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Phylogenetic patterns in the Uredinales

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Abstract. Rusts (Basidiomycetes: Uredinales) are a large, diverse group of obligate biotrophic fungi that include many important plant pathogens. Phylogenetic relationships within the group are poorly understood, with between two and 14 families being recognised on morphological and host taxonomic grounds. We report the first analysis of broad-scale relationships within the Uredinales using DNA sequence data from the small subunit gene of the rRNA operon. A major split is obvious between genera that have aecial stages on gymnosperm hosts and those with this stage on angiosperms. This finding is in accord with the early taxonomic two-family treatments of the Uredinales. Within the major clades, there is evidence of incongruency with morphologically defined families. *Racospermyces*, *Maravalia*, *Hemileia* and *Caeoma* are basal to the major phylogenetic sub-division and probably represent the most ancient rust lineages. Using these sequence data, we estimate that the rusts diverged from their closest relatives around 150 million years ago.

Additional keywords: molecular phylogeny.

Traditional taxonomy of the rusts at family level has been based primarily on the morphology of fungal structures such as teliospores (Dietel 1900) and pycnia (Arthur 1925) and, to a lesser extent, on host identity and life cycle stages (autoecious/heteroecious). However, the relative paucity of useful morphological characteristics has resulted in a lack of agreement as to the number, status and membership of major clades within the Uredinales. Following early studies (Tulasne and Tulasne 1847; Tulasne 1854), detailed work by Dietel (1900, 1928) recognised two families, Melampsoraceae and Pucciniaceae, with five and 15 tribes, respectively. Subsequent workers (Arthur 1905, 1906, 1925; Gäumann 1959, 1964; Gjaerum 1974; Wilson and Henderson 1966) raised some of these tribes to family status, abandoned others and added new tribes (Leppik 1972). A recent overview has recognised 14 discrete families (Cummins and Hiratsuka 1983) that collectively contain 100-plus genera, although no attempt was made to explain phylogenetic relationships among these families. Subsequently these authors (Cummins and Hiratsuka 2003) reduced the number of families to 13 (viz.: Chaconiaceae, Coleosporiaceae, Cronartiaceae, Melampsoraceae, Mikronegeriaceae, Phakopsoraceae, Phragmidiaceae, Pileolariaceae, Pucciniaceae, Pucciniastraceae, Pucciniosiraceae, Raveneliaceae, and Uropyxidaceae) by subsuming Sphaerophragmiaceae into Raveneliaceae. While the treatment in Cummins and Hiratsuka (1983) has not been universally accepted, and various authors have subsequently moved many taxa, the treatment was used in the present study as a template as it is one of the most recent that places nearly all genera into families. The only exception to this is a number of genera that cannot currently be placed in any family due to the apparent lack of a sexual state. These rusts include some important plant pathogens such as the coffee rust fungus, *Hemileia vastatrix* (*incertae sedis*) and examples from three anamorphic genera; *Caeoma*, *Peridermium* and *Uredo*. It must, however, be noted that although this study was performed using the 1983 edition of Cummins and Hiratsuka, the 2003 edition places *Hemileia* into the family Chaconiaceae.

DNA sequence data have provided a powerful means of resolving phylogenetic relationships in the fungi (Berbee and Taylor 1999; Taylor *et al.* 2000). However, until the recent publication by Maier *et al.* (2003) the only study that cast any light on higher-level classification within the Uredinales is one aimed primarily at the higher basidiomycetes as a whole

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(Swann and Taylor 1993). In that study, the Uredinales formed a monophyletic group within the basidiomycetes but, given the small number of rust species assessed (12 species covering seven different genera), no resolution among groups was possible. Subsequent molecular phylogenetic investigations of the Uredinales have been more focused on specific genera including selected *Puccinia* spp. parasitising cereals and grasses (Zambino and Szabo 1993), species of *Cronartium* and *Peridermium* (Moricca *et al.* 1996; Vogler and Bruns 1998), *Melampsoridium* species (Kurkela *et al.* 1999) and species in the *Puccinia monoica* complex (Roy *et al.* 1998). Sjamsuridzal *et al.* (1999) presented a study aimed at determining relationships among the rusts that infect ferns, but few other members of the Uredinales were included. Most recently, Maier *et al.* (2003) have presented a phylogeny of the rust fungi. This is the most complete study to date and included nine rust families. The focus of this study was Central European rusts and did not include any tropical taxa.

