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Research

Host jumps shaped the diversity of extant rust fungi (Pucciniales)

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Summary

- The aim of this study was to determine the evolutionary time line for rust fungi and date key speciation events using a molecular clock. Evidence is provided that supports a contemporary view for a recent origin of rust fungi, with a common ancestor on a flowering plant.
- Divergence times for > 20 genera of rust fungi were studied with Bayesian evolutionary analyses. A relaxed molecular clock was applied to ribosomal and mitochondrial genes, calibrated against estimated divergence times for the hosts of rust fungi, such as *Acacia* (Fabaceae), angiosperms and the cupressophytes.
- Results showed that rust fungi shared a most recent common ancestor with a mean age between 113 and 115 million yr. This dates rust fungi to the Cretaceous period, which is much younger than previous estimations. Host jumps, whether taxonomically large or between host genera in the same family, most probably shaped the diversity of rust genera. Likewise, species diversified by host shifts (through coevolution) or via subsequent host jumps. This is in contrast to strict coevolution with their hosts.
- *Puccinia psidii* was recovered in Sphaerophragmiaceae, a family distinct from Raveneliaceae, which were regarded as confamilial in previous studies.

Introduction

Rust fungi (Pucciniales, Pucciniomycotina) are the most species rich group of obligate, plant pathogenic fungi. They include many important plant pathogens such as *Puccinia graminis* (wheat stem rust), *Hemileia vastatrix* (coffee rust) and *Cronartium ribicola* (white pine blister rust). The divergence of rust fungi was thought to mirror the evolution of their host plants (Savile, 1976, 1979; Anikster & Wahl, 1979). Thus, ancestral species of rust have been considered pathogens of ferns (monilophytes) and gymnosperms, with succession to angiosperms (Cunningham, 1931; Leppik, 1953, 1965; Savile, 1976). Leppik (1965) hypothesized that the first ancestor of rust fungi may have evolved 200–300 million yr ago (Ma) on mosses (bryophytes) and ancient ferns (leptosporangiates, which excludes the Polypodiales), and have extant relatives in the genera *Eocronartium* and *Jola* (Platygloeales, Pucciniomycotina). This correlates with the evolutionary divergence times for ferns, gymnosperms and angiosperms at *c.* 394, 312 and 194 Ma (Magallón *et al.*, 2013).

Hart (1988) challenged the notion that ancestral hosts harbored ancestral parasites. This was based on a phylogenetic analysis with morphological characters from 30 genera of rust fungi. Hart (1988) suggested that ancestral rusts were autoecious (completed their life cycle on one host), short-cycled and evolved on angiosperms in tropical climates. He further proposed that the rusts on ferns and pines (Pinales) were derived, and groups now classified as the suborders Uredinineae *sensu* Aime (2006) and Melampsorineae *sensu* Aime (2006) diverged at the same time. Sjamsuridzal *et al.* (1999) determined that fern rusts were not ancestral in the Pucciniales with a molecular phylogenetic study that supported the conclusions of Hart (1988).

An evolutionary origin of rust fungi on angiosperms was supported by Aime (2006), who showed that some extant species, including *H. vastatrix* (on Rubiaceae), *Blastospora smilacis* (on Smilacaceae) and *Maravalia cryptostegiae* (on Apocynaceae), belonged to an ancestral family, Mikronegeriaceae (Pucciniales). This family is typified by *Mikronegeria*, which is heteroecious on gymnosperms and *Nothofagus* (Nothofagaceae, Fagales), or, in the case of *M. fuchsiae*, on a gymnosperm and *Fuchsia* (Onagraceae) (Crane & Peterson, 2007).

The most ancestral member of the Pucciniales recovered by Aime (2006) was *Caeoma torreyae* on a gymnosperm, *Torreya californica* (Taxaceae, Pinales). Peterson (1974) first proposed that *C. torreyae* was an ancestral rust that existed in the Mesozoic or early Cenozoic, between 66 and 250 Ma. *C. torreyae* occurs on *Torreya*, which diverged *c.* 138 Ma (Magallón *et al.*, 2013). The divergence time of the cupressophytes, which include *Torreya* and the aecial hosts of *Mikronegeria*, namely *Araucaria* and *Austrocedrus*, was estimated at 257 Ma (Magallón *et al.*, 2013).

Estimates for the age of rust fungi have varied considerably. Wingfield *et al.* (2004) estimated that rust fungi evolved as recently as 150 Ma on primitive angiosperms. This estimation was based on a set rate of nucleotide changes in the small subunit (SSU) region of ribosomal DNA. However, Aime (2006) estimated that rusts were an older group, *c.* 250 Ma, as this was similar to the ages of Araucariaceae and Taxaceae, and predated the break-up of the supercontinent Pangaea *c.* 138–160 Ma (Mao *et al.*, 2012).

A difficulty for molecular dating of fungi is the lack of fossil evidence used to calibrate divergence times for extant lineages (Berbee & Taylor, 2010). This is the case for rust fungi (Pucciniales), which are obligate pathogens and are mostly represented by fossils up to 70 Ma (Tiffney & Barghoorn, 1974; Savile, 1976). The underlying assumption of the present study is that the estimated times of host divergence, based on fossil evidence, provide a calibration point for the divergence times of parasites that share a coevolutionary relationship.

The aim of this study was to determine the evolutionary time line for the rust fungi and to date key speciation events with a molecular clock. The divergence dates of extant groups of rust fungi have never been studied with a molecular clock calibrated to definitive points in time. A Bayesian dating approach with three gene regions from ribosomal DNA and mitochondrial DNA was used to estimate the ages of monophyletic groups of rust fungi. The divergence times calculated in this study shed light on the common mechanisms for speciation in rust fungi.

