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ORIGINAL ARTICLE

Laying the foundation for a taxonomic review of *Puccinia coronata* s.l. in a phylogenetic context

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Abstract Intra-specific classification of *Puccinia coronata* has been controversial, with previous approaches falling into three major categories: 1. A two-species system, namely *P. coronifera* and *P. coronata*; 2. The same two-species system subdivided into many *formae speciales*, in which the host range of each is restricted to species within one genus of *Poaceae*; 3. A one-species system, *P. coronata*, subdivided into a few varieties with host ranges that may overlap. To re-assess these concepts in the context of multigene analyses and comparative morphological assessments, data were generated for a comprehensive set of herbarium and recently collected specimens, representing a broad range of hosts and geographic origins. Phylogenetic analyses of a combined data set of DNA sequences for four loci (BT, COI, ITS, and RPB2) revealed a high degree of genetic variation. Morphological differences among phylogenetic lineages were overlapping but nine lineages were differentiated using calculated means for teliospore and urediniospore length/width as well as measurements for the teliospore hilum and digitation. The taxon infecting *Avena* also comprises collections from a wide range of other grass hosts while other lineages, such as those on *Bromus* and *Agrostis*, were restricted in host association. Type specimen DNA sequences included in the analyses resolved the placement of five previously described varieties. Based on evidence of host specificity, morphology and multigene analyses, we recognized seven species, one of which was further divided into two varieties. Expanded descriptions, illustrations and a synoptic key are provided. A new series, *Puccinia* Series *Coronata*, was erected to accommodate all the lineages comprising *P. coronata sensu lato*.

Keywords Crown rust · Uredinales · Phylogeny · Cryptic species · Variety · *Forma specialis*

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Introduction

The telial stage of crown rust, *Puccinia coronata*, is readily identified based on the distinctive, yet morphologically variable, digitations adorning the upper cell of the teliospore – hence, the common name. Although well-known as causing disease on oats, the species also occurs on a broad range of other hosts, and is considered one of the most important and damaging diseases worldwide of cultivated oat (*Avena sativa*) and pasture grasses (Harder and Haber 1992; Potter et al. 1990; Simons 1970). In Canada, control of the disease mainly depends on the deployment of resistant cultivars, assisted by early planting strategies and fungicide applications (McCallum et al. 2007). For many years breeding for resistance was successful in providing a series of new cultivars with good rust resistance, but nearly all of the effective resistance genes used to date have now been overcome (for review see McCallum et al. 2007). In contrast to the detrimental effects to agriculture, *P. coronata* has also been investigated for beneficial purposes, such as a potential

biocontrol agent of wild oats (*Av. fatua*) on San Clemente Island, California (Carsten et al. 2000).

The macrocyclic lifecycle of the species includes uredinial and telial stages on a diverse range of grass species occurring worldwide and spanning more than 45 genera of *Poaceae* (Cummins 1971). Pycnia and aecia occur on shrubs in the buckthorn family (*Rhamnaceae*), which are widely distributed in the Northern Hemisphere and Middle East (Roelfs 1985). The future implementation of targeted disease management and breeding efforts, and the robust assessment of biological control potential, depend on a deeper understanding of the genetic and biological variation within this species complex.

Puccinia coronata was first described by Corda in 1837 from Reichenberg (now Liberec, Czech Republic) on *Luzula albida* (later redetermined as *Calamagrostis arundinaceae* or *Cal. villosa*). Early infection experiments using teliospores from a range of grasses to inoculate two aecial shrub hosts, *Rhamnus frangula* (the current name *Frangula alnus* is used in the following paragraphs; GRIN, http://www.ars-grin.gov/cgi-bin/npgs/html/tax_search.pl, Aug 2010) and *R. cathartica,* resulted in infection of one or the other host, but never both (Cornu 1880; Eriksson 1894; Klebahn 1892; Muhlethaler 1911; Nielsen 1877). Based on the initial evidence, Klebahn (1894) divided *P. coronata* into two species, i.e. *P. coronata* on *Fr. alnus* and *P. coronifera* on *R. cathartica*. Crown rust of oats (*Av. sativa*) was classified in *P. coronifera* because isolates did not infect *Fr. alnus* (Eriksson 1894; Muhlethaler 1911). In the same year, having recognized several specialized forms of crown rust, Eriksson (1894) divided them into four series, each series including multiple *formae speciales* (f. spp.) whereby each *forma specialis* (f. sp.) was restricted to a specific telial host genus. Later, Mühlethaler (1911) proposed different groupings based on his infection experiments. Nevertheless, the two species of Klebahn (1894) were accepted as distinct groups in both the Eriksson (1894) and Mühlethaler (1911) systems. These and subsequent studies resulted in the recognition of 13 f. spp.: *agropyri*, *agrostis*, *avenae, alopecuri, bromi, calamagrostis, festucae, glyceriae, holci, lolii, melicae, phalaridis* and *secalis* (Brown 1937; Eriksson 1894, 1908; Melhus et al. 1922; Peturson 1954).

