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FULL PAPER

Les J. Szabo

Deciphering species complexes: *Puccinia andropogonis* and *Puccinia coronata*, examples of differing modes of speciation

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Abstract Species of macrocyclic, heteroecious grass rusts often have been defined with wide host ranges and variation in spore morphology. Consequently, some are species complexes and contain genetically distinct forms. Molecular analyses, together with morphological and biological methods, provide powerful means to dissect these complexes. *Puccinia coronata* is a complex species that has a broad telial host range including more than 45 genera of grasses and a narrow aecial host range. Phylogenetic analysis of nuclear ribosomal internal transcribed spacer (ITS) DNA sequences from 15 aecial and telial collections grouped *P. coronata* into six distinct clades supporting separation of this complex into four distinct species. *Puccinia andropogonis*, a common rust of tall prairie grasses in North America, is also a complex species. However, in contrast to *P. coronata, P. andropogonis* has a narrow telial host range and a broad aecial host range. DNA sequence analysis grouped 15 collections of *P. andropogonis* into six distinct clades representing at least four distinct species. Speciation of *P. coronata* appears to have occurred primarily by radiation onto new telial hosts, whereas in *P. andropogonis* speciation appears to have occurred primarily by radiation onto new aecial hosts.

Key words Evolution · ITS · Phylogenetics · Species complex · Uredinales

Introduction

Phylogenetic relationships within and among rust species have historically been inferred based on range of host species and spore morphology; however, both approaches are problematic. Five spores stages may be produced in the life cycle of macrocyclic heteroecious grass rust, but relatively few morphological characters usually delineate species on a given family of hosts (Baum and Savile 1985). Identifying host range on telial and aecial hosts is difficult, and in many cases the aecial hosts are unknown. For rusts of cereals and grasses, this has resulted in broadly defined species. Many are described as having a large number of telial and/or aceial hosts, and species descriptions may include rather broad morphological variation (Arthur 1934; Cummins 1971).

Puccinia coronata Corda is widely distributed around the globe and is the causal agent of crown rust, an economically important disease on cereal and grass hosts. The common name, crown rust, is derived from the crown-like appendages on the apex of the teliospore. *Puccinia coronata* has a broad telial host range, including more than 45 genera of grasses, and a relatively narrow aecial host range, primarily on species of *Rhamnus* (Rhamnaceae) but also of *Berchemia* (Rhamnaceae) and *Elaeagnus* (Elaeagnaceae) (Cummins 1971; Urban and Marková 1994). *Puccinia coronata* has been subdivided into *formae speciales* based on telia host range (Eriksson and Henning 1894; Eriksson 1908; Brown 1937; Peturson 1954), leading to 13 subgroups. *P. coronata* f. sp. *avenae* on *Avena sativa* (oat) is the most economically important. However, overlap in host range among *formae speciales* has led to confusion (Eshed and Dinoor 1981). Others have used both morphological characters and host range to subdivide *P. coronata* into varieties; Cummins (1971) recognized five and Urban and Marková (1994) recognized four.

In contrast to *P. coronata*, the grass rust, *P. andropogonis* Schw., has a relatively narrow telial host range and broad aecial host range. Telial hosts of *P. andro-pogonis* include *Andropogon gerardii* (big bluestem) and *Schizachyrium scoparis* (little bluestem), primary grass species in North American tall-grass prairies. Aecial hosts are found in seven diverse host families (Fabaceae, Hippocostanaceae, Oxalidaceae, Polygalaceae, Rutaceae, Santalaceae, and Scrophulariacea) in a total of 17 host genera (Arthur 1934; Cummins 1971). A common characteristic of the tall-grass

L.J. Szabo (✉)
Cereal Disease Laboratory, USDA Agricultural Research Service, Department of Plant Pathology, University of Minnesota, 1551 Lindig Street, St. Paul, MN, USA
Tel. +1-612-625-3780; Fax +1-651-649-5055
e-mail: lszabo@umn.edu