Materials and Methods

Taxon selection

Representative species were selected from genera for which there are sequence data on GenBank for the large subunit (LSU) and SSU regions of ribosomal DNA (rDNA), and cytochrome c oxidase subunit 3 (CO3) of mitochondrial DNA (Table 1). Sequence data for tropical rust fungi on angiosperms obtained in a study on Australian rust fungi (Shivas *et al.*, 2014) were included and uploaded to GenBank (Table 1). These genera included *Achrotelium*, *Ceratocoma*, *Coleosporium*, *Cystopsora*, *Hemileia*, *Phragmidium*, *Sphaerophragmium*, *Thekopsora* and *Uromyces*.

Phylogenetic analyses

The SSU, LSU and CO3 sequences were aligned with the MAFFT algorithm (Katoh *et al.*, 2009) in SATe-II (Liu *et al.*, 2012). *Eocronartium* and *Helicobasidium* were selected as outgroup taxa based on the phylogenetic study by Aime (2006). The three alignments were concatenated and run as partitioned datasets with maximum likelihood and Bayesian inference as phylogenetic criteria. GTRGAMMA was specified as the model of evolution in both criteria. Maximum likelihood was implemented as a search criterion in RAxML (Stamatakis, 2014). The RAxML analyses were run with a rapid Bootstrap analysis (command -f a) using a random starting tree and 1000 maximum likelihood bootstrap replicates. A Markov chain Monte Carlo search in a Bayesian analysis was conducted with MrBayes (Ronquist & Huelsenbeck, 2003). Four runs, each consisting of four chains, were implemented for 2.6 million generations until the standard deviation of split frequencies was < 0.008. The cold chain was heated at a temperature of 0.25. Substitution model parameters were sampled every 1000 generations and trees were saved every 1000 generations. Convergence of the Bayesian analysis was confirmed using AWTY (Nylander *et al.*, 2008) (available at: ceb.csit.fsu.edu/awty/). A burn-in of 25% was used and 8000 trees were summarized for the final topology. The maximum likelihood and Bayesian analyses were run three times to test accuracy. Alignments and trees were uploaded to TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S17850).

Molecular dating analyses

Bayesian evolutionary analysis by sampling trees was implemented in BEAST 2 (Bouckaert *et al.*, 2014), which allowed estimation of the divergence times of monophyletic groups of rust fungi from their most recent common ancestors. The LSU, SSU and CO3 regions were run as separate partitions with $GTR + I + \Gamma$ and HKY as substitution site models, a gamma category count of four, and estimated parameters. A lognormal relaxed clock, which proposes that nucleotide changes occur at variable rates within lineages or in particular genes, was used as the clock model, and the Yule model was used as a tree prior for a constant birth-death, which is appropriate for trees with different species (Drummond & Bouckaert, 2015). Outgroups used in the phylogenetic analyses were excluded from the BEAST analyses as recommended by Drummond & Bouckaert (2015). A congruent topology obtained from maximum likelihood and Bayesian inference was fixed as a topology for the BEAST analyses. Two separate BEAST analyses that differed at the calibration of Pucciniales were run for 30 million generations, with trees logged every 1000 generations. Tracer v.1.6.0 (Rambaut *et al.*, 2014) was used to determine the behavior of the chains and to test the confidence of estimated parameters with the effective sample sizes (ESS). The mean node ages and 95% highest posterior density (HPD) values were summarized and annotated on the final topologies with TreeAnnotator v2.2.0 (Drummond *et al.*, 2012).

Calibration of nodes

Nodes were calibrated in BEAUTi 2 (Bouckaert *et al.*, 2014) based on mean divergence times for the hosts of rust fungi. A normal distribution was selected for the data as recommended by Ho (2007) and Drummond & Bouckaert (2015). The age of the rust node was calibrated so the mean age of host divergence was the maximum age in the 97.5% quantile of the distribution of the prior (priors are defined in Table 2 and calibrated nodes are shown in Fig. 1). This is weighted to host-tracking rather than cospeciation, and assumes a lag time between the evolution of the parasite and its host (Roy, 2001). Nodes within *Endoraecium* were calibrated based on evidence that these rusts coevolved with

Table 1 Species of rust, specimen number, host and GenBank numbers of taxa included in the analyses