The appropriateness of the two species system was questioned by Melhus et al. (1922) and Dietz (1926) because of studies demonstrating cross-infection between *P. coronata* and *P. coronifera* and their respective hosts. Melhus et al. (1922) reviewed experimental data published by Treboux (1912, 1914) indicating that aecial spores from *Fr. alnus* infected *Av. sativa*, while those from *R. cathartica* infected *Agrostis stolonifera*, *Cal. arundinacea*, and *Phalaris arundinacea*, documented hosts of *P. coronata* not *P. coronifera*. Eshed and Dinoor (1980) noted that during this period of time, the concept of *forma specialis* broadened from implying a pathogenic form specific for one host genus to include specificity for multiple genera, which may overlap.

In the early 1930s, Fraser and Ledingham (1933) completed a comprehensive study of crown rust in Canada, observing that the aecial state occurred on only four species of shrubs in the Prairie Provinces, i.e. *R. cathartica, R. alnifolia, Lepargyraea canadensis* and *Elaeagnus commutata*. Based on aecial (I) characteristics (absence or presence of hypertrophy of the tissue, arrangement of aecial cups and color of aecia), and in vitro inoculation experiments with various grasses to document development of the uredinial (II) and telial (III) stages, four varieties were designated: "*Puccinia coronata Avenae*" (aecial state I on *R. cathartica*, II and III on *Avena* spp.), "*Puccinia coronata Calamagrostis*" (I on *R. alnifolia*, II and III mainly on *Calamagrostis* spp. and *Scolochloa festucacea*), "*Puccinia coronata Bromi*" (I on *L. canadensis* [current name: *Shepherdia canadensis,* GRIN Aug 2010], II and III on *Bromus* spp.), "*Puccinia coronata Elaeagni*" (I on *E. commutata*, II and III on *Cal. elongata* [name was not found in GRIN]).

In the early 1970s, Cummins (1971) published a new classification based on detailed studies that focused on the minute morphological differences among urediniospores and teliospores and de-emphasized host association. Five varieties were recognized including *P. coronata* var. *avenae sensu* Fraser and Ledingham (1933). The other three varieties of Fraser and Ledingham (1933) were combined within the type variety, *P. coronata* var. *coronata*, a broad and inclusive taxon found on *Berchemia, Rhamnus* and *Elaeagnus* (I), and grasses in 10 different tribes (II, III). The three additional varieties were: *P. coronata* var. *gibberosa* (Lagerh.) Joerst. (on *Festuca altissima* in Europe); *P. coronata* var. *rangiferina* (Ito) Cumm. (on *Cal. arundinacea* and *Cal. epigeios* in China and Japan); and *P. coronata* var. *himalensis* Barcl. (on grasses in four different tribes) characterized by having small thin-walled urediniospores.

More recent research emphasizing spore morphology, geographic distribution and natural occurrence on various hosts in Europe was summarized by Urban and Marková (1993). Their classification divided the species complex into three varieties: *P. coronata* var. *coronata* (I on *R. cathartica* and *Fr. alnus*, II and III on a wide range of grasses), *P. coronata* var. *avenae* (I commonly on *Rhamnus* spp., II and III mainly on *Avena*) and *P. coronata* var. *intermedia* (I on *R. pumilus* Turra [written as *R. pumila* Turra in the article], II and III on *Calamagrostis* and *Sesleria*). They pointed out that *P. coronata* var. *avenae* also likely occurs on wild oats in nature.

The contradictions among these classifications resulted from an emphasis on plastic morphological characters, insufficient sampling, uncertainties about host ranges and difficulties in confirming host alterations in the life cycle. DNA-based studies could be used to resolve some of these

contradictions. Recent analyses by Szabo (2006) of rDNA sequences for 16 specimens provided molecular evidence that *P. coronata* is a species complex comprising multiple phylogenetic lineages. Based on those results, six subspecies were proposed but not named. Sampling was limited to seven of the 45 reported telial host genera and only one specimen on *Avena* was included.