Table 1. Rust collections used in this study

Rust, host	Collections[a]	Location	GenBank accession no.
Puccinia andropogonis			
Telial host			
Andropogon gerardii	HSZ0027	USA, Wisconsin, Fish Lake	DQ344518
	HSZ0219	USA, Wisconsin, Fish Lake	DQ344517
	HSZ0224	USA, Minnesota, CCNHA	DQ344512
	HSZ0237	USA, Minnesota, CCNHA	DQ344511
	HSZ0574	USA, Wisconsin, Fish Lake	DQ344515
	HSZ0576	USA, Wisconsin, Fish Lake	DQ344514
Schizachyrium scoparis	HSZ0217	USA, Wisconsin, Grantsburg	DQ344507
	HSZ0225	USA, Minnesota, Afton	DQ344505
	HSZ0562	USA, Minnesota, Afton	DQ344510
Aecial host			
Castilleja coccinea	HSZ0388	USA, Minnesota, Afton	DQ344509
	HSZ0389	USA, Minnesota, Afton	DQ344508
Comandra umbellata	HSZ0263	USA, Wisconsin, Fish Lake	DQ344513
Lupinus perennis	HSZ0264	USA, Wisconsin, Fish Lake	DQ344519
Penstemon gracilis	HSZ0262	USA, Wisconsin, Fish Lake	DQ344506
Zanthoxylum americanum	HSZ0265	USA, Wisconsin, Fish Lake	DQ344516
Puccinia coronata			
Telial host			
Arrhenatherum elatius	PRC 190	Czech Republic, Bohemia	DQ355443
Avena sativa	CDL 93MN437	USA, Minnesota	AY114290
Bromus erectus	PRC 194	Slovakia	DQ355449
	PRC 196	Czech Republic, Bohemia	DQ355450
B. inermis	HSZ1400	USA	DQ355448
	HSZ1401	USA	DQ355446
Calamagrostis canadensis	CDL 91WI9526	USA, Wisconsin	L08694[b]
Elytrigia repens	CDL 73MN873	USA, Minnesota	DQ414723
	CDL MN01	USA, Minnesota	DQ414724
Holcus lanatus	PRC 200	Ireland	DQ355444
Lolium perenne	PRC 203	Czech Republic, Bohemia	DQ355441
Aecial host			
Rhamnus catharticus	HSZ0757	USA, Minnesota	DQ355445
	HSZ0760	USA, Minnesota	DQ355447
	HSZ0761	USA, Minnesota	DQ355442
	HSZ1309	USA, New York	DQ355452
R. saxatilis	PRC 247	Slovakia	DQ355451

[a] Prefix designations and sources for collections: CDL, USDA ARS Cereal Disease Laboratory; HSZ, L.J. Szabo; PRC, J. Marková, Charles University, Prague, Czech Republic
[b] Zambino and Szabo (1993)

prairie is the rich diversity of plant species where several telial and aecial hosts are often found in close proximity. Arthur (1934) subdivided *P. andropogonis* into nine varieties based primarily on aecial hosts but also on the number and placement of uredi-niospore germ pores. More recently, Cummins (1953) proposed four variants of *P. andropogonis* based on urediniospore morphology.

DNA sequence analysis of nuclear ribosomal internal transcribed spacer (ITS) region has been used to resolve phylogenetic relationships among closely related rust fungi (Zambino and Szabo 1993; Pfunder et al. 2001; Vogler and Bruns 1998; Weber et al. 2003). Phylogenetic analysis of the *P. coronata* complex (based on partial ITS sequence data) resulted in three distinct groups: *Calamagrostis* type, *Avena*/*Lolium* type, and *Elytrigia repens* (=*Agropyron repens*) type (Zambino and Szabo 1993). To expand on this study, the complete ITS region was cloned and sequenced from representatives of two of these three groups as well as from additional collections of *P. coronata*. A parallel study was carried out on collections of the *P. andropogonis* complex.

