

Polyphyly and two emerging lineages in the rust genera *Puccinia* and *Uromyces*

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Abstract

The phylogenetic validity of *Puccinia* and *Uromyces*, *Pucciniaceae*, and closely related genera was evaluated using nucLSU rDNA sequences. Using a wide range of rust species with different life cycles and with different host specificities, *Puccinia* and *Uromyces* were shown to be highly polyphyletic and to also include representatives of the genera *Aecidium*, *Cumminsia*, *Dietelia*, *Endophyllum*, *Miyagia*, and *Uredo*. Furthermore, the structure of the phylogenetic data did not reflect previous sub-generic delimitations based on teliospore pedicel structure, but rather suggests that at least two major lineages have evolved within *Puccinia/Uromyces*: Rusts with telial states on *Poaceae* were exclusively found in one of these groupings and those with telial states on *Cyperaceae* resided in the other lineage. This might suggest that the two lineages evolved in close association with these host groups in different biomes.

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Introduction

Puccinia and *Uromyces* are by far the two largest genera of rust fungi (*Pucciniales*), currently including some 4000 and 600 described species, respectively (Cummins & Hiratsuka 2003). These genera have a world-wide distribution and they constitute the vast majority of the rust flora on all six continents (compare McAlpine, 1906, Tranzschel, 1939, Doidge, 1950, Gäumann, 1959 and Hennen et al., 2005).

Puccinia and *Uromyces* cannot be distinguished by the morphology of their spermogonia, aecia or uredinia or the respective spore types produced within these structures. Thus, the generic definitions were simply based on the number of the teliospore cells, which are one-celled in *Uromyces* and two-celled in *Puccinia*. This simplistic scheme is complicated by the fact, that there are species having both one- and two-celled teliospores and sometimes three- and four-celled spores. In these cases, the species have typically been relegated to *Puccinia*, and the one-celled teliospores have been referred to as mesospores (Gäumann 1926). For these reasons and because of the homogeneity in the morphology of the sori and spores other than teliospores, it has repeatedly been noted that *Puccinia* and *Uromyces* are not natural or monophyletic genera (Tulasne, 1854, Sydow and Sydow, 1904, Sydow and Sydow, 1910, Arthur, 1934, Guyot, 1938 and Leppik, 1959).

Arthur (1906) made the first attempt at splitting the genera *Puccinia* and *Uromyces* into smaller and more manageable taxa based solely on life-cycle characteristics. This approach was legitimately criticised by Sydow (1921) and Dietel, 1922a and Dietel, 1922b, because Dietel (1899) had previously demonstrated the phylogenetic connection between a macrocyclic (*P. coronata*) and a microcyclic rust (*P. mesneriana*) based on teliospore morphology. Details of this concept were greatly elaborated by Tranzschel (1904), and Tranzschel's law stating that microcyclic autoecious rusts can be linked phylogenetically to macrocyclic heteroecious rusts and their telia will occur on the former aecial host thus emerged. Because of Tranzschel's law, it became evident that a life-cycle based generic concept must lead to placement of very closely related species into different genera. Arthur (1934) consequently abandoned his life-cycle concept of genera and retained *Puccinia* and *Uromyces* based on their classical circumscription.

Phylogenetically, however, he treated them as if they were one big genus.

Below the genus level the robustness of the teliospore pedicel has been used to group species of both *Puccinia* and *Uromyces* (Fischer, 1904, Klebahn, 1914 and Gäumann, 1959). Arthur (1934) defined the subgenera *Bullaria* with fragile pedicels and consequently dehiscent teliospores and *Eu-Puccinia* with firm pedicels and consequently persistent teliospores, and also included *Uromyces* in this subgeneric definition.

Besides the giant genera *Puccinia* and *Uromyces*, there are various closely related satellite genera that have been described based on peculiarities of their life-cycles or teliospore morphology. Thus, *Endophyllum* (Léveillé 1826) is defined by a special life-cycle or ontogeny, in which the *Puccinia*-type aeciospores germinate to produce basidia. The same holds true for *Dietelia* (Hennings 1897), which forms part of the presumably polyphyletic *Pucciniosiraceae* that includes only endocyclic representatives. *Miyagia* (Miyabe 1913) has been treated as a discrete genus because of the presence of paraphyses around the telia. *Cumminsiella* was split from *Uropyxis*, where it had originally been accommodated due to similar teliospore morphology, because of its *Puccinia*-like spermogonial and uredinial morphology (Arthur 1933).

Contemporary evidence based on molecular phylogenetic studies using different gene regions has accumulated in support of early evidence contesting the monophyly of *Puccinia* and *Uromyces*. Thus, in an ITS-based study, it was found that *Puccinia hordei*,

which produces both one- and two-celled teliospores, is more closely related to *Uromyces scillarum* than to other *Puccinia* species “and may be closely related to *Uromyces* leaf rusts on *Hordeum*” (Zambino & Szabo 1993). With the help of nucLSU rDNA data, it was shown that both *Puccinia* and *Uromyces* are polyphyletic, but also encompass *Cumminsella* and *Endophyllum* (Maier *et al.* 2003). Likewise, using nucSSU rDNA data, it has been demonstrated that *Miyagia* and *Dietelia* belong to the monophyletic *Puccinia-Uromyces* cluster (Wingfield *et al.* 2004). The fact that *Endophyllum* is embedded in *Puccinia* and that *Puccinia* and *Uromyces* are polyphyletic could also be deduced from another phylogenetic study using ITS sequences (Wood & Crous 2005). However, none of these DNA sequence-based studies have adequately considered the broader implications of their results. This is because the questions addressed in these studies were specific to particular groups of species and none of the studies included a large number of representatives of *Uromyces* and *Puccinia*.