Taxon	Specimen	Host of specimen	GenBank number		
			LSU	SSU	CO3
Achrotelium ichnocarpi Syd.	BRIP 55634	*Ichnocarpus frutescens*	**KT199393**	**KT199381**	**KT199404**
Caeoma torreyae Bonar		*Torreya californica*	AF522183[1]	AY123284[2]	NA
Ceratocoma jacksoniae (Henn. ex McAlpine) Buriticá & J.F. Hennen	BRIP 57762	*Davesia* sp.	**KT199394**	**KT199382**	**KT199405**
Chrysomyxa cassandrae (Gobi) Tranzschel	NA	*Picea glauca*	FJ666455[3]	NA	FJ666432[3]
Chrysomyxa ledi (Alb. & Schwein.) de Bary	DAOM 149959	*Rhododendron palustre*	FJ666468[3]	NA	FJ666445[3]
Chrysomyxa ledicola Lagerh.	NA	*Rhododendron groenlandicum*	FJ666446[3]	NA	FJ666423[3]
Chrysomyxa nagodhii P.E. Crane	NA	*Picea mariana*	FJ666461[3]	NA	FJ666438[3]
Chrysomyxa pyrolae Rostr.	NA	*Pyrola* sp.	FJ666466[3]	NA	FJ666443[3]
Coleosporium tussilaginis (Pers.) Lév.	BRIP 56944	*Senecio* sp.	**KT199395**	**KT199383**	**KT199406**
Cystopsora notelaeae Syd.	BRIP 58325	*Notelaea microcarpa*	**KT199396**	**KT199384**	**KT199407**
Dasyspora amazonica Beenken	BPI 0116382	*Xylopia amazonica*	JF263460[4]	JF263496[4]	JF263512[4]
Dasyspora echinata Beenken & Berndt	PUR N6196	*Xylopia emarginata*	JF263462[4]	JF263497[4]	JF263513[4]
Dasyspora gregaria (Kunze) Henn.	ZT Myc 3397	*Xylopia cayennensis*	JF263477[4]	JF263502[4]	JF263518[4]
Dasyspora guianensis Beenken	ZT Myc 3413	*Xylopia benthamii*	JF263479[4]	JF263503[4]	JF263519[4]
Dasyspora mesoamericana Beenken	PUR 42390	*Xylopia frutescens* var. *frutescens*	JF263480[4]	JF263504[4]	JF263520[4]
Dasyspora nitidae Beenken	ZT Myc 3409	*Xylopia nitida*	JF263484[4]	JF263505[4]	JF263521[4]
Dasyspora segregaria Beenken	PMA MP4941	*Xylopia aromatica*	JF263488[4]	JF263507[4]	JF263523[4]
Dasyspora winteri (Pazschke) Beenken	S F30078	*Xylopia sericea*	JF263492[4]	JF263508[4]	JF263524[4]
Endoraecium acaciae Hodges & D.E. Gardner	BPI 871098	*Acacia koa*	DQ323916[5]	DQ323917[5]	NA
Endoraecium auriculiforme McTaggart & R.G. Shivas	BRIP 56548	*Acacia auriculiformis*	KJ862298[6]	NA	KJ862432[6]
Endoraecium carnegiei McTaggart & R.G. Shivas	BRIP 57924	*Acacia dealbata*	KJ862301[6]	NA	KJ862435[6]
Endoraecium disparrimum McTaggart & R.G. Shivas	BRIP 55626	*Acacia disparrima*	KJ862304[6]	KJ862403[6]	KJ862437[6]
Endoraecium falciforme McTaggart & R.G. Shivas	BRIP 57583	*Acacia falciformis*	KJ862306[6]	KJ862405[6]	KJ862439[6]
Endoraecium irroratum McTaggart & R.G. Shivas	BRIP 57286	*Acacia irrorata*	KJ862312[6]	KJ862407[6]	KJ862442[6]
Endoraecium koae (Arthur) M. Scholler & Aime	BPI 871071	*Acacia koa*	DQ323918[5]	DQ323919[5]	NA
Endoraecium maslinii McTaggart & R.G. Shivas	BRIP 57872	*Acacia daphnifolia*	KJ862314[6]	KJ862408[6]	KJ862444[6]
Endoraecium parvum Berndt	BRIP 57524	*Acacia leiocalyx*	KJ862316[6]	KJ862409[6]	KJ862445[6]
Endoraecium peggii McTaggart & R.G. Shivas	BRIP 55602	*Acacia holosericia*	KJ862308[6]	NA	KJ862440[6]
Endoraecium phyllodiorum (Berk. & Broome) Berndt	BRIP 57516	*Acacia aulacocarpa*	KJ862324[6]	KJ862411[6]	KJ862447[6]
Endoraecium podalyriifolium McTaggart & R.G. Shivas	BRIP 57576	*Acacia podalyriifolia*	KJ862334[6]	KJ862414[6]	KJ862449[6]
Endoraecium tierneyi (J. Walker & R.G. Shivas) M. Scholler & Aime	BRIP 27071	*Acacia harpophylla*	KJ862335[6]	KJ862415[6]	KJ862450[6]
Endoraecium tropicum McTaggart & R.G. Shivas	BRIP 56557	*Acacia tropica*	KJ862337[6]	KJ862417[6]	KJ862452[6]
Endoraecium violae-faustiae Berndt	BRIP 55601	*Acacia aulacocarpa*	KJ862338[6]	KJ862418[6]	KJ862453[6]
Eocronartium muscicola (Pers.) Fitzp.	NA	NA	AF014825[1]	DQ241438[7]	NA
Gerwasia rubi Racib.	BRIP 58369	*Rubus* sp.	**KT199397**	NA	**KT199408**
Hamaspora acutissima P. Syd. & Syd.	BRIP 55606	*Rubus moluccanus*	**KT199398**	**KT199385**	**KT199409**
Helicobasidium purpureum (Tul.) Pat.	TUB 011542	*Carpinus betulus*	AY254180[8]	D85648[9]	NA
Hemileia vastatrix Berk. & Broome	BRIP 61233	*Coffea robusta*	**KT199399**	DQ354565[10]	**KT199410**
Hemileia sp.	BRIP 57470	Rubiaceae	**KT199400**	**KT199386**	**KT199411**
Maravalia cryptostegiae (Vestergr.) Y. Ono	BRIP 56898	*Cryptostegia grandiflora*	**KT199401**	**KT199387**	**KT199412**
Masseeëlla capparis (Hobson bis ex Cooke) Dietel	BRIP 56844	*Flueggea virosa*	JX136798[11]	NA	**KT199413**
Melampsora abietis-canadensis C.A. Ludw.	NA	*Tsuga canadensis*	FJ666512[3]	NA	FJ666542[3]
Melampsora aecidioides (DC.) J. Schröt.	NA	*Populus alba*	FJ666520[3]	NA	FJ666550[3]
Melampsora medusae f.sp. *tremuloides* Shain	NA	*Populus tremuloides*	FJ666517[3]	NA	FJ666547[3]
Melampsora pinitorqua Rostr.	NA	*Pinus sylvestris*	FJ666523[3]	NA	FJ666553[3]
Phakopsora annonae-sylvaticae Beenken	PUR 87311	*Annona sylvatica*	KF528008[12]	KF528038[12]	KF528046[12]
Phakopsora cherimoliae (Lagerh.) Cummins	NA	*Annona cherimola*	KF528011[12]	KF528040[12]	KF528048[12]
Phakopsora crucis-filii (Dianese, R.B. Medeiros & L.T.P. Santos) Beenken	ZT Myc 48990	*Annona paludosa*	KF528016[12]	KF528041[12]	KF528049[12]
Phakopsora myrtacearum McTaggart, Maier, Jol. Roux, M.J. Wingf.	PREM 61155	*Eucalyptus grandis*	KP729473[13]	NA	**KT199414**
Phakopsora pistila (Buriticá & J.F. Hennen) Beenken	ZT Myc 48992	*Annona sericea*	KF528026[12]	KF528043[12]	KF528051[12]
Phakopsora rolliniae (W.T. Dale) Beenken	ZT Myc 49000	*Annona exsucca*	KF528034[12]	KF528045[12]	KF528054[12]
Phragmidium barnardii Plowr. & G. Winter	BRIP 56945	*Rubus multibracteatus*	**KT199402**	NA	**KT199415**
Phragmidium potentillae (Pers.) P. Karst.	BRIP 60089	*Acaena novae-zelandiae*	**KT199403**	NA	**KT199416**
Puccinia lagenophorae Cooke	BRIP 57563	*Emilia sonchifolia*	KF690696[15]	**KT199388**	**KT199417**