Materials and methods

Collections

The rust collections used in this study, their hosts, and their geographical origins are listed in Table 1. Fifteen samples of *Puccinia andropogonis* were collected from the northern plains of the United States (Minnesota and Wisconsin) in sites representing both disturbed sites and natural habitats. Fifteen samples of *P. coronata* were collected from central United States and Europe in sites representing cultivated fields, disturbed sites, and natural habitats.

Polymerase chain reaction and DNA sequencing

DNA was extracted from either dried urediniospores collected from infected host material or from dried infected host leaf tissue as described by Anikster et al. (2004). In some cases, an OminiPrep (Genotech, St. Louis, MO, USA) DNA extraction kit was used instead of the CTAB DNA

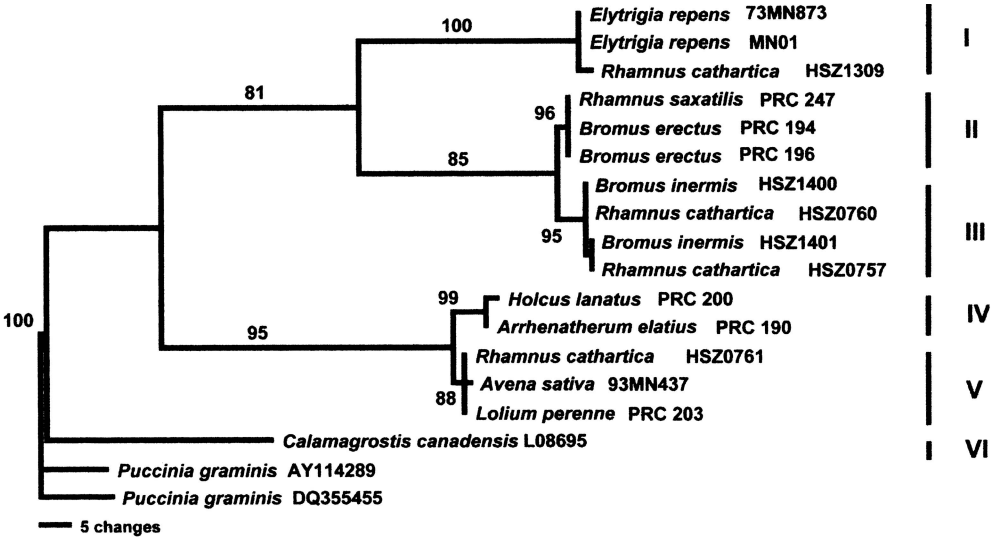

Fig. 1. Parsimony tree from the analysis of nuclear ribosomal internal transcribed spacer (ITS) region sequence data of *Puccinia coronata*. Phylogenetic analysis resulted in three optimal trees, each with a tree length of 185 steps (CI = 0.9081, HI = 0.0919, RI = 0.9424, and RC = 0.8518), one of which is shown. *Numbers above or below branches* indicate percentage of congruent clusters in 1000 bootstrap trials, and only values above 80% are shown. Clades are indicated along the *right-hand side*. Two DNA sequences of *P. graminis* (AY114289, DQ355455) were used as an outgroup

extraction method. Nuclear ribosomal internal transcribed spacer (ITS) region and the 5′-end of the large subunit were amplified using polymerase chain reaction (PCR), and amplification products were cloned (Anikster et al. 2004). Primer pairs used for amplification were ITS1F (Gardes and Bruns 1993) and RUST1 (Kroop et al. 1995). DNA sequencing reactions were performed using a Thermo Sequenase Primer Cycle sequencing kit (GE Healthcare Bio-Sciences, Piscataway, NJ, USA) and analyzed on an automated DNA sequencer (LI-COR, Lincoln, NE, USA). At least three clones were sequenced for each sample, and the DNA sequence was assembled and edited with Sequencer (Gene Codes, Ann Arbor, MI, USA).

