.

The riddle of Cyttaria.—Subgenus *Nothofagus*, which hosts *Cyttaria*, is sister of subgenus *Brassospora*, which does not host *Cyttaria*. Following Hill (1996), Swenson et al. (2001) discuss the riddle of the presence of subgenus *Brassospora* in New Caledonia and New Guinea. The riddle is its absence elsewhere, according to Heads (2006). *Cyttaria* presents a third riddle: Why is it present on subgenera *Lophozonia* and *Nothofagus* but absent on subgenera *Brassospora* and *Fuscospora*? The two subgenera that host *Cyttaria* are not sister taxa and they are not devoid of *Cyttaria* (FIGS. 1, 2). Furthermore, although the current distribution of subgenus *Brassospora* is restricted to tropical latitudes in New Caledonia and New Guinea, its species once were more widespread, occurring in Antarctica, Australia and South America by the late Maastrichtian (Dettmann et al. 1990). Subgenus *Fuscospora*, whose extant species co-occur with *Cyttaria* hosts in the other two subgenera, also was widespread, occurring in Antarctica, Australia and South America by at least the mid-Paleocene (Dettmann et al. 1990). In fact the four extant subgenera were distributed widely from Australasia to Antarctica and South America by the mid-Eocene (Dettmann et al. 1990).

With respect to subgenera *Brassospora* and *Fuscospora* the absence of *Cyttaria* remains a riddle. It is unknown whether the ancestors of subgenera *Brassospora* and *Fuscospora* simply escaped colonization by *Cyttaria* or whether *Cyttaria* went extinct on the ancestors of these subgenera. No fossils have been attributed to *Cyttaria* (Korf 1983), but if the fossil record of *Nothofagus* is any indication of what happened to *Cyttaria* extensive extinction likely contributed to the current distribution of *Cyttaria*. The biogeographic reconstructions by Linder and Crisp (1995) and Swenson et al. (2001), which predict extinct lineages within at least the three major lineages of *Nothofagus* (*Fuscospora*, *Lophozonia* and *Brassospora-Nothofagus*), demonstrate the effect of extinction on the interpretation of biogeographic patterns observed from extant species (see also Cook and Crisp 2005, Manos 1997).

Long distance dispersal from Australia to New Zealand.—In agreement with biogeographic, phylogenetic and taxonomic studies of *Nothofagus* (Cook and Crisp 2005, Dettmann et al. 1990, Hill and Jordan

1993, Hill and Read 1991, Knapp et al. 2005, Linder and Crisp 1995, Manos 1997, Martin and Dowd 1993) and *Cyttaria* (Sanmartín and Ronquist 2004), this study found a closer relationship between Australian and New Zealand *Cyttaria* taxa (FIGS. 1, 2) associated with subgenus *Lophozonia* than would be predicted based on the breakup sequence of southern Gondwana. Some suggest that this common pattern is a result of propagules being carried by west wind drift, which began in conjunction with Antarctic circumpolar current (~35–28 mya), both of which could account for dispersal across the great expanse of the Tasman Sea (e.g. Sanmartín et al. 2007, Winkworth et al. 2002). The divergence between Australian and New Zealand species of extant subgenus *Lophozonia* was estimated by Cook and Crisp (2005) at 40–14 mya, and Knapp et al. (2005) estimated divergence at 31–16 mya. Thus, according to this hypothesis, earlier *Fuscospora* and *Lophozonia* fossil pollen from New Zealand represent extinct lineages. Our divergence estimates infer the separation of Australian and New Zealand clades at 44.6–28.5 mya, consistent with the long distance dispersal of *Cyttaria* between Australia and New Zealand.

The fossil record suggests that several species of *Nothofagus* traveled from Australia to New Zealand via transoceanic long distance dispersal (Hill 2001; Pole 1994, 2001). Although all pollen types were present at some point in New Zealand, only one *Nothofagus* pollen type, now extinct, was present before New Zealand drifted from Gondwana, and representatives of subgenus *Lophozonia*, Australasian hosts of *Cyttaria*, did not appear in New Zealand until the Early Eocene (Dettmann et al. 1990).

Conclusions.—Because it was widespread in southern Gondwana before the drift of Australia, New Zealand and South America *Nothofagus* reflects the major events that occurred during the formation of the current biota of these regions, including vicariance, extinction and transoceanic long distance dispersal. This study demonstrates how, in turn, obligate associates of *Nothofagus* such as *Cyttaria* can function ''sufficiently accurate as taxonomists'' (Korf 1983) and perhaps hold keys to understanding more about their hosts.

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