Table 1 (Continued)

Taxon	Specimen	Host of specimen	GenBank number		
			LSU	SSU	CO3
Puccinia myrsiphylli (Thüm.) G. Winter	BRIP 57782	*Asparagus asparagoides*	**KM249854**	NA	**KT199418**
Puccinia psidii G. Winter	BRIP 57793	*Rhodamnia angustifolia*	KF318449[14]	KF318457[14]	**KT199419**
Puccinia stylidii McAlpine	BRIP 60107	*Stylidium armeria*	KJ622215[15]	**KT199389**	**KT199420**
Puccinia ursiniae R.G. Shivas	BRIP 57993	*Ursinia anthemoides*	KF690705[15]	**KT199390**	**KT199421**
Sphaerophragmium acaciae (Cooke) Magnus	BRIP 56910	*Albizzia* sp.	KJ862350[6]	KJ862429[6]	KJ862462[6]
Sphenorchidium polyalthiae (Syd. & P. Syd.) Beenken & A.R. Wood	ZT HeRB 251	*Polyalthia longifolia*	JF263493[4]	JF263509[4]	JF263525[4]
Thekopsora minima (Arthur) P. Syd. & Syd	BRIP 57654	*Vaccinium corymbosum*	KC763340[16]	**KT199391**	**KT199422**
Uromyces lomandracearum J. Walker & van der Merwe	BRIP 59022	*Lomandra* sp.	**KM249862**	**KT199392**	**KT199423**
Uromycladium acaciae (Cooke) P. Syd. & Syd.	BRIP 60092	*Acacia terminalis*	KR994853[17]	KR994932[17]	KR995046[17]
Uromycladium sp.	BRIP 59239	*Acacia mearnsii*	KR994852[17]	KR994931[17]	KR995045[17]
Uromycladium falcatarium Doungsa-ard, McTaggart & R.G. Shivas	BRIP 57447	*Falcataria moluccana*	KJ632973[18]	KJ633013[18]	KJ639059[18]
Uromycladium fusisporum (Cooke & Massee) Savile	BRIP 57526	*Acacia salicina*	KJ632991[18]	KJ633031[18]	KJ639075[18]
Uromycladium naracoortensis Berndt	MEL 2359562	*Acacia iteaphylla*	KR994880[17]	KR994958[17]	KR995071[17]
Uromycladium notabile (F. Ludw.) McAlpine	BRIP 59234	*Acacia dealbata*	KJ632992[18]	KJ633030[18]	KJ639076[18]
Uromycladium simplex McAlpine	BRIP 59214	*Acacia pycnantha*	KJ632990[18]	KJ633029[18]	KJ639078[18]
Uromycladium tepperianum (Sacc.) McAlpine	BRIP 56928	*Acacia leiocalyx*	KJ632981[18]	KJ633017[18]	KJ639073[18]
Uromycladium tepperianum	BRIP 57860	*Acacia saligna*	KJ632988[18]	KJ633027[18]	KJ639069[18]

NA, sequences were not available. [Correction added after online publication 13 October 2015: in the 'Host of specimen' column, *Pinus alba* and *Pinus tremuloides* have been corrected to *Populus alba* and *Populus tremuloides*, respectively.] GenBank numbers obtained for this study are shown in bold font. [1]T. D. Bruns & T. M. Szaro (unpublished); [2]Wingfield *et al.* (2004); [3]Vialle *et al.* (2009); [4]Beenken *et al.* (2012); [5]Scholler & Aime (2006); [6]McTaggart *et al.* (2015); [7]Henk & Vilgalys (2007); [8]Lutz *et al.* (2004); [9]S. Kuninaga (unpublished); [10]Aime (2006); [11]Liberato *et al.* (2014); [12]Beenken (2014); [13]Maier *et al.* (2015); [14]Pegg *et al.* (2014); [15]McTaggart *et al.* (2014); [16]McTaggart *et al.* (2013); [17]C. Doungsa-ard (unpublished); [18]Doungsa-ard *et al.* (2015). LSU, large subunit region of ribosomal DNA (rDNA); SSU, small subunit region of rDNA; CO3, cytochrome c oxidase subunit 3 of mitochondrial DNA.