The taxa included in this study comprised samples of 64 distinct species of the Uredinales representing 34 genera [31 teleomorphic (including one *incertae sedis*) and three anamorphic] and 12 families of the taxa recognised by Cummins and Hiratsuka (1983) (Table 1). More than one species was studied for 12 of the teleomorphic genera and one of the anamorphic genera. Eight genera phylogenetically closely related to rust fungi were used as outgroup taxa: *Eocronartium, Helicobasidium, Helicogloea*, *Insolibasidium*, *Leucosporidium*, *Naohidea*, *Septobasidium* and *Sporidiobolus*. Where possible, two or more species from each genus were included in the study.

DNA was isolated from small spore samples from each of the rust isolates (Table 1) in two laboratories (Canberra, ACT, Australia, and Berkeley, California, USA). Different DNA isolation and small subunit (SSU) rDNA amplification protocols were followed in each of these laboratories. In Canberra, DNA was extracted from all specimens (fresh and dried) as described by Murray and Thompson (1980) and followed by PCR amplification using universal primers (NS1 and NS4, NS3 and NS8) known to amplify basidiomycete SSU rDNA sequences (White *et al.* 1990). PCR amplicons were sequenced in both directions using the same primers that were used to amplify the fragments. Sequencing reactions were done using an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase, FS (Perkin-Elmer, Warrington, UK). DNA sequences were determined using an ABI PRISM 377 automated DNA sequencer.

In Berkeley, DNA was extracted from fresh specimens using the DNA miniprep procedure of Lee and Taylor (1990). DNA from herbarium specimens was prepared via a microprep crushing procedure as detailed by Taylor and Swann (1994). The 18S rDNA gene was then PCR amplified using the following primers: NS1, NS2, NS3, NS4, NS5, NS6, NS7, NS8, CNS26, NS19, NS20, NS21, CNS3.6R, BASID3 and MB2. The sequences of all these primers have been previously published and may be found on the Bruns Lab website at: http://plantbio.berkeley.edu/~bruns/primers.html#18s. PCR products were sequenced with the same primers in one of two ways; manual sequencing using ^{35}S labelled dCTP as described by Bruns *et al.* (1990), and automated fluorescent sequencing with a PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Perkin-Elmer Corporation, Warrington, UK), with subsequent data collection on an ABI Model 373A DNA Sequencer, as described in Kretzer *et al.* (1996).

All the DNA sequences were subjected to BLAST search to ensure plant sequence data were not accidentally included. Similarly, potentially unrelated fungal contaminants were screened out by a combination of multiple DNA isolations and placement in the phylogenetic tree. The material processed in the two laboratories was largely complementary although in a few cases the same species were sequenced. These duplicate sequences served to validate the DNA sequences of the complete dataset. In all cases where there was duplication, the most complete sequence was used in the analysis. Occasionally, duplicate sequences have been used as this strengthened the analysis.

Alignment of nucleotide sequences was performed manually. Small subunit rDNA sequence data were combined for Phylogenetic Analysis using Parsimony (PAUP) (Swofford 1998). A heuristic search was performed with gaps treated as missing, with tree-bisection-reconnection (TBR). Branches were collapsed if the maximum length was zero. The 'MulTrees' option was in effect and topological constraints were not enforced. The dataset consisted of 84 taxa and 1826 characters, 1081 characters were constant with 297 variable characters being parsimony-uninformative. The aligned dataset has been submitted to TreeBase (http://www.treebase.org/treebase/).

All sequences obtained have been submitted to GenBank together with details regarding the origin of the rust samples. DNA sequences for eight very closely related non-rust fungal genera were used as outgroup taxa (*Eocronartium, Helicobasidium, Helicogloea*, *Insolibasidium*, *Leucosporidium*, *Naohidea*, *Septobasidium* and *Sporidiobolus*). *Helicobasidium, Helicogloea* and *Leucosporidium* sequences were obtained from GenBank and SSU rDNA sequences for the remainder of the outgroup taxa were generated for this study. However, the resolution of the DNA sequence data was not sufficient to determine relationships between species in the different genera and a different region of the rust genome will need to be targeted to determine these relationships.