Alignment and phylogenetic analysis

DNA sequences were initially aligned using the program Clustal W (Thompson et al. 1994) and then hand edited using the multiple sequence editor in MacVector (version 7.2.3; Accelrys, San Diego, CA, USA). Phylogenetic analysis of the data sets included the complete ITS1, 5.8S, and ITS2 region (*P. andropogonis,* 700 characters, 1–719 minus 191–194 and 235–249; *P. coronata*, 649 characters) using an heuristic parsimony program (PAUP version 4.04b10; Swofford 2001) with random stepwise addition option with 10 replicas. Support for the nodes of the trees was determined by analysis of 1000 bootstrap replicas. DNA sequence alignments and trees have been submitted to TreeBASE.

Results

Puccinia coronata

Approximately 1250 bp was sequenced for each sample including the 3′-end of the nuclear ribosomal, the complete ITS region (ITS1, 5.8S, and ITS2), and the 5′-end of the 28S providing an aligned sequence of 1308 characters. Variation in the 5′-end of the 28S was low and therefore not included in the phylogenetic analysis. Of the 649 aligned characters analyzed, 508 were constant, 34 were variable but uninformative, and 107 characters were parsimony-informative. Parsimony analysis of ITS sequence data from 15 collections resulted in three optimal trees, each with a tree length of 185 steps, one of which is shown in Fig. 1. Only minor variation of the terminal structure occurred between the three trees.

DNA sequences from 15 collections of *P. coronata* grouped into six well-supported clades with bootstrap values of 88% or greater. Clade I consisted of DNA sequences from one collection of *Rhamnus catharticus* (aecial host, HSZ1309) and two collections of *Elytrigia repens* (73MN873 and MN01), collected from the United States. All three sequences were nearly identical. Clade II consisted of one collection from *R. saxatilis* (aecial, PRC 247) and two from *Bromus erectus* (telial, PRC 194, PRC 196), collected from the Czech Republic and Slovakia. All three sequences were identical. Clade III consisted of two collections from *R. catharticus* (aecial host, HSZ0757 and HSZ0760) and two from *Bromus inermis* (telial host, HSZ1400 and HSZ1401), collected from the United States. Clades II and III are closely related and formed a well-supported branch with 85% bootstrap value. Clade IV consisted of one collection from *Arrhenatherum elatius* (telial host, PRC 190) and one from *Holcus lanatus* (telial host, PRC 200) collected from the Czech Republic and Ireland, respectively. The two sequences were nearly identical. Clade V consisted of collections from *R. catharticus* (aecial host, HSZ0761), *Avena sativa* (telial host, 93MN437), and *Lolium perenne* (telial host, PRC 203) collected from the United States and the Czech Republic. All three sequences were nearly identical. Clades IV and V are closely related and formed a well-supported branch (95% bootstrap). Clade VI consisted of the partial ITS sequence of *P. coronata* collected from the telial host *Calamagrostis canadensis* used in the analysis by Zambino and Szabo (1993).