In this study, we consider the phylogenetic relationships between the genera *Puccinia*, *Uromyces*, *Cumminsella*, *Miyagia*, *Dietelia* and *Endophyllum* in considerably greater detail than has previously been attempted. The primary focus of interest is to find characters that correlate with natural groupings in the *Puccinia/Uromyces* complex, which eventually could help in promote a better understanding of this diverse and important group of rust fungi. Therefore, *Puccinia* and *Uromyces* species infecting a variety of plant families and displaying many different life cycle strategies were sampled.

Materials and methods

Sample collection and identification

The European samples included in this study were mainly collected by W.M., and then identified using light microscopy. Most of the southern African specimens were collected and identified by M.M. (Mennicken and Oberwinkler, 2004, Mennicken *et al.*, 2005a, Mennicken *et al.*, 2005b and Mennicken *et al.*, 2005c). Specimens that were used in this study, with additional information on host species, life-cycle, geographic origin and GenBank accession numbers can be found in Table 1.

Table 1.

Species and specimens used in the present study

Rust species	Host species	I-host	III-host	GenBank accession no.	Voucher	Geographical origin
<i>Aecidium</i> sp.	<i>Elaeagnus parvifolia</i>	<i>Elaeagnaceae</i>	?	DQ917721	WM 3523	As
<i>Chrysomyxa empetri</i>	<i>Empetrum nigrum</i>	(<i>Pinaceae</i>)	(<i>Ericaceae</i>)	DQ917750	CFB 22242	NA
<i>Cumminsiella mirabilissima</i>	<i>Mahonia aquifolium</i>	<i>Berberidaceae</i>	<i>Berberidaceae</i>	(AF426206)	TUB 014955	CE
<i>Dietelia mesoamericana</i>	<i>Mikania micrantha</i>	<i>Asteraceae</i>	–	DQ917691	IMI 393070	CA
<i>Endophyllum euphorbiae-sylvaticae</i>	<i>Euphorbia amygdaloides</i>	<i>Euphorbiaceae</i>	–	(AF426200)	HeRB C-82 in ZT	CE
<i>Endophyllum sempervivi</i>	<i>Sempervivum tectorum</i>	<i>Crassulaceae</i>	–	DQ917747	TUB 014957	CE
<i>Gymnosporangium sabinae</i>	<i>Pyrus communis</i>	(<i>Rosaceae</i>)	(<i>Cupressaceae</i>)	(AF426209)	TUB 014958	CE
<i>Miyagia pseudosphaeria</i>	<i>Sonchus cf. oleraceus</i>	–	<i>Asteraceae</i>	DQ917704	RSA 173	Saf
<i>Miyagia pseudosphaeria</i>	<i>Sonchus cf. oleraceus</i>	–	<i>Asteraceae</i>	DQ917705	RSA 125	Saf
<i>Puccinia actaeae-agropyri</i>	<i>Actaea spicata</i>	<i>Ranunculaceae</i>	<i>Poaceae</i>	DQ917746	TUB 014959	CE

Rust species	Host species	I-host	III-host	GenBank accession no.	Voucher	Geographical origin
<i>Puccinia aegopodii</i>	<i>Aegopodium podagraria</i>	–	<i>Apiaceae</i>	DQ917698	TUB 014960	CE
<i>Puccinia arenariae</i>	<i>Dianthus barbatus</i>	–	<i>Caryophyllaceae</i>	DQ917731	TUB 014961	CE
<i>Puccinia arenariicola</i> var. <i>caricis-montanae</i>	<i>Carex alba</i>	<i>Asteraceae</i>	<i>Cyperaceae</i>	DQ917686	FO 3195	CE
<i>Puccinia aridariae</i>	<i>Mesembryanthemum guerichianum</i>	?	<i>Aizoaceae</i>	DQ917724	RSA 153	SAf
<i>Puccinia aridariae</i>	<i>Aridaria noctiflora</i>	?	<i>Aizoaceae</i>	DQ917725	RSA 155	SAf
<i>Puccinia asarina</i>	<i>Asarum europaeum</i>	–	<i>Aristolochiaceae</i>	DQ917732	TUB 014962	CE
<i>Puccinia bardanae</i>	<i>Arctium lappa</i>	–	<i>Asteraceae</i>	DQ917703	TUB 014963	CE
<i>Puccinia bistortae</i>	<i>Polygonum bistorta</i>	<i>Apiaceae</i>	<i>Polygonaceae</i>	DQ917697	TUB 014964	CE
<i>Puccinia calthicola</i>	<i>Caltha palustris</i>	<i>Ranunculaceae</i>	<i>Ranunculaceae</i>	DQ917701	TUB 014965	CE
<i>Puccinia caricinia</i> var. <i>ribesii-diversicoloris</i>	<i>Carex flacca</i>	<i>Grossulariaceae</i>	<i>Cyperaceae</i>	DQ917695	TUB 014966	CE
<i>Puccinia caricina</i> var. <i>ribesii-ferrugineae</i>	<i>Carex ferruginea</i>	<i>Grossulariaceae</i>	<i>Cyperaceae</i>	DQ917694	TUB 014967	CE
<i>Puccinia caricina</i> var.	<i>Carex pendula</i>	<i>Grossulariaceae</i>	<i>Cyperaceae</i>	DQ917693	TUB	CE