The phylogenetic analysis shows that the rust fungi are a closely related monophyletic group that can be divided into two distinct clades that largely reflect early taxonomic divisions of the Uredinales into two families (Dietel 1900,

Table 1. Fungal species, host of origin, host and alternate host, GenBank number, collector and collection identifier

Species	Source host if known	Autoecious or heteroecious	Aecial host	Telial host	GenBank[A]	Source or reference[A]
Caeoma torreyae	*Torreya californica* (gymnosperm)	Unknown	Gymnosperm	Unknown	AY123284	T. Szaro, University of California, USA
Chrysomyxa arctostaphyli	*Picea engelmannii* (gymnosperm)	Heteroecious	Gymnosperm	Angiosperm	AY123285	T. Szaro, University of California, USA
Coleosporium asterum 1	*Aster* sp. (angiosperm)	Heteroecious	Gymnosperm	Angiosperm	AY123286	T. Szaro, University of California, USA
Coleosporium asterum 2	*Aster* sp. (angiosperm)	Heteroecious	Gymnosperm	Angiosperm	AY123287	T. Szaro, University of California, USA
Coleosporium paederiae	*Paederia foetida*[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY125390	D. Gardner, University of Hawaii, USA
Cronartium ribicola	*Ribes* sp. (angiosperm)	Heteroecious	Gymnosperm	Angiosperm	M94338	Bruns *et al.* (1992)
Dasturella divina	*Oxytenanthera* (angiosperm)	Heteroecious	Angiosperm	Angiosperm	AY123288	T. Szaro, University of California, USA
Dietelia portoricensis 1	*Mikania micrantha*[B] (angiosperm)	Heteroecious only 0&III known[C]	Aecia not produced but spermogonia are observed	Angiosperm	AY125389	H. Evans, CABI UK [ex Costa Rica]
Dietelia portoricensis 2	*Mikania micrantha*[B] (angiosperm)	See above			AY125414	H. Evans, CABI UK [ex Mexico]
Gerwasia rubi	*Rubus alceifolius*[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY125388	T. Le Bourgeois, CIRAD
Gymnoconia nitens	Unknown	Autoecious	Angiosperm	Angiosperm	U41565	Swann and Taylor (1995)
Gymnoconia peckiana	*Rubus articus*[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY125407	L. Ericson, Umeå University, Sweden
Gymnosporangium clavipes	Unknown	Heteroecious	Angiosperm	Gymnosperm	U41566	Swann and Taylor (1995)
Gymnosporangium juniperi-virginianae	*Juniperus* sp. (gymnosperm)	Heteroecious	Angiosperm	Gymnosperm	AY123289	T. Szaro, University of California, USA
Gymnosporangium libocedri	*Calocedrus decurrens* (gymnosperm)	Heteroecious	Angiosperm	Gymnosperm	AY123290	T. Szaro, University of California, USA
Hemileia vastatrix	*Gardenia jasminoides*[B] (angiosperm)	Heteroecious	Stage not known[D]	Angiosperm	AY125422	T. Coutinho, FABI, South Africa
Hyalopsora aspidiotus	*Gymnocarpium dryopteris*[B] (fern)	Heteroecious	Gymnosperm	Fern	AY123291	L. Ericson, Umeå University, Sweden
Kuehneola uredinis	*Rubus argutus*[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY123310	D. Gardner, University of Hawaii, USA
Maravalia cryptostegiae	*Cryptostegia grandiflora*[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY125404	J.J. Burdon, CSIRO, Australia
Melampsora epitea	*Salix* sp. (angiosperm)	Heteroecious	Gymnosperm	Angiosperm	AY123293	T. Szaro, University of California, USA
Melampsora euphorbiae 1	*Euphorbia peplus*[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY125393	J.J. Burdon, CSIRO, Australia
Melampsora euphorbiae 2	*Euphorbia peplus* (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY123294	T. Szaro, University of California, USA
Melampsora hypericorum	*Hypericum* sp. (angiosperm)	Unknown	Unknown	Angiosperm	AY123311	T. Szaro, University of California, USA
Melampsora larici-populina	*Populus canadensis*[B] (angiosperm)	Heteroecious	Gymnosperm	Angiosperm	AY125395	L. Ericson, Umeå University, Sweden
Melampsora lini	*Linum marginale*[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY125396	J.J. Burdon, CSIRO, Australia
Melampsora occidentalis	*Populus* sp. (angiosperm).	Heteroecious	Gymnosperm	Angiosperm	AY123295	T. Szaro, University of California, USA
Melampsora ricini	*Ricinus communis*[B] (angiosperm)	Autoecious	Stage not known[D]	Angiosperm	AY125394	M. Priest, NSW Agriculture, Australia
Melampsora sp.	*Salix* sp. (angiosperm)	Heteroecious	Gymnosperm	Angiosperm	AY123296	T. Szaro, University of California, USA