their host species in the genus *Acacia* (McTaggart *et al.*, 2015). The divergence dates for species of *Acacia* were determined by Miller *et al.* (2013), and five calibration points were provided for *Endoraecium* and *Uromycladium* (21.2 Ma maximum age in 97.5% quantile), and species of *Endoraecium* monophyletic on subclade Botrycephaleae (7.0 Ma maximum age in 97.5% quantile), Juliflorae (11.0 Ma maximum age in 97.5% quantile) and Plurinerves (10.3 Ma maximum age in 97.5% quantile) (Table 2). The Pucciniales were calibrated to the maximum mean divergence age of angiosperms (193.76 Ma maximum age in 97.5% quantile) determined by Magallón *et al.* (2013). This calibration is based on a most recent common ancestor of rust fungi evolving on angiosperms (Hart, 1988). A second analysis was made with the Pucciniales calibrated to the divergence age of the cupressophytes, which are hosts of the most ancestral species of rust, *C. torreyae* and *Mikronegeria* spp. (Aime, 2006). The cupressophytes were calibrated between 136 and 256 Ma, which included the divergence of *Torreya* as the lowest age, and the mean age of the cupressophytes determined by Magallón *et al.* (2013) as the upper age. The .xml files are available from the corresponding author.

Results

Phylogenetic analyses

Bayesian inference and maximum likelihood recovered congruent topologies (Fig. 1). The topologies reflected the familial classification recognized by Aime (2006), who recovered eight phylogenetically supported families. There were three exceptions: the Pucciniastraceae and Sphaerophragmiaceae were recognized as distinct families, and the position of *Uromycladium* was unresolved.

Thekopsora minima was recovered sister to the Melampsoraceae, and its inclusion in the Coleosporiaceae *sensu* Aime (2006) would make this family polyphyletic. It was treated in the Pucciniastraceae *sensu* Cummins & Hiratsuka (2003). *Puccinia psidii* was recovered in Sphaerophragmiaceae *sensu* Cummins & Hiratsuka (1983), a family distinct from the Raveneliaceae as considered by more recent authors (Cummins & Hiratsuka, 2003; Wingfield *et al.*, 2004). *Uromycladium* was recovered sister to genera in Phakopsoraceae and Raveneliaceae. Cummins & Hiratsuka (2003) considered *Uromycladium* a member of the Pileolariaceae, and a monophyletic Pileolariaceae *sensu stricto* containing *Pileolaria* and *Uromycladium* was proposed by Aime (2006). Doungsa-ard *et al.* (2015) determined that *Uromycladium* was sister to Pileolariaceae, and the results of the present study indicated that it had an unresolved familial position. *Cystopsora notelaeae* and *Achrotelium ichnocarpi* were recovered in an ancestral family of rust fungi, Mikronegeriaceae. This is the first molecular evidence to determine the systematic position of these genera, which were placed in the Pucciniaceae and Chaconiaceae by Cummins & Hiratsuka (2003). *Hamaspora* and *Gerwasia* were recovered sister to *Phragmidium* in Phragmidiaceae, which all occur on members of the Rosaceae. *Ceratocoma*, which previously had an uncertain familial position, was well resolved within Pucciniaceae.

Table 2 Calibration ages, mean ages of most recent common ancestor (MRCA) and 95% highest posterior density (HPD) ranges for selected clades and taxa

Clade/taxon	Calibration (Ma)	Calibrated to divergence of angiosperms		Calibrated to divergence of cupressophytes	
		Mean age of MRCA (Ma)	95% HPD (Ma)	Mean age of MRCA (Ma)	95% HPD (Ma)
Pucciniales calibrated to mean age of angiosperms	2.5% quantile = 107, 5% quantile = 114, Median = 150, 95% quantile = 186, 97.5% quantile = 193	115.01	78.54–150.01	NA	NA
Pucciniales calibrated to divergence of cupressophytes	2.5% quantile = 136, 5% quantile = 146, Median = 196, 95% quantile = 246, 97.5% quantile = 256	NA	NA	112.92	69.55–160.95
Uromycladium	2.5% quantile = 10.8, 5% quantile = 11.6, Median = 16.0, 95% quantile = 20.4, 97.5% quantile = 21.2	16.71	12.42–21.18	16.26	11.84–20.59
Endoraecium	2.5% quantile = 10.8, 5% quantile = 11.6, Median = 16.0, 95% quantile = 20.4, 97.5% quantile = 21.2	18.52	14.54–22.57	17.96	13.92–22.03
E. auriculiformum *E. disparrimum* *E. parvum* *E. peggii* *E. phyllodiorum* *E. tropicae* *E. violae-faustiae*	2.5% quantile = 7.04, 5% quantile = 7.36, Median = 9.0, 95% quantile = 10.6, 97.5% quantile = 11.0	8.85	7.08–10.68	8.75	6.91–10.55
E. acaciae *E. koae* *E. tierneyi*	2.5% quantile = 6.34, 5% quantile = 6.66, Median = 8.3, 95% quantile = 9.94, 97.5% quantile = 10.3	7.38	5.42–9.36	7.37	5.40–9.33
E. carnegiei *E. falciforme* *E. irroratum* *E. maslinii* *E. podalyriifolium*	2.5% quantile = 1.0, 5% quantile = 1.48, Median = 4.0, 95% quantile = 6.52, 97.5% quantile = 7.0	4.88	2.94–6.91	2.70	1.60–3.84

The mean ages and 95% HPD are provided for two analyses calibrated to the divergence times of angiosperms or cupressophytes.
NA, not applicable. Ma, million yr ago.
Shortened genus *E.* refers to *Endoraecium*.