Rust species	Host species	I-host	III-host	GenBank accession no.	Voucher	Geographical origin
<i>ribesii-pendulae</i>					014968	
<i>Puccinia cf. helianthi</i>	<i>Helianthus annuus</i>	<i>Asteraceae</i>	<i>Asteraceae</i>	DQ917711	WM 3524	SAf
<i>Puccinia circaeae</i>	<i>Circaea lutetiana</i>	–	<i>Onagraceae</i>	DQ917716	TUB 014969	CE
<i>Puccinia coronata</i>	<i>Rhamnus cathartica</i>	<i>Rhamnaceae</i>	<i>Poaceae</i>	DQ917741	TUB 014970	CE
<i>Puccinia dioicae</i> var. <i>dioicae</i>	<i>Carex davalliana</i>	<i>Asteraceae</i>	<i>Cyperaceae</i>	DQ917687	TUB 014971	CE
<i>Puccinia extensicola</i> var. <i>linosyridi-caricis</i>	<i>Carex humilis</i>	<i>Asteraceae</i>	<i>Cyperaceae</i>	DQ917685	TUB 014972	CE
<i>Puccinia firma</i>	<i>Carex firma</i>	<i>Asteraceae</i>	<i>Cyperaceae</i>	DQ917696	TUB 014973	CE
<i>Puccinia galeniae</i>	<i>Galenia crystallina</i>	?	<i>Aizoaceae</i>	DQ917729	RSA 162	SAf
<i>Puccinia galeniae</i>	<i>Aizoon canariense</i>	?	<i>Aizoaceae</i>	DQ917730	RSA 148	SAf
<i>Puccinia gigantea</i>	<i>Epilobium angustifolium</i>	–	<i>Onagraceae</i>	(AF426198)	TUB 014974	CE
<i>Puccinia graminis</i> f. sp. <i>tritici</i>	<i>Triticum aestivum</i>	<i>Berberidaceae</i>	<i>Poaceae</i>	(L08728)		NA
<i>Puccinia hieracii</i>	<i>Hieracium murorum</i>	–	<i>Asteraceae</i>	DQ917688	TUB 014975	CE
<i>Puccinia impatientis</i>	<i>Adoxa moschatellina</i>	<i>Adoxaceae</i>	<i>Balsaminaceae</i>	DQ917700	TUB	CE

Rust species	Host species	I-host	III-host	GenBank accession no.	Voucher	Geographical origin
					014976	
<i>Puccinia knersvlaktensis</i>	<i>Mesembryanthemum nodiflorum</i>	–	<i>Aizoaceae</i>	DQ917727	RSA 33	Saf
<i>Puccinia knersvlaktensis</i>	<i>Mesembryanthemum nodiflorum</i>	–	<i>Aizoaceae</i>	DQ917726	RSA 176	Saf
<i>Puccinia lagenophorae</i>	<i>Bellis perennis</i>	<i>Asteraceae</i>	<i>Asteraceae</i>	DQ917692	TUB 014977	CE
<i>Puccinia luzulae-maximae</i>	<i>Luzula sylvatica</i>	?	<i>Juncaceae</i>	DQ917689	TUB 014978	CE
<i>Puccinia malvacearum</i>	<i>Alcea rosea</i>	<i>Malvaceae</i>	<i>Malvaceae</i>	(AF426208)	TUB 014979	CE
<i>Puccinia menthae</i>	<i>Mentha x piperita</i>	<i>Lamiaceae</i>	<i>Lamiaceae</i>	DQ917712	TUB 014980	CE
<i>Puccinia mesembryanthemi</i>	<i>Psilocaulon leptarthron</i>	<i>Aizoaceae</i>	<i>Aizoaceae</i>	DQ917728	RSA 166	Saf
<i>Puccinia otzeniani</i>	<i>Lampranthus otzenianus</i>	<i>Aizoaceae</i>	<i>Aizoaceae</i>	DQ917742	RSA 164	Saf
<i>Puccinia oxyriae</i>	<i>Oxyria digyna</i>	?	<i>Polygonaceae</i>	DQ917735	TUB 014981	NE
<i>Puccinia poarum</i>	<i>Tussilago farfara</i>	<i>Asteraceae</i>	<i>Poaceae</i>	DQ917748	TUB 014982	CE
<i>Puccinia polygoni-</i>	<i>Persicaria amphibia</i>	<i>Geraniaceae</i>	<i>Polygonaceae</i>	DQ917702	FO 47837	CE

Rust species	Host species	I-host	III-host	GenBank accession no.	Voucher	Geographical origin
<i>amphibii</i>						
<i>Puccinia punctiformis</i>	<i>Cirsium arvense</i>	<i>Asteraceae</i>	<i>Asteraceae</i>	DQ917706	TUB 014983	CE
<i>Puccinia saxifragae</i>	<i>Saxifraga hieracifolia</i>	–	<i>Saxifragaceae</i>	DQ917734	TUB 014984	NE
<i>Puccinia senecionis</i>	<i>Senecio cacaliaster</i>	–	<i>Asteraceae</i>	DQ917699	TUB 014985	CE
<i>Puccinia senecionis-acutiformis</i>	<i>Senecio ovatus</i>	<i>Asteraceae</i>	<i>Cyperaceae</i>	DQ917690	TUB 014986	CE
<i>Puccinia silvatica</i>	<i>Taraxacum officinale</i> agg.	<i>Asteraceae</i>	<i>Cyperaceae</i>	DQ917707	TUB 014987	CE
<i>Puccinia silvatica</i>	<i>Carex brizoides</i>	<i>Asteraceae</i>	<i>Cyperaceae</i>	DQ917708	TUB 014988	CE
<i>Puccinia substriata</i>	<i>Pennisetum glaucum</i>	<i>Solanaceae</i>	<i>Poaceae</i>	DQ917743	TUB 014989	SAf
<i>Puccinia tetragoniae</i>	<i>Tetragonia echinata</i>	?	<i>Aizoaceae</i>	DQ917733	RSA 106	SAf
<i>Puccinia urticata</i> var. <i>urticae-acutae</i>	<i>Carex acuta</i>	<i>Urticaceae</i>	<i>Cyperaceae</i>	DQ917719	TUB 014990	CE
<i>Puccinia urticata</i> var. <i>urticae-acutiformis</i>	<i>Carex acutiformis</i>	<i>Urticaceae</i>	<i>Cyperaceae</i>	(AF426202)	TUB 014991	CE
<i>Puccinia urticata</i> var. <i>urticae-biporulae</i>	<i>Carex pallescens</i>	<i>Urticaceae</i>	<i>Cyperaceae</i>	DQ917717	TUB 014992	CE