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Table 1. (*Continued*)

Species	Source host if known	Autoecious or heteroecious	Aecial host	Telial host	GenBank[A]	Source or reference[A]
Melampsora sp.	Unknown	Unknown	Unknown	Angiosperm	AY123297	T. Szaro, University of California, USA
Melampsorella caryophyllacearum	*Stellaria uliginosa*[B] (angiosperm)	Heteroecious	Gymnosperm	Angiosperm	AY123298	L. Ericson, Umeå University, Sweden
Melampsoridium betulinum	*Betula pubescens*[B] (angiosperm)	Heteroecious	Gymnosperm	Angiosperm	AY125391	L. Ericson, Umeå University, Sweden
Melampsoridium sp.	*Betula* sp. (angiosperm)	Unknown	See above		AY123299	T. Szaro, University of California, USA
Milesina laeviuscula	*Polypodium glycyrrhiza* (fern)	Heteroecious	Gymnosperm	Fern	AY123300	T. Szaro, University of California, USA
Miyagia pseudosphaeria	*Sonchus arvensis*[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY125411	L. Ericson, Umeå University, Sweden
Nyssopsora echinata	Unknown	Autoecius? (only stages II&III known[C])	Angiosperm	Angiosperm	NU77061	Swann and Taylor (1995)
Peridermium ephedrae	*Ephedra trifurca* (gymnosperm)	Unknown	Unknown	Gymnosperm	AY123317	T. Szaro, University of California, USA
Peridermium harknessii	*Pinus contorta* (gymnosperm)	Autoecious	Gymnosperm	Unknown	M94339	Bruns *et al.* (1992)
Phakopsora jatrophicola	*Jatropha* sp.[B] (angiosperm)	Unknown	Unknown	Angiosperm	AY125398	H. Evans, CABI, UK
Phakopsora pachyrhizi 1	*Glycine max*[B] (angiosperm)	Autoecious	Stage not known[D]	Angiosperm	AY125399	J.J. Burdon, CSIRO, Australia
Phakopsora pachyrhizi 2	*Glycine max* (angiosperm)	Autoecious	Stage not known[D]	Angiosperm	AY123301	T. Szaro, University of California, USA
Phakopsora tecta	*Commelina diffusa*[B] (angiosperm)	Autoecious	Unknown	Angiosperm (uredinia only)	AY125397	D. Gardner, University of Hawaii, USA
Phragmidium barnardii	*Rubus triphyllus*[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY125406	J.J. Burdon, CSIRO, Australia
Phragmidium rubi-idaei	*Rubus idaeus*[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY125405	J.J. Burdon, CSIRO, Australia
Phragmidium tuberculatum	*Rosa* sp. (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY123313	T. Szaro, University of California, USA
Pileolaria brevipes	*Toxicodendron diversiloba* (angiosperm)	Autoecious	Unknown	Angiosperm	AY123314	T. Szaro, University of California, USA
Prospodium tuberculatum	*Lantana camara*[B] (angiosperm)	Autoecious	Stage not known[D]	Angiosperm	AY221466	H. Evans, CABI, UK [ex Brazil]
Puccinia cardui-pycnocephali	*Carduus pycnocephalus*[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY125410	J.J. Burdon, CSIRO, Australia
Puccinia graminis tritici	*Triticum aestivum*[B] (angiosperm)	Heteroecious	Angiosperm	Angiosperm	AY125409	C. Wellings, PBI, Australia
Puccinia hordei	*Hordeum vulgare*[B] (angiosperm)	Heteroecious	Angiosperm	Angiosperm	AY125412	J.J. Burdon, CSIRO, Australia
Puccinia lagenophorae	*Senecio minimus*[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY125413	J.J. Burdon, CSIRO, Australia
Puccinia menthae	*Mentha* sp.[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY123315	T. Szaro, University of California, USA
Puccinia pelargonii zonalis 1	*Pelargonium* sp.[B] (angiosperm)	Autoecious	Stage not known[D]	Angiosperm	AY125421	J.J. Burdon, CSIRO, Australia
Puccinia pelargonii zonalis 2	*Pelargonium* sp. (angiosperm).	Autoecious	Stage not known[D]	Angiosperm	AY123316	T. Szaro, University of California, USA
Pucciniastrum areolatum	*Picea abies*[B] (gymnosperm)	Heteroecious	Gymnosperm	Angiosperm	AY125392	L. Ericson, Umeå University, Sweden
Pucciniastrum epilobii 1	*Epilobium* sp.[B] (angiosperm)	Heteroecious	Gymnosperm	Angiosperm	AY123304	T. Szaro, University of California, USA
Pucciniastrum epilobii 2	*Fuchsia magellanica*[B] (angiosperm)	Heteroecious	Gymnosperm	Angiosperm	AY123303	D. Gardner, University of Hawaii, USA
Pucciniastrum goeppertianum	*?Abies grandis* (gymnosperm)	Heteroecious	Gymnosperm	Angiosperm	AY123305	T. Szaro, University of California, USA