The objectives of the present study were to analyze data from four gene regions for a broader sampling of *P. coronata*, including multiple representatives from a wider host and geographic range, re-assess the published classification systems, and to investigate the following questions. Is the pathogen that causes crown rust on oats a phylogenetically distinct group within the *P. coronata* complex and what is its host range? Will increased sampling reveal additional intra-specific taxa and can the lineages be differentiated morphologically? We present a framework for a new classification for the species based on a review of previous taxonomic treatments and the synthesis of molecular, morphological and biological data.

Material and methods

Fungal specimens

Fungal specimens were obtained either from herbaria or as new collections from the field. Twelve international herbaria loaned material (see Table 1 footnotes for list) and all new collections were deposited in the National Mycological Herbarium in Ottawa, Canada (DAOM). Herbarium voucher numbers, plant host, provenance, year collected and Genbank accession numbers for 156 specimens sequenced in this study are listed in Table 1.

Genomic DNA extraction

Small pieces of infected plant material, 1–4×0.1–0.8 cm, were selected for DNA extraction and surface-sterilized by gentle wiping using a Kimwipe (KimTech Inc., Louisville, USA) sprayed with 75 % ethanol. For small batches of samples, E.Z.N.A. Fungal DNA Extraction Kit (VWR) was used as described in Liu and Hambleton (2010); larger batches of samples were processed in a 96-well plate format using the QIAGEN DNeasy 96 Plant Kit (QIAGEN Inc., Toronto, Canada) as per the manufacturer's protocol.

PCR amplification and sequencing

We targeted short amplicons due to the challenge to PCR-amplify long fragments for DNA from dried herbarium specimens, in which the DNA degradation from poor initial drying methods as well as specimen aging could be inhibiting factors. Four gene regions were sampled. The nuclear rDNA internal transcribed spacer (ITS) region and a portion of the β-tubulin (BT) gene, between introns 5 and 7 (Ayliffe et al. 2001), were amplified and sequenced using the method described in Liu and Hambleton (2010). Exons one and two of the mitochondrial cytochrome oxidase subunit I (COI) gene were amplified by primers P360f (GCTAAGGATA TTGCCATTCTATAT) and P360r (TCCATCCYGTYCCT GCYCC) designed by M. Allaire (http://www.boldsystems.org/views/primerlist.php), with annealing temperature at 51°C for 30 sec.

For the nuclear RNA Polymerase II subunit 2 (RPB2) gene, semi-nested PCR was performed using newly designed primers to amplify a fragment beginning in exon 7 downstream from primer RPB2-7 F (Liu et al. 1999). First round amplification was with primers RPB2-187f (CGATCCTGTGYTAY TCGGGMTAYAACCA) and RPB2-853r2 (GCATCRC CYTCRTTVCCKGWG), and the second round with the forward primer RPB2-492f (CGGATGAAGACRCAYACK AARCG) and the same reverse primer. Protocols were as same as those for amplifying BT (Liu and Hambleton 2010).

If no DNA fragment was amplified, the PreCR Repair Mix (New England BioLabs, Ipswich, USA) was used as per the manufacturer's instructions to attempt DNA repair, in case DNA degradation was the reason for the failed PCR amplifications.

Phylogenetic analyses

Sequences were edited using Sequencher™ 4.7 (Gene Codes Corporation, Ann Arbor, USA) and compiled using BioEdit Sequence Alignment Editor 7.0.5.3 (Hall 1999). The compiled sequences were submitted to a web server (http://mafft.cbrc.jp/alignment/server/index.html) for alignment by MAFFT ver.5 (Katoh et al. 2005) and the model FFT-NS-I (iterative refinement method) was selected. The sequence alignments were manually adjusted to correct obvious misalignments by the computer algorithm.

Data matrices were subjected to parsimony analysis in PAUP* 4.0b10 (Swofford 1998). Heuristic searches with random stepwise addition of 100 replicates and tree bisection-reconnnection (TBR) branch swapping were conducted. A limit of 1,000,000 rearrangements was set for each replicate. Bootstrapping analyses were set with 1000 replicates with full heuristic search of random stepwise-addition of 10 replicates and a limit of 10,000 rearrangements per replicate. Gaps were treated as missing data. Parsimony analyses were conducted for each locus separately and for the combined data set. In order to evaluate the level of congruence of individual loci, we estimated Partitioned Bremer Support for the branches of the tree based on the combined data set (Baker et al. 1998) using TreeRot ver. 3 (Sorenson and Franzosa 2007).