Rust species	Host species	I-host	III-host	GenBank accession no.	Voucher	Geographical origin
<i>Puccinia urticata</i> var. <i>urticae-hirtae</i>	<i>Carex hirta</i>	<i>Urticaceae</i>	<i>Cyperaceae</i>	DQ917718	TUB 014993	CE
<i>Puccinia urticata</i> var. <i>urticae-inflatae</i>	<i>Carex rostrata</i>	<i>Urticaceae</i>	<i>Cyperaceae</i>	DQ917720	TUB 014994	CE
<i>Puccinia virgaureae</i>	<i>Solidago virgaurea</i>	–	<i>Asteraceae</i>	DQ917709	TUB 014995	CE
<i>Puccinia windhoekensis</i>	<i>Coccinia rehmannii</i>	<i>Cucurbitaceae</i>	<i>Cucurbitaceae</i>	DQ917710	Na 152	Saf
<i>Trachyspora intrusa</i>	<i>Alchemilla vulgaris</i> agg.	(<i>Rosaceae</i>)	(<i>Rosaceae</i>)	(AF426220)	TUB 014996	CE
<i>Uredo guerichiani</i>	<i>Mesembryanthemum guerichianum</i>	?	<i>Aizoaceae</i>	DQ917722	RSA 29	Saf
<i>Uredo guerichiani</i>	<i>Mesembryanthemum guerichianum</i>	?	<i>Aizoaceae</i>	DQ917723	RSA 153	Saf
<i>Uromyces aloes</i>	<i>Aloe arborescens</i>	–	<i>Asphodelaceae</i>	DQ917740	WM 3290	Saf
<i>Uromyces caricis-sempervirentis</i>	<i>Carex sempervirens</i>	<i>Campanulaceae</i>	<i>Cyperaceae</i>	DQ917714	GZU 10-94	CE
<i>Uromyces cf. ixiae</i>	<i>Lapeirousia</i> sp.	?	<i>Iridaceae</i>	DQ917737	RSA 211	Saf
<i>Uromyces cf. ixiae</i>	<i>Hesperantha</i> sp.	?	<i>Iridaceae</i>	DQ917736	RSA 213	Saf
<i>Uromyces dactylidis</i>	<i>Ranunculus acris</i>	<i>Ranunculaceae</i>	<i>Poaceae</i>	DQ917745	TUB 014997	CE

Rust species	Host species	I-host	III-host	GenBank accession no.	Voucher	Geographical origin
<i>Uromyces ficariae</i>	<i>Ranunculus ficaria</i>	–	<i>Ranunculaceae</i>	(AF426204)	TUB 014998	CE
<i>Uromyces gageae</i>	<i>Gagea lutea</i>	–	<i>Liliaceae</i>	(AF426208)	TUB 014999	CE
<i>Uromyces ixiae</i>	<i>Babiana tubulosa</i>	?	<i>Iridaceae</i>	DQ917738	RSA 191	Saf
<i>Uromyces ixiae</i>	<i>Babiana cf. sambucina</i>	?	<i>Iridaceae</i>	DQ917739	RSA 8	Saf
<i>Uromyces junci</i> (filed under <i>Tuberculina</i> sp.)	<i>Pulicaria dysenterica</i>	<i>Asteraceae</i>	<i>Juncaceae</i>	(AF426203)	GZU 11-98	CE
<i>Uromyces lycoctoni</i>	<i>Aconitum napellus</i>	–	<i>Ranunculaceae</i>	DQ917749	TUB 015000	CE
<i>Uromyces otaviensis</i>	cf. <i>Ipomoea verbascoidea</i>	<i>Convolvulaceae</i>	<i>Convolvulaceae</i>	DQ917715	Na 305	Saf
<i>Uromyces pisi</i>	<i>Euphorbia cyparissias</i>	<i>Euphorbiaceae</i>	<i>Fabaceae</i>	(AF426201)	TUB 015001	CE
<i>Uromyces poae</i>	<i>Ranunculus ficaria</i>	<i>Ranunculaceae</i>	<i>Poaceae</i>	DQ917744	TUB 015002	CE
<i>Uromyces scutellatus</i>	<i>Euphorbia cyparissias</i>	–	<i>Euphorbiaceae</i>	DQ917713	TUB 015003	CE
<i>Uromyces viciae-fabae</i>	<i>Vicia pannonica</i>	<i>Fabaceae</i>	<i>Fabaceae</i>	(AF426199)	TUB 015004	CE

Only the varietal epithets of the rust taxa were used in the phylogenetic trees. These varietal names also represent widely used species synonyms (see **Gäumann 1959**). GenBank accession numbers of sequences that had been published previously (**Maier et al., 2003** and **Zambino and Szabo, 1993**) are given in parentheses. Herbarium acronyms: CFB (Northern Forestry Centre, Canadian Forest Service, Edmonton, Alberta); FO (F. Oberwinkler, private herbarium); GZU (Karl-Franzens-Universität Graz, Austria); IMI (CABI Bioscience, Egham, UK) M (Botanische Staatssammlung München, Germany); PREM (Plant Protection Research Institute, Pretoria, South Africa); TUB (Eberhards-Karls-Universität Tübingen, Germany); WM (W. Maier, private herbarium) Z + ZT (Combined herbaria of the Universität Zürich and of the ETH Zürich). Na, RSA (collection numbers of collections made by Mennicken in Namibia and South Africa, respectively. These are housed in PREM and Z + ZT, and PREM and M, respectively. RSA 153 displays double infections with *Puccinia aridariae* and *Uredo guerichiani*. Host relationships of the rusts at the family level are given in the columns I-host and III-host. “I” refers to aecial host, “III” to telial host. Heteroecious rusts can be identified by two different plant family names in these two columns. Autoecious macrocyclic rusts have the same family name presented twice in these two columns. “–” refers to those species lacking an aecial host and, thus, a short-cyclic rust. “?” refers to an unknown part of the life cycle. The host relationships of the outgroup species are cited in parentheses. Acronyms used for geographical origin: As (Asia), CA (Central America), CE (Central Europe), NA (North America), NE (Northern Europe), SAf (Southern Africa).