Molecular dating analyses

The BEAST analyses run with GTR + I + Γ as a nucleotide substitution model had ESS values < 200 for the posterior and prior parameters, and rates of nucleotide change when viewed in Tracer. These results were not used in the final estimate of ages because they indicate that the rates of change for GTR had low confidence.

The BEAST analyses run with HKY as a nucleotide substitution model had ESS values > 200 for all parameters when viewed in Tracer. The final topology with mean ages and 95% HPD range was obtained from the HKY analyses (Fig. 2). The 95% HPD values for discussed clades are included in Table 2.

The mean node ages were in agreement between the two analyses calibrated to the ages of angiosperms and cupressophytes (Fig. 1). The 95% HPDs were all slightly younger in the analysis calibrated to cupressophytes than that calibrated to angiosperms. The largest difference between the two calibrations occurred for the most recent common ancestor of the Puccinales. The mean age of this node was 115 Ma with 79–150 95% HPD calibrated to angiosperms, and 113 Ma with 70–161 95% HPD calibrated to cupressophytes. These ages were 43 Ma younger than the calibrated mean age for angiosperms and 94 Ma younger than the oldest calibrated age for cupressophytes.

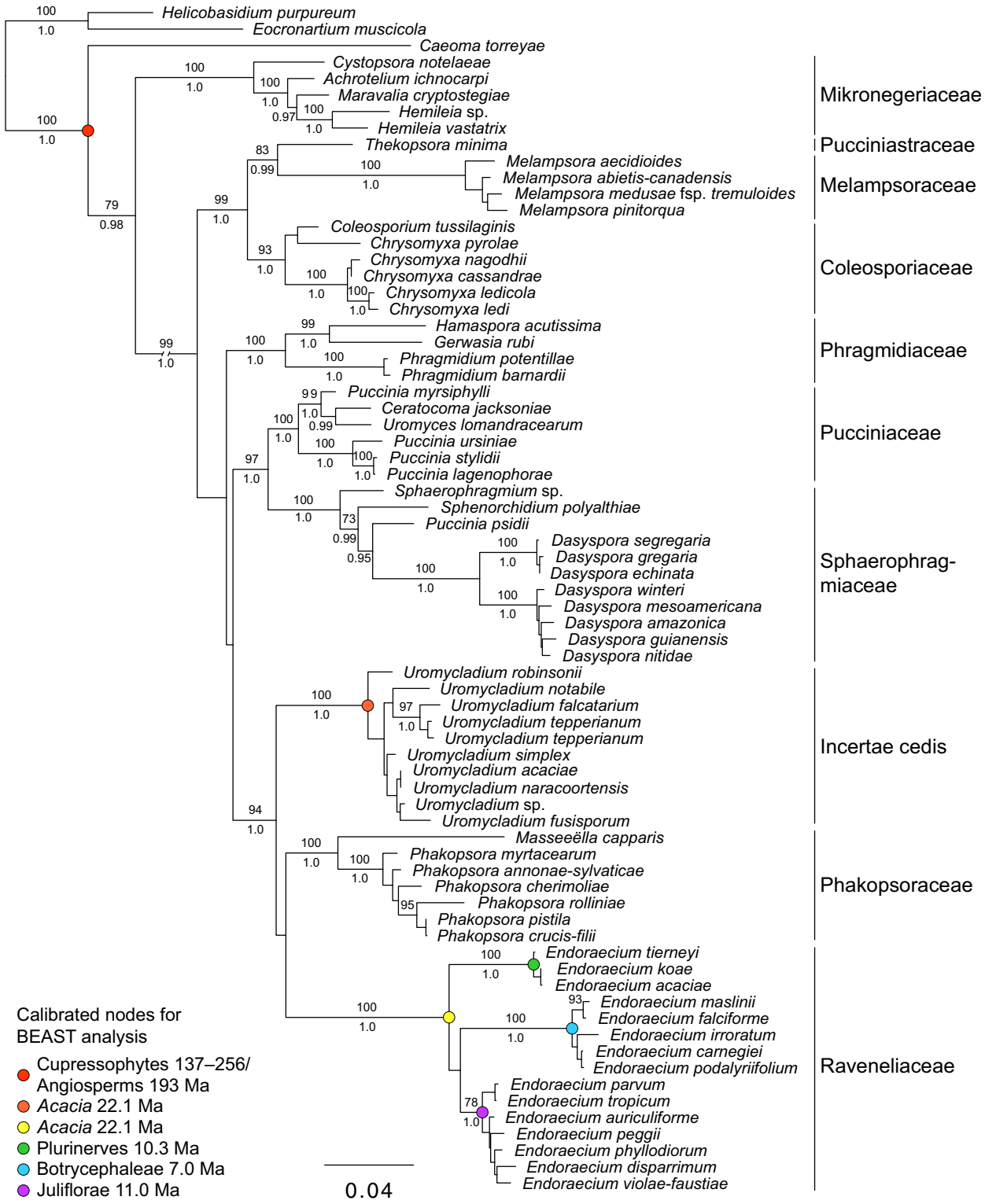

Fig. 1 Phylogram obtained from a maximum likelihood search in RAxML with the large subunit (LSU), small subunit (SSU) and cytochrome c oxidase subunit 3 (CO3) gene regions. Bootstrap values are from 1000 maximum likelihood replicates above nodes and posterior probability values are summarized from 8000 converged trees in a Bayesian search below nodes. This topology was fixed for the BEAST analyses (Fig. 2), and calibrated to the estimated divergence ages of host plants shown at selected nodes. Ma, million yr ago.