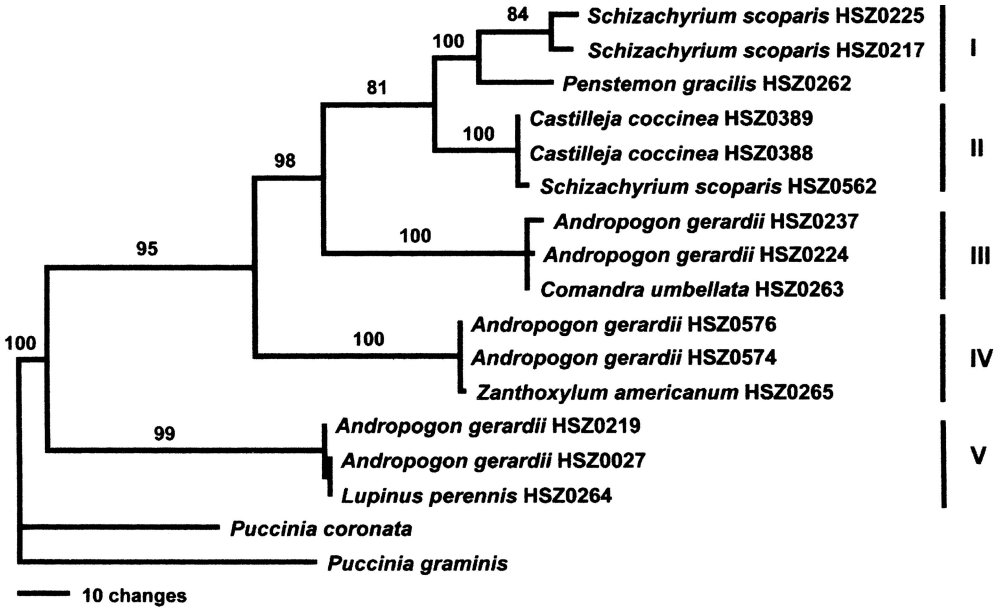

Fig. 2. Parsimony tree from the analysis of nuclear ribosomal ITS region sequence data of *Puccinia andropogonis*. Phylogenetic analysis resulted in four optimal trees, each with a tree length of 288 steps (CI = 0.8785, HI = 0.1215, RI = 0.9081, and RC = 0.7978), one of which is shown. *Numbers above or below branches* indicate percentage of congruent clusters in 1000 bootstrap trials, and only values above 80% are shown. Clades are indicated along the *right-hand side*. DNA sequences of *P. coronata* (AY114290) *P. graminis* (DQ344520) were used as an outgroup

Puccinia andropogonis

Approximately 1250 bp was sequenced for each collection, including the 3′-end of the nuclear ribosomal, the complete ITS region (ITS1, 5.8S, and ITS2), and the 5′-end of the 28S. Variation in the 5′-end of the 28S was low, and therefore these data were not included in the phylogenetic analysis. Of the 700 aligned characters analyzed, 507 were constant, 74 were variable but parsimony-uninformative, and 119 characters were informative. Parsimony analysis of the ITS sequence data from 15 collections resulted in four optimal trees, each with a tree length of 288 steps, one of which is shown in Fig. 2. Only minor variation on the terminal structure occurred among the four trees.

DNA sequences from 15 *P. andropogonis* collections grouped into five well-supported clades with bootstrap values of 99% or greater. All collections were made from the United States in two states (Minnesota and Wisconsin). Clade I consisted of one collection from *Penstemon gracilis* (aecial host, Scrophulariacea, HSZ0262) and two collections from *Schizachyrium scoparis* (telial host, HSZ0217 and HSZ0225). All three sequences were similar but showed variation between the collections, with the *P. gracilis* sequence being the most different. Each of the collections was from a different site. Clade II consisted of two collections from *Castilleja coccinea* (aecial host, Scrophulariacea, HSZ0389 and HSZ0388) and one collection from *S. scoparis* (telial host, HSZ0562). The three collections were from a single site (Afton, Minnesota, USA), the only site in which *C. coccinea* was found infected with *P. andropogonis.* All three sequences were nearly identical. Clade III consisted of one collection from *Comandra umbellata* (aecial host, Santalaceae, HSZ0263) and two collections from *Andropogon gerardii* (telial host, HSZ0224 sand HSZ0237). All three sequences were nearly identical. Clade IV consisted of one collection from *Zanthoxylum americanum* (aecial host, Rutaceae, HSZ0265) and two collections from *A. gerardii* (telial host, HSZ0574 and HSZ0576). All three sequences were nearly identical. The three collections came from the same site (Fish Lake, WI, USA). Eight of the 15 collections came from this site and are grouped into four of the five clades. Clade V consisted of one collection from *Lupinus perennis* (aecial host, Fabaceae, HSZ0264) and two collections from *A. gerardii* (telial host, HSZ0027 and HSZ0219). The three collections came from the same site (Fish Lake, WI, USA). All three sequences were nearly identical.