DNA-isolation, PCR and DNA-sequencing

DNA was isolated from the rust spores that were lifted from fruiting structures on infected tissue using insect pins, under a dissecting microscope. Whenever possible these spores were taken from single rust sori to avoid contamination of possible infections by multiple rust species. Spores were crushed between two microscope slides or with the help of a tissue lyser (Retsch Mixer Mill 301, Haan, Germany) by shaking the spores in an Eppendorf tube together with a steel bead 3 mm diam for 3 min at 30 Hz. The crushed spores were subsequently suspended in lysis buffer from the Qiagen Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's protocols. PCR and direct

sequencing of both strands of the 5' end of the large subunit of the ribosomal gene cluster was performed using the primer pair NL1 and NL4, LR 0R (Moncalvo *et al.* 1995) and LR 5 or LR 6 (Vilgalys & Hester 1990). PCR, PCR product purification and cycle sequencing settings were as described previously (Maier *et al.*, 2003 and Ritz *et al.*, 2005). DNA sequence electrophoresis was done on automated DNA sequencers (ABI 373stretch and ABI PRISM 3100TM, Perkin-Elmer, Warrington, UK).

DNA-sequencing and phylogenetic analyses

Contigs of the double stranded nucleotide sequences were produced, proof-read and edited with the help of Sequencher 4.5 (Gene Codes Corporation, Ann Arbor, MI). From the obtained sequences an alignment was produced with the help of MAFFT 5.66 (Katoh *et al.* 2005) using the iterative refinement method and the following settings: the Needleman-Wunsch algorithm active, 2 tree rebuilding steps, 1000 iterations and the program's default values for gap opening and gap extension penalties. No further manual manipulation of the alignment was performed. The model of DNA substitution best fitting the data was determined with the help of the Akaike Information Criterion (Akaike 1974) implemented in Modeltest 3.7 (Posada & Crandall 1998). As a result GTR + I + G (Tavare, 1986 and Rodríguez *et al.*, 1990) was chosen for the following analyses.

Phylogenetic estimations based on the obtained alignment were derived with the help of PAUP 4.0b10 (Swofford 2001) using Neighbour Joining (NJ (Saitou & Nei 1987) and with MrBayes 3.1.1 (Ronquist & Huelsenbeck 2003), respectively, using Metropolis Coupled Monte Carlo Markov Chains (MC³) to approximate the Bayesian posterior probability distribution. Branch support for neighbour joining was determined by 1000 bootstrap replicates. MC³ was run over one million and six million generations, respectively, starting from default (flat) values for the *prior* settings. Every 100th generation was sampled resulting in 10 001 and 60 001 trees. Of these the first 2001 and 40 001 trees, respectively, were discarded as burn-in and the posterior probability was estimated from the remaining 8000 and 20.000 trees, after the chains had converged to stationarity. Because MrBayes internally runs two independent analyses at once, two independent results for both, the one and six million generations were obtained. All

phylogenetic trees were rooted with *Trachyspora intrusa*, *Gymnosporangium sabinae* and *Chrysomyxa empetri*.

Results

Data structure

The phylogenetic trees obtained are based on the D1/D2 region of the nuclear large subunit of the ribosomal genes. The final alignment contained 550 characters, of which 179 were variable and 100 parsimony informative. The final alignment is deposited at TreeBASE (SN 2941; study accession number = S1606, matrix accession number = M2889). In total 79 specimens representing 70 species were included in these analyses.

Phylogenetic trees

The tree topologies obtained by Neighbour Joining (Fig 1) and Bayesian phylogenetic inference (Fig 2) are largely congruent for supported clades. The main difference being that in the Bayesian phylogeny, many of the non-supported groups are presented as polytomies.



Fig 1. Phylogram obtained by a Neighbour Joining analysis using GTR + I + G as DNA substitution model. Bootstrap values above 60 % obtained by 1000 replicates are given

above branches. Black squares behind species names refer to persistent teliospores (“*Eu-Puccinia*”); circles refer to dehiscent teliospores (“*Bullaria*”). “A” stands for the “African clade” discussed in the text. Only the variety epithets of the rust taxa were used in this tree (see the caption of **Table 1**).