Continued overleaf

Table 1. (*Continued*)

Species	Source host if known	Autoecious or heteroecious	Aecial host	Telial host	GenBank[A]	Source or reference[A]
Racospermyces koae	*Acacia koa*[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY125387	D. Gardner, University of Hawaii, USA
Racospermyces digitata	*Acacia koa*[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY125400	D. Gardner, University of Hawaii, USA
Tranzschelia discolor	*Prunus domestica*[B] (angiosperm)	Heteroecious	Angiosperm	Angiosperm	AY125403	J.J. Burdon, CSIRO, Australia
Triphragmium ulmariae 1	*Filipendula ulmaria*[B] (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY125401	L. Ericson, Umeå University, Sweden
Triphragmium ulmariae 2	*Filipendula ulmaria*[B]	Autoecious	Angiosperm	Angiosperm	AY125402	L. Ericson, Umeå University, Sweden
Unknown rust	*Daviesia mimosoides*[B]	Unknown	Unknown	Angiosperm	AY125408	J.J. Burdon, CSIRO, Australia
Uredinopsis sp.	*Abies concolor* (gymnosperm)	Heteroecious	Gymnosperm	Pteridosperm (all known *Uredinopsis* have stage III on ferns[C])	AY123306	T. Szaro, University of California, USA
Uredo vetus	*Selaginella* sp. (club moss)	Unknown	Unknown	Bryophyte	AY123318	T. Szaro, University of California, USA
Uromyces appendiculatus	*Phaseolus* sp. (angiosperm)	Autoecious	Angiosperm	Angiosperm	AY123307	T. Szaro, University of California, USA
Uromycladium notabile 1	*Acacia* sp.[B] (angiosperm)	Autoecious	Stage not known[D]	Angiosperm	AY125416	J.J. Burdon, CSIRO, Australia
Uromycladium notabile 2	*Acacia (trachyphloia*?)[B]	Autoecious	Stage not known[D]	Angiosperm	AY125417	J.J. Burdon, CSIRO, Australia
Uromycladium tepperianium 1	*Acacia* sp.[B]	Autoecious	Stage not known[D]	Angiosperm	AY125418	J.J. Burdon, CSIRO, Australia
Uromycladium sp.	*Acacia* sp.[B]	Autoecious	Stage not known[D]	Angiosperm	AY125415	J.J. Burdon, CSIRO, Australia
Uromycladium tepperianium 2	*Acacia* sp.[B]	Autoecious	Stage not known[D]	Angiosperm	AY125419	J.J. Burdon, CSIRO, Australia
Uromycladium tepperianium 3	*Acacia* sp.[B]	Autoecious	Stage not known[D]	Angiosperm	AY125420	J.J. Burdon, CSIRO, Australia
Eocronartium muscicola	Unidentified moss	Not in the Uredinales			AY123323	T. Szaro, University of California, USA
Helicobasidium corticioides	Unknown	Not in the Uredinales			HCU75303	E.C. Swann, D.J. McLaughlin and E.M. Frieders
Helicobasidium purpureum	Unknown	Not in the Uredinales			RHZCBS163T	S. Kuninaga
Helicogloea variabilis	Unknown	Not in the Uredinales			HVU78043	E.C. Swann, D.J. McLaughlin and E.M. Frieders
Insolibasidium deformans	*Lonicera* sp.	Not in the Uredinales			AY123292	T. Szaro, University of California, USA
Leucosporidium scottii	None – not a pathogen	Not in the Uredinales			X53499	Hendriks *et al.* (1991)
Naohidea sebacea	None – not a pathogen	Not in the Uredinales			AY123302	T. Szaro, University of California, USA
Septobasidium canescens	Quercus agrifolia	Not in the Uredinales			AY123320	T. Szaro, University of California, USA
Sporidiobolus sp.	None – not a pathogen	Not in the Uredinales			AY123321	T. Szaro, University of California, USA