Discussion

Phylogenetic analysis of the complete ITS region clearly demonstrated that *P. coronata* is a species complex consisting of at least six distinct clades (see Fig. 1). This result confirms the analysis by Zambino and Szabo (1993), which separated *P. coronata* into three distinct groups composed of collections from *Calamagrostis canadensis* (clade VI), *Elytrigia repens* (=*Agropyron repens*, clade I), and *Avena sativa* and *Lolium perenne* (clade V). The latter group was previously shown to also include collections from *Alopecurus aequalis* and *Festuca elatior*. The three new groups consist of collections from the telial hosts *Holcus lanatus* and *Arrhenatherum elatius* (clade IV), *Bromus erectus* (clade II), and *Bromus inermis* (clade III). Four of the six clades also contain collections from *Rhamnus* aecial hosts, providing the first molecular confirmation of the linkage between telial and aecial hosts.

Recently, collections of *P. coronata* from *B. inermis* (clade III) were described and shown to be distinct from

other *formae speciales* and varieties based primarily on host range, teliospore morphology, and being self-fertile (Delgado et al. 2001; Anikster et al. 2003). This new morphotype was provisionally designated *P. coronata* f. sp. *bromi* sensu Mühlethaler by Delgado et al. (2001) and has only been found in North America. In Europe, crown rust (*P. coronata* var. *coronata*) has been described on several species of brome grass, including *B. erectus, B. inermis*, and *B. ramosus* (Urban and Marková 1994). The genetic connection between aecia on *R. cathartica* and uredinia/telia on *B. erectus* and *B. inermis* has been demonstrated (reviewed by Urban and Marková 1994). Two European collections of *P. coronata* from *B. erectus* formed a distinct but closely related clade (II) to *P. coronata* f. sp. *bromi* sensu Mühlethaler from *B. inermis* (clade III). The teliospores of the collections from *B. erectus* have shorter apical projections (Y. Anikster, personal communication) than *P. coronata* f. sp. *bromi*. The molecular results presented here combined with morphological data suggest that the crown rust on *Bromus* is a separate species (*P. coronata* species 1, PcSP1) and should be subdivide into at least two subspecies. Molecular and host range studies indicate that *R. cathartica* and *R. saxatilis* are aecial hosts of PcSP1.

Fraser and Ledingham (1933) described crown rust (*P. coronata* var. *bromi*) of brome grass from Canada. Host range studies demonstrated that the aecial host was *Lepargyraea canadensis* rather than *R. cathartica*, and telial hosts did not include *B. inermis*. At present it is not clear what the relationship is between *P. coronata* var. *bromi* sensu Fraser and Ledingham and the two brome rusts, *P. coronata* var. *coronata* and *P. coronata* f. sp. *bromi* sensu Mühlethaler, examined in this study. Molecular and morphological studies are needed to clarify this issue.

As previously described, molecular analysis placed collections of *P. coronata* from *A. sativa* and *L. perenne* in a distinct group (Zambino and Szabo 1993), which is confirmed by these results (clade V). Based on morphological characters and host range, crown rust on *A. sativa* was classified as *P. coronata* var. *avenae* by Cummins (1971) and Urban and Marková (1994). Anikster et al. (2003) compared morphological and biological characteristics of *P. coronata* var. *avenae* and *P. coronata* f. sp. *bromi* sensu Mühlethaler and showed that these two crown rusts were distinctly different; characters included teliospore size, teliospore morphology, pycniospore DNA content, promycelium morphology, and substomatal vesicle morphology. In addition to *L. perenne*, DNA sequence analysis indicated that crown rust on *A. aequalis* and *F. elatior* be included with *P. coronata* var. *avenae* (Zambino and Szabo 1993). In contrast to the molecular data, crown rust on *L. perenne*, *A. aequalis*, and *F. elatior* were included in *P. coronata* var. *coronata* rather than in *P. coronata* var. *avenae* (Urban and Marková 1994). This classification is based primarily on differences in urediniospore morphology.