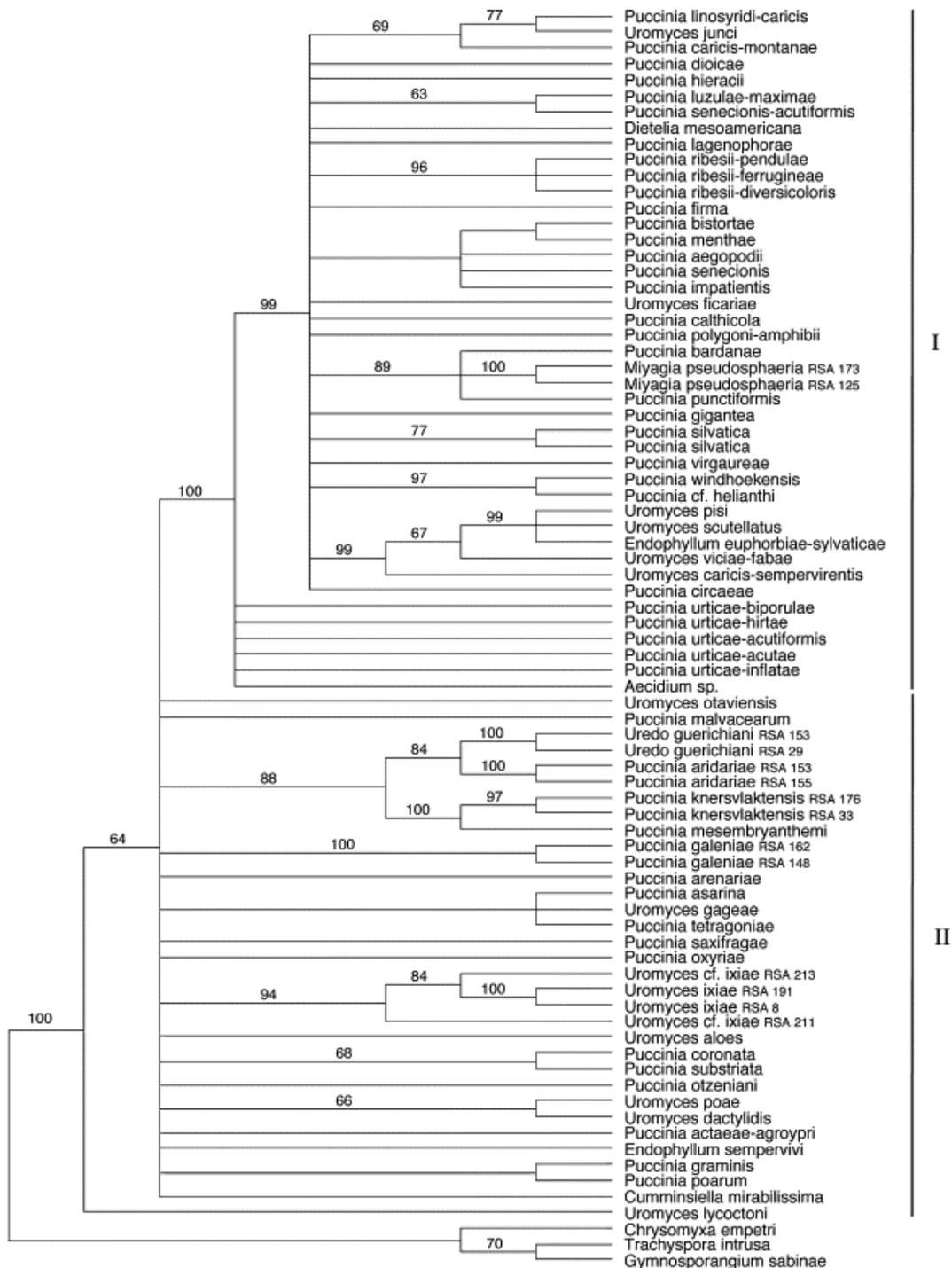


Fig 2. Majority-rule-consensus tree derived from 20000 trees sampled from the stationary phase of a Bayesian Monte Carlo Markov Chain analysis with GTR + I + G as nucleotide substitution model. *A posteriori* probabilities greater than 60 % are given above branches. Two highly supported larger groupings were obvious in both phylograms (Fig 1 and Fig 2). These are the in-group as a whole (98 % bootstrap/100 % *a posteriori* probability), comprising *Aecidium*, *Cumminsiiella*, *Dietelia*, *Endophyllum*, *Miyagia*, *Puccinia*, and *Uromyces*, and the group of species designated as cluster “I” (91 %/100 %). Only in the Bayesian analyses a large subcluster of cluster I was also highly supported (99 %). This subcluster was lacking the taxa of *Puccinia urticata* and the *Aecidium* sp. sampled. Cluster “II” is then defined as all the species that do not reside in cluster I, but belong to the supported ingroup. Cluster II is however not statistically supported as a monophyletic group itself. Representatives of *Aecidium*, *Endophyllum*, *Miyagia*, *Puccinia*, and *Uromyces* resided in cluster I, while cluster II included representatives of *Cumminsiiella*, *Endophyllum*, *Puccinia*, *Uredo*, and *Uromyces*. *Puccinia*, *Uromyces*, and *Endophyllum* were clearly polyphyletic, and *Puccinia/Uromyces* species with *Cyperaceae* and *Juncaceae* as telial hosts were found only in Group I. In contrast, species with *Poaceae* as telial hosts were found only in Group II. Species with dehiscent (“*Bullaria*”) as opposed to non-dehiscent (“*Eu-Puccinia*”) teliospores, did not correspond to either of the two large clusters accommodating the species included in this study.

Species circumscription as reflected by the phylogenetic data

In most cases where several collections of the same species were sequenced, the sequences were identical or differed only in one base pair in the gene region being studied (e.g. *Miyagia pseudosphaeria*, *Puccinia aridaria*, *Uromyces ixiae* (RSA 8, RSA 191 in the phylogenetic trees), and *Puccinia silvatica*. In the case of *P. silvatica*, sequences were obtained from both the aecial and telial hosts. In a limited number of cases, for example *Uromyces* cf. *ixiae* (RSA 211, RSA 213) compared to *Uromyces ixiae* (RSA 8, RSA 191) differences based on the DNA sequence data were found. It is probable that each of these represent separate taxa and, in this case, undescribed species.

Discussion

Results of this study provide clear evidence that *Cumminsiella*, *Dietelia*, *Endophyllum*, *Miyagia*, *Puccinia*, *Uromyces* and, at least parts of the anamorph genera *Aecidium* and *Uredo* represent a highly supported monophyletic group of genera. The phylogenetic trees emerging from comparisons of DNA sequence data, however, do not support the generic boundaries of the species that we have included in the study.