[A] References to GenBank sequences: Bruns *et al.* (1992); Hendriks *et al.* (1991); S. Kuninaga [Phylogenetic analysis in *Rhizoctonia solani* based on 18S rDNA sequences (unpublished – GenBank)]; E.C. Swann, D.J. McLaughlin and E.M. Frieders [A new paradigm in basidiomycete classification as illustrated by the enigmatic sedge parasite *Kriegeria eriophori* and relatives (unpublished – GenBank)].

[B] Species for which material is held by J.J. Burdon; all other sequence information obtained from GenBank or submitted by T. Szaro.

[C] 0 stands for the spermogonial stage, II stands for the uredinial stage, III stands for the telial stage.

[D] Indicates that this stage has never been observed in this species and probably does not exist.

1928). Both clades contain heteroecious and autoecious species. An interesting feature of the smaller clade (clade 1; Fig. 1) is that it includes a number of heteroecious species with gymnosperm aecial hosts that alternate with either an angiosperm, or in the case of the genera *Hyalopsora*, *Milesina* and *Uredinopsis*, a pteridophyte as the telial host. Typical examples of heteroecious species alternating between gymnosperms and angiosperms include *Cronartium*

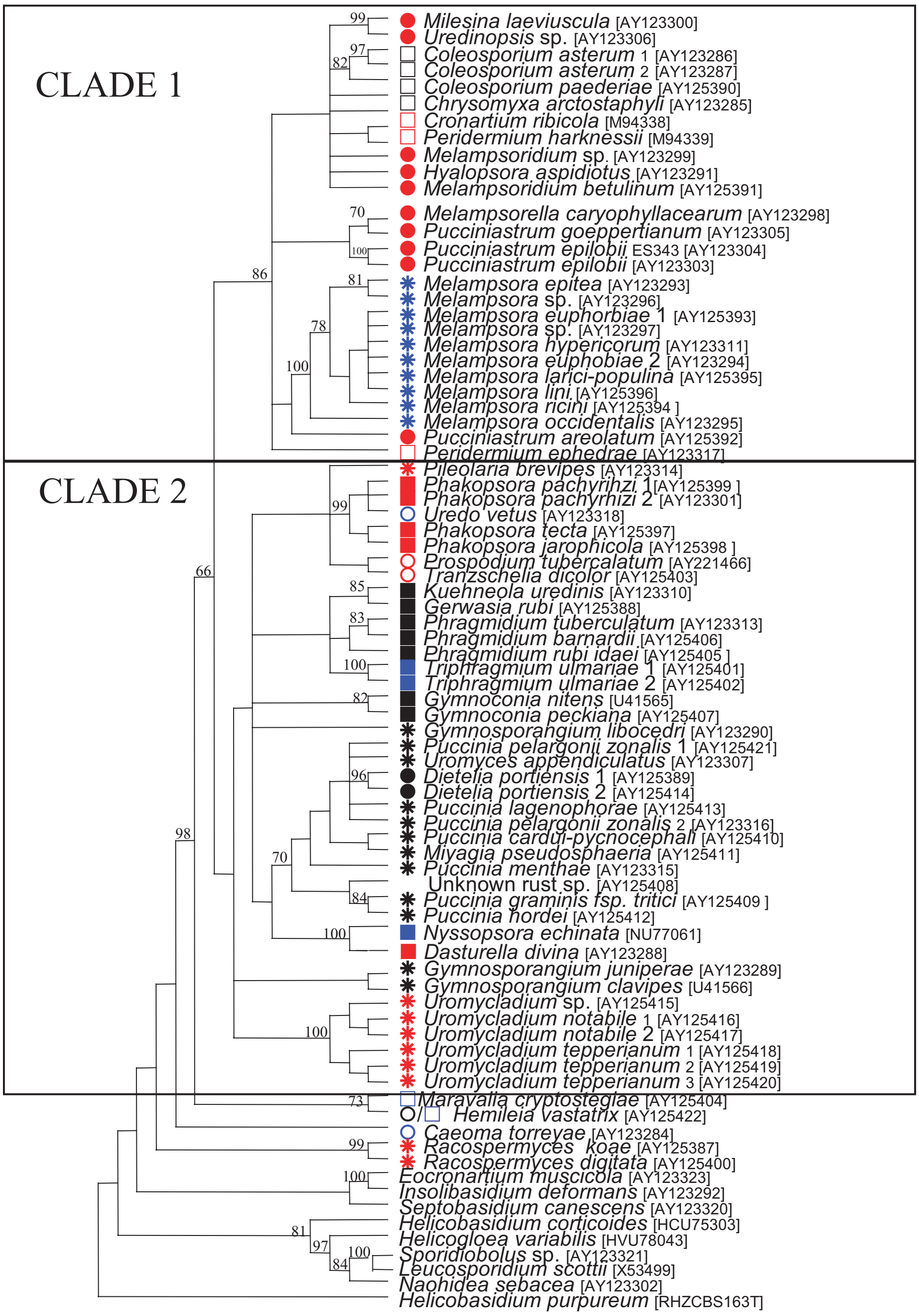

Fig. 1. A consensus phylogram of 44000 most parsimonious trees generated using the heuristic search option in PAUP from SSU sequence of the rRNA operon from 73 different rusts covering 34 genera and eight closely related genera. Bootstrap values over 66% are indicated above the branch points. GenBank numbers for all the sequences used in this study are indicated after the species name. Clades 1 and 2 are indicated by two boxes on the diagram. Symbols indicate rust family designations: ■ Phragmidiaceae, 🟧 Phakopsoraceae, 🟦 Sphaerophragmiaceae = Raveneliaceae, ■ Coleosporiaceae, 🟧 Cronartiaceae, 🟦 Chaconiaceae, ● Pucciniosiraceae, 🟠 Pucciniastraceae, 🟠 Uropyxidaceae, * Pucciniaceae, * Pileolariaceae, * Melampsoraceae, ● *Incertae sedis*, 🔵 anamorphic genera.

ribicola and *Melampsoridium betulinum* that respectively alternate between *Pinus strobus* and *Ribes* spp., and between *Larix* spp. and *Betula pubescens.* However, individual autoecious species within this clade, for example *Melampsora lini*, may complete their entire life cycle on an angiosperm host.