P. coronata samples collected from the telial hosts *Arrhenatherum elatius* and *Holcus lanatus* formed a well-supported clade (IV) that is closely related to clade V. This molecular result is supported by taxonomic classification of Urban and Marková (1994). *P. coronata* var. *avenae* is subdivided into *P. coronata* var. *avenae* f. sp. *avenae* and *P. coronata* var. *avenae* f. sp. *graminicola*. The latter includes telial hosts *A. elatius* and *H. lanatus*. Based on the genetic similarities between these rust collections (clades IV and V) and the genetic distance between the clades IV/V and the other clades, *P. coronata* var. *avenae* should be considered a separate species, PcSP2. This new species would include crown rust on *A. aequalis*, *A. elatius*, *A. sativa*, *F. elatior*, *H. lanatus*, and *L. perenne*. PcSP2 should be divided into at least two subgroups corresponding to clades IV and V. Additional morphological studies are needed to verify the molecular data.

Schwinghamer (1955) described a new morphotype of *P. coronata* collected from *E. repens* (=*Agropyron repens*) designated *P. coronata* f. sp. *agropyri*. Phylogenetic analysis of two collections of crown rust from *E. repens* supports the morphological and host range studies. Jin and Steffenson (1999) considered that *P. coronata* f. sp. *agropyri* as the same as *P. coronata* var. *hordei* based on morphological and host range data. Based on the genetic and morphological data, *P. coronata* f. sp. *agropyri* should be considered a separate species, PcSP3. Preliminary ITS sequence analysis indicates that crown rust collections from *E. repens* and *Hordeum vulgare* are very similar (Szabo, unpublished data). Additional molecular analysis needs to be done to confirm this relationship.

The single collection of crown rust from *Calamagrostis canadensis* formed a separate clade (VI) and was basal compared to the rest of the *P. coronata* samples. Taxonomic classification has placed this rust as part of *P. coronata* var. *coronata* (Cummins 1971; Urban and Marková 1994). Teliospores of *P. coronata* on *C. canadensis* are morphologically distinct from teliospores of *P. coronata* var. *hordei* (PcSP3) and *P. coronata* var. *avena*e (PcSP2) (Jin and Steffenson 1999). Crown rust has been reported on several species of *Calamagrostis*. Cummins (1971) designated collections of crown rust from *C. epigeios* and *C. arundinacea* as a separate variety, *P. coronata* var. *rangiferina*. Although only a partial ITS sequence is available, molecular analysis indicates that *P. coronata* on *C. canadensis* represents a new species (PcSP4). Clearly, additional collections of crown rust from *Calamagrostis* need to be analyzed to verify this placement and determine the relationship of *P. coronata* var. *rangiferina* to PcSP4.

In contrast to *P. coronata*, *P. andropogonis* has a narrow telial host range and a broad aecial host range. Collections from five aecial hosts and two telial hosts were examined, and phylogenetic analysis grouped these samples into five distinct clades correlating with aecial hosts (see Fig. 2). Arthur (1934) separated *P. andropogonis* into nine varieties, five of which are represented in this study.

Collections from *Schizachyrium scoparis* (telial), *Penstemon gracilis* (aecial), and *Castilleja coccinea* (aecial) formed two closely related clades (I, II). Each clade contained samples from *S. scoparis* and samples from either *P. gracilis* (clade I) or *C. coccinea* (clade II). The genetic relationship between aecia on *Penstemon* and uredinia/telia on *S. scoparis* (=*Andropogon scoparis*) has been demonstrated

(Arthur 1900, 1907; Mains 1933); however, the genetic connection between aecia on *C. coccinea* and uredinia/telia on *S. scoparis* has not. Arthur (1934) designated these two groups *P. andropogonis* var. *pentstemonis* (clade I) and *P. andropogonis* var. *micropuncta* (clade II).