In this study, the two largest rust genera, *Puccinia* and *Uromyces*, emerged as polyphyletic. While this result was not unexpected, the degree of the polyphyly was surprisingly high. From the phylogenetic analyses, it is clear that the number of cells in the teliospore (one- versus two-celled), which is the basis of the separation between *Puccinia* and *Uromyces*, does not have phylogenetic significance. This supports the view of e.g. Anikster & Wahl (1979: 369) that *Puccinia* and *Uromyces* are “only artificially separated from each other”. Thus, the transition from either one- to two-celled teliospores, or *vice versa* or transitions in both directions must have occurred frequently within closely related groups. The taxonomic value of this character is also flawed by species displaying intermediate forms that were included in the present study. Thus, one- and two-celled teliospores are displayed by *Puccinia aridariae* and *Miyagia pseudosphaerica*, or one, two, and three-celled by *P. galeniae*, or two-, three- and four-celled teliospores by *P. knersvlaktensis*.

Phylogenetic trees showed that *Cumminsiella* clearly belongs to the *Puccinia/Uromyces* cluster, which has previously been suggested (Maier *et al.* 2003). Arthur (1933) recognized that *Cumminsiella* needed to be segregated from *Uropyxis*, which accommodated its representatives, because of its strikingly different spermogonial and aecial morphology. It then became clear that based on these characters *Cumminsiella* was very similar to *Puccinia*, from which it differs only by having two as opposed to one germ pore per teliospore. It must, however, be noted that certain *Puccinia* species also display two germ pores per cell (e.g. *Puccinia abutili* or *Puccinia cephalandrae*; Mennicken *et al.* 2005b). All representatives of *Cumminsiella* are autoecious, most of them macrocyclic and they are naturally restricted to *Mahonia* and *Berberis* in the Americas (Baxter, 1957 and McCain and Hennen, 1982). Considering these features collectively, we hypothesize that *Cumminsiella* is a monophyletic group within

Puccinia/Uromyces, because the species included in this genus are morphologically and biologically strongly homogenous.

The observation that *Miyagia* is part of *Puccinia/Uromyces* supports and enhances the findings of Wingfield *et al.* (2004). In that study, *Miyagia* clustered with representatives of *Puccinia*, *Uromyces*, and *Dietelia* with moderate support. More precisely, *M. pseudosphaeria* formed part of a cluster of autoecious *Puccinia* species parasitizing *Asteraceae* in the present study. This corresponds well with the fact that the genus *Miyagia* includes three species on *Asteraceae* and it “differs from *Puccinia* only in the peridiate [formed by palisade-like paraphyses] uredinia and telia” (Cummins & Hiratsuka 2003). Nevertheless, soral paraphyses represent a variable character within *Puccinia* species complexes (Savile, 1984 and Anikster *et al.*, 2004). This fact and the phylogenetic placement of *Miyagia* suggest that soral paraphyses are not phylogenetically useful and it is obvious that the validity of *Miyagia* should be questioned.

Consistent with observations regarding *Miyagia*, results of this study suggest that the three species of *Corbulopsora*, which also display uredinial and telial peridia and are parasitic on *Asteraceae*, will reside in the *Puccinia/Uromyces* cluster. *Corbulopsora* can be interpreted as a one-celled (“*Uromyces*-type”) variant of *Miyagia*. Cummins (1940) who erected the genus treated it under *Miyagia* in the first edition of the “Illustrated Genera of Rust Fungi” (Cummins 1959) but the genus was kept separate in the second and third editions of this work (Cummins and Hiratsuka, 1983 and Cummins and Hiratsuka, 2003).

Results of this study show that both the endo-cyclic genera, *Endophyllum* and *Dietelia*, are clearly part of *Puccinia/Uromyces*, and that *Endophyllum* is polyphyletic. *E. sempervivi* resides in the phylogenetic Group II, whereas *E. euphorbiae-sylvaticae* clustered with the heteroecious-macrocytic *U. pisi* and the microcytic *U. scutellatus* in Group I. *U. pisi* alternates between *Euphorbia* and the genera *Lathyrus* and *Pisum* (*Fabaceae*), while both *U. scutellatus* and *E. euphorbiae-sylvaticae* are short-cyclic on *Euphorbia*. This phylogenetic relationship has previously been predicted based on morphological traits and host relationships of these rust fungi by Jørstad (1952) who proposed to transfer *E. euphorbiae-sylvaticae* to *U. euphorbiae-sylvaticae*. Also from a phylogenetic point of view, *Endophyllum* as a whole would need to be included in

Puccinia/Uromyces, because it merely represents a special (endo) life cycle form of *Puccinia/Uromyces* as was clearly stated by Tranzschel (1910).

The fact that *Dietelia* is part of the *Puccinia/Uromyces* cluster is consistent with previous results based on 18SrDNA sequence data (Wingfield *et al.* 2004). Despite the fact that *Dietelia* resides in *Puccinosiraceae* as suggested by Cummins & Hiratsuka (2003) and not in the *Pucciniaceae*, this is not a surprising result, because *Dietelia* has the same spermogonial type as *Puccinia* and *Uromyces* and it is very similar to *Endophyllum*. The characters used to distinguish *Dietelia* from *Endophyllum* are subtle and include compact versus powdery aecia (aecioid telia according to the ontogenetic concept) and smooth versus ornamented aeciospores (teliospores in the ontogenetic concept) (Buriticá & Hennen 1980). Using the latter character to distinguish between the two genera was further obscured by the demonstration of verrucose aeciospores in *D. codiae* (Boerema *et al.* 1994). Following the argument already presented for *Endophyllum* and based on a phylogenetic species concept, *Dietelia* also would need to be merged with *Puccinia/Uromyces*.

It is no surprise that the sampled representatives of *Aecidium* and of *Uredo* belong to the *Puccinia/Uromyces* cluster. The vast majority of species in these anamorph genera can be expected to belong here.