In marked contrast, is the larger clade containing those genera whose aecial hosts are invariably angiosperms (clade 2; Fig. 1). Within clade 2 are species that are autoecious on dicotyledons (for example *Phragmidium rubi-idaei* on *Rubus idaeus*), and others that are heteroecious alternating between monocotyledonous and dicotyledonous hosts (for example, *Puccinia graminis* f.sp. *tritici* alternating between *Triticum aestivum* and *Berberis* spp.). With the exception of the genus *Gymnosporangium*, the telial hosts of all genera in this clade are also angiosperms. *Gymnosporangium* is the only genus of rusts in which gymnosperms serve as the telial rather than the aecial host (the reverse of the pattern in clade 1). *Gymnosporangium* is also different from other conifer rusts in that its telial hosts are in the *Cupressaceae* and the aecial hosts predominantly in the *Rosaceae*. The other conifer rusts have conifer hosts (aecial) in other families of the Pinophyta. This genus is clearly unrelated to other conifer rusts and its placement within clade 2 is consistent with the notion that the telial host is phylogenetically informative.

Within the two major clades, the SSU rDNA sequence data provided substantially less resolution, although it was apparent that in both clade 1 and clade 2 there is considerable evidence of polyphyly and a need for major revision of the taxonomic boundaries of the individual families delineated by Cummins and Hiratsuka (1983). Of the four families falling within clade 1 (Coleosporiaceae, Cronartiaceae, Melampsoraceae and Pucciniastraceae), the family Melampsoraceae with its single genus *Melampsora* is clearly monophyletic while the Pucciniastraceae (represented by *Pucciniastrum, Melampsorella, Melampsoridium* and *Hyalopsora*) is apparently polyphyletic. A similar situation occurs in clade 2 in which representatives of seven of Cummins and Hiratsuka's (1983) families fall (Phakopsoraceae, Phragmidiaceae, Pileolariaceae, Pucciniaceae, Pucciniosiraceae, Sphaerophragmiaceae and Uropyxidaceae). While genera in the Pucciniaceae (*Miyagia*, *Puccinia* and *Uromyces*) grouped together reflecting their close affinities, genera from other families (*Dietelia*, and an unnamed rust from the legume genus *Daviesia*) were embedded in this grouping.