In the tall-grass prairies of North America, it is common to find both telial hosts (*A. gerardii* and *S. scoparis*) and several aecial hosts (*P. gracilis, Comandra umbellata*, and *Lupinus perennis*) within a meter of each other and each infected with *P. andropogonis.* In each case, DNA sequence analysis identified the rust on *S. scoparis* as *P. andropogonis* var. *pentstemonis*, further supporting the genetic separation of this group (Szabo, unpublished data). In addition, at the single site that rust was found on *C. coccinea*, *P. andropogonis* was also found on *C. umbellata*. All samples of rust analyzed from *S. scoparis* were *P. andropogonis* var. *micropuncta*. Based on the genetic and biological data, *P. andropogonis* on *S. scoparis* with aecia on *P. gracilis* or *C. coccinea* should be considered a new species (*Puccinia andropogonis* species 1, PaSP1). Further analysis needs to be done to determine whether *P. andropogonis* var. *pentstemonis* and *P. andropogonis* var. *micropuncta* represent subgroups of the same species (PaSP1) or different species.

Collections from *A. gerardii* (telial), *C. umbellata* (aecial), *Lupinus perennis* (aecial), and *Zanthoxylum americanum* (aecial) formed three well-supported and distinct clades (III, IV, V). Each clade contained collections from *A. gerardii* and either *C. umbellata* (clade III), *Z. americanum* (clade IV), or *L. perennis* (clade V). The genetic relationship between aecia on *Comandra* and uredinia/telia on *A. gerardii* (=*Andropogon furcatus*), as well as between aecia on *Z. americanum* and uredinia/telia on *A. gerardii*, has been demonstrated (Arthur 1904, 1906; Davis 1929). DNA sequence analysis presented here represents the first direct confirmation of the genetic connection between aecia on *L. perennis* and uredinia/telia on *A. gerardii*. These three clades correspond with three varieties defined by Arthur (1934): clade III (*C. umbellata*), *P. andropogonis* var. *pustulata*; clade IV (*Z. americanum*), *P. andropogonis* var. *zanthoxyli*; clade V (*L. perennis*), *P. andropogonis* var. *onobrychidis*. Based on the genetic distance between each of these clades and the distinct aecial hosts, *P. andropogonis* var. *pustulata*, *P. andropogonis* var. *zanthoxyli*, and *P. andropogonis* var. *onobrychidis* should be considered separate species – PaSP2, PaSP3, and PaSP4, respectively. Additional morphological studies are needed to verify the molecular data.

Speciation in the *P. andropogonis* complex appears to have occurred via jumps involving both telial and aceial hosts. From the phylogenetic data presented, *A. gerardii* was likely the telial host of the ancestral form. Speciation occurred via successive aecial host jumps to *Z. americanum* (PaSP3, clade IV) and then *C. umbellata* (PaPS2, clade III). The next speciation event appears to include a telial host jump to *S. scoparis* followed by aecial host jumps to *C. coccinea* (PaSP1, clade I) and *P. gracilis* (PaSP1, clade II). Additional collections of *P. andropogonis* representing varieties not included in this study must be analyzed to establish a better understanding of this species complex and its evolution.

In contrast to *P. andropogonis*, speciation in *P. coronata* appears to have resulted from radiation primarily onto new telial hosts. Phylogenetic analysis places the rust on *C. canadensis* basal to the other member of this complex, indicating that it is more closely related to the ancestral form. Speciation then appears to have occurred along two distinct lineages; one radiated out onto *E. repens* (PcSP2, clade 1) and *Bromus* sp. (PcSP1, clade 2), whereas the second radiated out onto *A. sativa*, *L. perenne*, *A. elatius*, and *H. lanatus* (PcSP3, clades IV, V). It is interesting to note that these two lineages follow host taxonomic divisions: lineage 1 contains members of the Triticodea (*Bromus* and *Elytrigia*) and lineage 2 contains members of the Aveneae (*Avena*, *Arrhenatherum*, and *Holcus*) and Poeae (*Lolium*). It is not clear what role aecial hosts have played in the speciation process because *R. cathartica* is an aecial host for members of the *P. coronata* complex found in both lineages. Additional collections of *P. coronata* complex must be analyzed to better understand this species complex.

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