Sub-generic classification and the host relationships of phylogenetic groupings

Whether teliospores are borne on fragile versus robust pedicels correlates with phylogenetic groupings was also enquired. The relatively random distribution of this feature when plotted on a phylogenetic tree (Fig 1), however, suggests that it is a variable convergent character that can change in closely related groups. The homoplasious nature of this character had been postulated by Savile, 1954 and Savile, 1971, who gave a plausible ecological-evolutionary explanation for the observation that in closely related species-groups both types of teliospore pedicels can be found. Sub-generic classifications that had been based on this character, like the sub-genera *Bullaria* and *Eu-Puccinia* (Arthur 1934) do therefore not contain phylogenetic information.

The two large groups that were found in the present study correlate with the biology of the rust species and thus might represent true sub-generic monophyla. Rusts parasitizing

Poaceae in their uredinial and telial stages were found in various sub-clusters of species residing in cluster II, but never in cluster I. In contrast, the rusts that are parasitic on *Cyperaceae* or *Juncaceae* in their uredinial and telial stages were found only in several sub-clusters of species residing in cluster I of the phylogenetic tree. This pattern is supported by a high bootstrap support for group I, but not for group II, and a comparable pattern has also emerged in another study by van der Merwe *et al.* (2007) based on other gene regions and a different species sampling. Despite lacking support for the monophyly of group II, this pattern of association could be interpreted in support of the suggestion that *Puccinia/Uromyces* radiated mainly and independently on *Poaceae* and *Cyperaceae* and *Juncaceae*, respectively, as proposed by Savile (1976). While the *Poaceae* diversified mainly in dry grassland biomes, the *Cyperaceae* and *Juncaceae* radiated in a similar fashion predominantly in wet grassland biomes. Thus, the main-diversifications of *Puccinia* and *Uromyces* could have occurred through a mixture of radiation with *Poaceae* and *Cyperaceae/Juncaceae*, respectively, and frequent jumps to co-occurring new plant hosts in the respective biomes.

Also several smaller groupings observed in the phylogenetic trees correlate with the families on which these rusts occur, while the grouping of other species indicate that host jumps are likely to have been common within *Puccinia/Uromyces*, as was postulated before (Savile, 1971, Savile, 1990 and Roy, 2001). One possible example of this intricate relationship between host specificity and host jump, can be found in the group comprising *Uromyces pisi*, *U. scutellatus*, *E. euphorbiae-sylvaticae*, *U. viciae-fabae* and *U. caricis-sempervirentis* within Group I. This group is only highly supported by the MCMC analyses (99 %), nevertheless it is monophyletic in both the MCMC and NJ trees. *U. caricis-sempervirentis* displays a host shift between *Phyteuma* (*Campanulaceae*) and *Carex* (*Cyperaceae*), while the other species in this group alternate between *Euphorbiaceae* and *Fabaceae* (*U. pisi*), are macrocyclic-autoecious on *Fabaceae* (*U. viciae-fabae*) or are short-cyclic on *Euphorbiaceae* only (*U. scutellatus*, *Endophyllum euphorbiae-sylvaticae*). The current data, however, precluded speculation as to the direction of the presumed host jump in an ancestor of this group.

Geographical patterns

Because the majority of species sampled in this study are of European origin, it is pertinent to briefly consider the clustering of species from other geographic origins with them. Within Group II, a cluster exclusively comprising southern African rust species can be found. Although not statistically supported in the NJ tree, a large part of this group is supported by the MCMC phylogram. All species in this group are parasitic on *Aizoaceae* and thus, it is not only geographic origin but also the host specificity of this group that is reflected by the phylograms. In this context, it is especially important to note that *Puccinia otzeniani*, which is also parasitic on *Aizoaceae* in southern Africa, is not part of this group. Thus, rusts on the *Aizoaceae* have originated from different lineages within group II and are only partly monophyletic. The majority of rusts sampled from southern Africa are part of Group II, which might reflect the fact that large parts of this area are dominated by grasslands and savannas, where *Poaceae* are especially frequent, and that *Cyperaceae*, more common in wet lands, are much less frequent. However, there was one southern African representative residing in Group I, *P. windhoekensis*, suggesting that its origin was from the “*Cyperaceae*-rust group”.

This is the first study based on a considerable taxon sampling using species from a broad range of host families and different geographic origins that has attempted to explore the phylogenetic structure of *Puccinia* and *Uromyces* and satellite genera. Intriguing phylogenetic patterns have emerged from the analyses including some that might have been expected and others that are surprising. Nonetheless, the polytomies in the Bayesian consensus tree and many statistically unsupported groupings in general show that various results of this study must be regarded as preliminary. The observed polytomies can be interpreted as reflection of the fact that less than 2 % of the 4500 or so species residing in *Puccinia/Uromyces* have been sampled. In addition, it is important to consider that the phylogeny is based on sequences of a single gene region, and the value of this gene region especially lies in detecting larger phylogenetic lineages within *Puccinia/Uromyces*. For these and for practical reasons no name changes have been attempted at this stage. However, the results should serve as a basis for further studies and for large-scale collaborations that will be necessary to address the questions raised here in more detail.

Addendum

Additional evidence to support this study is the research presented by van der Merwe *et al.* (2007). Their study came to our attention only after the experimental part of the present study had been completed. They observe the same major groupings as we report in the present study. However, van der Merwe *et al.* (2007) used different gene regions and a different subset of species. As the two studies reflect similar results, we have chosen to submit both studies simultaneously in order that they would be published in the same journal issue.

During the review process of the present paper, a combined nuc rDNA SSU/LSU study was published dealing with higher-level relationships of the rust fungi (Aime 2006). This study provides additional support for the view that *Aecidium*, *Cumminsia*, *Dietelia*, *Miyagia*, *Puccinia* and *Uromyces* have a common origin. *Puccinosira* and *Sphenospora* also formed part of that clade, and *Puccinia* and *Uromyces* were again shown to be polyphyletic.

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Appendix. Supplementary material



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