Discussion

When rust fungi first evolved and how they diversified into one of the most important groups of plant pathogens with over 8000 described species and 120 genera on ferns, gymnosperms and angiosperms has never been resolved. This is the first study in which a molecular clock has been used to estimate an evolutionary timescale for rust fungi. Our results indicate that rust fungi shared

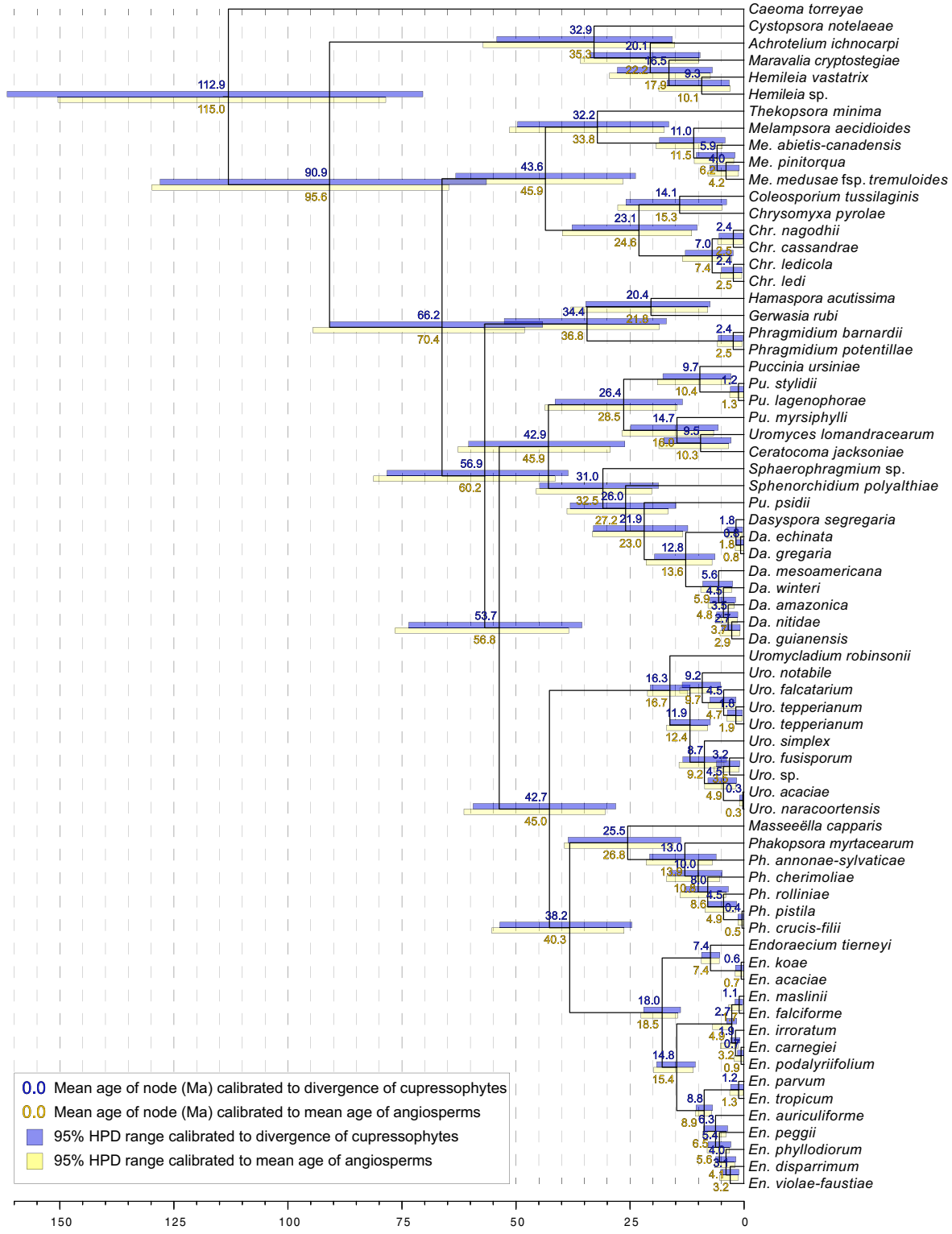

Fig. 2 Phylogram obtained from a BEAST analysis with the Pucciniales calibrated to the divergence age of the cupressophytes. The mean divergence ages calibrated to cupressophytes are above the node (blue), and those calibrated to angiosperms are below the node (yellow). The 95% highest posterior density (HPD) values calibrated to cupressophytes are above the node (blue), and those calibrated to angiosperms are below the node (yellow). Ma, million yr ago; HPD, highest posterior density.

a most recent common ancestor between 70 and 161 Ma, with a mean age of 113–115 Ma. This is more recent than most other estimates that lie between 150 and 300 Ma (Leppik, 1965; Savile, 1976; Wingfield *et al.*, 2004; Aime, 2006). This revised age provides evidence that host jumps, rather than coevolution, were the main speciation events that drove the evolution of rust fungi.