The most extreme example of the incongruence between taxonomic relationships based on morphology (Cummins and Hiratsuka 1983) and the SSU data was provided by the two genera of the family Pileolariaceae (*Pileolaria* and *Uromycladium*). These two genera are apparently unrelated based on the SSU data (Fig. 1). It is important to note that only 13 of the genera studied represent the type species and it is possible that the familial placement of the remainder could be inaccurate and thus result in the heterogeneous placement of these species in the phylogram.

In the most recent version of Cummins and Hiratsuka (2003), which was published after the completion of this study, the number of families has been reduced to 13 by recognising Sphaerophragmiaceae as being a synonym of Raveneliaceae. This fact is reflected in Fig. 1 of this study. Furthermore, the genus *Hemileia* is no longer considered *incertae sedis* but has been placed in the family Chaconiaceae. This is in agreement with the results presented in this study as the genus phylogenetically most closely related to *Hemileia* is *Maravalia*, which is considered to be in the same family.

The identity of the most evolutionarily primitive of the rusts has been the subject of argument with many authors arguing for those occurring on ferns as they occur on the most primitive of extant hosts (Arthur 1934; Gäumann 1964; Savile 1955, 1979). This argument has been countered by Leppik (1953, 1955) who asserted that while the most primitive rusts occurred on ferns, these were tropical in origin. The molecular phylogeny presented here supports part of the latter view. The rusts occurring on ferns (*Hyalopsora, Milesina* and *Uredinopsis*) all group in clade 1. In contrast, the genera *Maravalia, Hemileia* and *Racospermyces* (which are typically tropical and have non-dormant teliospores) are basal to the clade 1/clade 2 split and can, therefore, be considered to be the most ancient rust lineages. In this respect, the close relationship between *Hemileia* and *Maravalia* revealed in the phylogram (Fig. 1) confirms the connection between these two genera made by Evans (1993), and supports the proposition that these two genera are more primitive because of their simple teliospores and unexpanded or partially expanded life-cycles (Ono and Hennen 1983). An additional species, *Caeoma torreyae*, was also among the basal group of rusts (Fig. 1). The generic affinities of this species are unknown (*Caeoma* is an anamorphic genus) but are particularly intriguing given that it is the only species in the basal group with a gymnosperm host (*Torreya* sp. a member of Taxaceae). The apparently primitive nature of *C. torreyae* was commented on by Peterson (1974).

The genus *Atelocauda* was, until recently, used for two of the *Acacia* rust species used in this study. *Atelocauda incrustans*, which is the type of the genus, has been moved to the family *Raveneliaceae* (Walker 2001) while the *Acacia* species have been placed in a new genus *Racospermyces*. The familial position of the newly established genus *Racospermyces* has however, not been established. The fact that this group of fungi is unique is supported by the data presented in this study.

The sequence data generated here were added to the SSU database used to infer phylogeny and timing of fungal divergences (Berbee and Taylor 2001). Using this dataset and the calibration of 1.26% substitution per lineage per 100 Ma suggested by Berbee and Taylor (2001), we estimate that the rusts (as represented by the most primitive taxon examined here — *Racospermyces*) diverged ~150 million years ago. This is about the same time that angiosperms are thought to have evolved in the plant kingdom (Stewart and Rothwell 1993). These dates suggest that it is possible that the rusts could have evolved initially on primitive angiosperms.

This study is the most comprehensive molecular phylogeny on rusts to have been completed to date. It provides evidence that many previously hypothesised relationships within the rusts are invalid and is in general agreement with the study of Maier *et al*. (2003). More importantly, it provides more conclusive evidence as to which of the rusts are most ancient and presents a serious challenge to the idea that the most ancient rusts also have phylogenetically ancient hosts. The sequences presented in this study provide the groundwork for further studies on the phylogeny of rust fungi.

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