The divergence ages of 70–161 Ma for rust fungi estimated in this study reflect the host ages of ancestral plant species. For example, the gymnosperm *Torreya* diverged *c.* 137 Ma (Magallón *et al.*, 2013). Its rust, *C. torreyae*, was recovered as sister to all other species of rust and is of uncertain familial position. *Nothofagus*, the telial host of *Mikronegeria*, diverged 13–113 Ma (Sauquet *et al.*, 2012). The aecial stage of *M. fagi* and the aecial rusts *A. balansae* and *A. fragiforme* occur on Araucariaceae, which diverged *c.* 243 Ma (Magallón *et al.*, 2013). These rusts were not included in the present study, but could be ancestral in the Pucciniales, as was first proposed by Aime (2006).

The earliest probable fossil record of a rust was recorded in the Pennsylvanian period (299–323 Ma) by Tiffney & Barghoorn (1974), which is not consistent with the findings of the current study. This fossil was identified as a species of *Teleutosporites* (*Uromyces*) on *Lepidodendron*, an extinct club moss. Based on the estimated age of rust fungi here and on host taxonomy, this species could be a member of the Platygloeales (Pucciniomycotina), which parasitize species of moss and are sister to the Pucciniales (Aime *et al.*, 2006). Leppik (1965) and Hennen & Buriticá (1980) considered these fungi as extant ancestors of rust fungi, with unexpanded life cycles.

The revised age for rust fungi found in this study dictates that host jumps, rather than coevolution, were the main speciation events that drove the diversification of rust fungi on angiosperms, gymnosperms and ferns between 70 and 161 Ma. The reasoning is that the mean divergence ages of these plant hosts are between 194 and 394 Ma, and rust fungi were simply not present at that time. Hart (1988) first hypothesized that host jumps drove the diversification of rust fungi. Genera of rust fungi probably arose from host jump events and then diversified by cospeciation or taxonomically small host shifts. Strict examples of coevolution are seen in species of *Endoraecium* that infect *Acacia* (McTaggart *et al.*, 2015) and between genera in the Phragmidiaceae on hosts in Roseaceae, as shown in the present study. Host jumps were seen in genera such as *Phakopsora* (Maier *et al.*, 2015), *Puccinia* (van der Merwe *et al.*, 2008; McTaggart *et al.*, 2014) and *Uromycladium* (Doungsa-ard *et al.*, 2015), and within genera of the Mikronegeriaceae and Sphaerophragmiaceae, as seen in the present study. These findings support the hypothesis that hosts and their parasites are not always the result of long term coevolution (de Vienne *et al.*, 2013).

The results of the present study show that families, genera and species of rust fungi within the two suborders Uredinineae *sensu* Aime (2006) and Melampsorineae *sensu* Aime (2006) diverged *c.* 38–46, 22–37 and 0.3–17 Ma, respectively. This was not consistent with a study on the time tree of life (Hedges *et al.*, 2015), which estimated that families, genera and species in the Basidiomycotina diverged *c.* 111, 98 and 6 Ma, respectively (Hedges *et al.*, 2015). Hedges *et al.* (2015) determined that speciation was clock-like. However, parasites have shorter generations and can make taxonomically large host jumps, which means that less time is required for speciation.

The nature of the compatible reactions that have allowed rust fungi to make large jumps between taxonomically diverse hosts is not known. There are well known pathways of host jumps for rust fungi between pines and ferns (Savile, 1979), Ranunculales and Poales (van der Merwe *et al.*, 2008), Asterales and Cyperaceae (van der Merwe *et al.*, 2008), and Annonaceae and Myrtaceae (Maier *et al.*, 2015). Savile (1971) considered that ecological proximity was another requirement for successful host jumps. A genomic approach that compares genes in closely related species that have lost or gained a life cycle stage (e.g. rusts related by Tranzschel's law) or changed hosts may shed light on the requirements for compatible host–parasite interactions.

Rust fungi are mostly host-specific, and this has been a basic assumption for descriptions of new taxa. Molecular phylogenetic studies have shown that narrow host ranges are common for rust fungi. But there are some notable exceptions, such as in the cases of *Puccinia lagenophorae* (Scholler *et al.*, 2011) and *Uromycladium notabile* (C. Doungsa-ard, unpublished) on multiple species, and *P. psidii* on multiple genera (Pegg *et al.*, 2014). The aecial stages of heteroecious rust fungi considered in this study, namely *Coleosporium*, *Chrysomyxa* and *Thekopsora*, are confined to one or two host genera, with a wider host range observed in the telial stage (Cummins & Hiratsuka, 2003). Baum & Savile (1985) highlighted the frequency of host jumps that heteroecious rusts make when they alternate hosts each year. Perhaps there is more plasticity in the host range of rust fungi, particularly in the telial stage, and when rusts are exposed to novel host populations that have not developed resistance. An example is a plastic aecial rust, *Cronartium ribicola*, which was introduced to North America and spread to native species of *Pinus* (Kinloch, 2003).

Rust fungi are a species-rich and important group of plant pathogens worldwide. This study has shown that rust fungi evolved in a much shorter time period than was previously estimated. Host jumps explain how rust fungi have become widespread pathogens on a wide variety of plants in < 160 Ma. There are various practical implications. For example, narrow host specificity is a tacit requirement, if not assumption, for the use of rust fungi as biological control agents. In light of evidence showing that rust fungi have diversified by frequent host jumps, biological control programs may need to apply greater amounts of caution before rusts are introduced into naïve ecosystems.

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Author contributions

A.R.M. and M.J.W. planned and designed the research. A.R.M., M.A.vdN. and R.G.S. performed experiments, and analysed data.

A.R.M., R.G.S., M.A.vdN., J.R., B.D.W. and M.J.W. wrote the manuscript.

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