Table 1 List of fungal specimens used in the phylogenetic analyses

Species Name and Voucher Number[a]	Clade	PCA[b]	Host	Country	Year	ITS[c]	BT	COI	RPB2
P. coronata var. *avenae* f. sp. *avenae* (Clade V): Wide host range; Cosmopolitan									
BR 8665	V	no	*Agropyron coninum*	Belgium	1993	HM131278	—	—	—
DAOM 240187	V	yes	*Avena cf fatua*	Canada	2006	HM131285	HM147304	HM147429	HM147374
K(M): 77013	V	yes	*Avena cf sterilis*	Greece	2000	HM131289	HM147297	HM147422	—
DAOM 240064	V	yes	*Avena satica*	Canada	2006	HM057140	HM068002	HM147405	HM147354
(S)reg.nr. F34373	V	no	*Avena sativa*	Argentina	2000	HM131257	—	—	—
(S)reg.nr. F35766	V	yes	*Avena sativa*	Sweden	2003	**HM131259**	HM147294	HM147419	HM147365
BP 88887	V	no	*Avena sativa*	Hungary	1988	HM131266	—	HM147416	—
BP 91484	V	yes	*Avena sativa*	Hungary	1986	HM131267	HM147292	HM147415	HM147363
BPI 0195485	V	no	*Avena sativa*	Australia	1981	**HM131269**	—	HM147418	—
BPI 843832	V	no	*Avena sativa*	Germany	1948	**HM131274**	—	—	—
BPI 871766	V	yes	*Avena sativa*	USA	2004	**HM131276**	HM147293	HM147417	HM147364
PUR 22125 (LT)	V	no	*Avena sativa*	Canada	1923	HM131256	—	—	—
PUR N1190	V	no	*Avena sativa*	USA	2001	**HM131292**	HM147323	—	—
PUR N5418	V	no	*Avena sativa*	Panama	1973	HM131300	—	HM147390	—
DAOM 240180	V	no	*Avena sp.*	Canada	2005	HM131280	HM147316	—	—
DAOM 240181	V	no	*Avena sp.*	Canada	2005	HM131281	—	—	—
DAOM 240182	V	no	*Avena sp.*	Canada	2005	HM131282	—	—	—
DAOM 240184	V	no	*Avena sp.*	Canada	2006	HM131283	—	HM147421	HM147370
DAOM 240186	V	no	*Avena sp.*	Canada	2006	HM131284	HM147303	—	—
DAOM 240190	V	no	*Avena sp.*	Canada	2006	HM131286	—	—	—
PUR N1365	V	no	*Dactylis glomerata*	USA	1997	HM131293	—	HM147386	—
BR 47770-46	V	no	*Festuca arundinacea*	Belgium	1994	**HM131277**	—	—	—
PUR N1542	V	no	*Festuca sp.*	USA	1997	**HM131296**	—	—	—
B 70 0012413	V	no	*Glyceria maxima*	Austria	1981	HM131260	—	HM147428	—
BP 87217	V	no	*Glyceria maxima*	Hungary	1980	HM131264	—	—	HM147358
BP 88886	V	no	*Glyceria maxima*	Hungary	1993	**HM131265**	—	HM147410	—
BP 91486	V	no	*Glyceria maxima*	Hungary	1986	HM131268	—	—	—
B 70 0012414	V	no	*Glyceria sp.*	Austria	1983	HM131261	—	—	—
BPI 60255	V	no	*Hordeum sp.*	Columbia	1960	HM131270	—	HM147433	—
BP 87214	V	no	*Lolium multiflorum*	Hungary	1982	**HM131263**	—	HM147411	—
BPI 60333	V	yes	*Lolium perenne*	Afghanistan	1976	HM131271	HM147307	HM147434	—
DAOM 181681	V	no	*Lolium perenne*	Canada	1981	HM131279	HM147317	—	HM147344
PUR F19478	V	no	*Lolium perenne*	Chile	1970	HM131290	—	—	—
BPI 60405	V	no	*Phalaris stenoptera*	Argentina	1958	HM131272	—	—	—
PUR N436	V	no	*Poa pratensis*	USA	1992	HM131299	—	—	—
PUR N1982	V	no	*Poa sp.*	USA	1991	HM131298	—	—	—

Table 1 (continued)

Species Name and Voucher Number[a]	Clade	PCA[b]	Host	Country	Year	ITS[c]	BT	COI	RPB2
(S)reg.nr. F35463	V	no	*Rhamnus cathartica*	Sweden	2003	HM131258	HM147295	—	HM147366
BPI 747668	V	no	*Rhamnus cathartica*	USA	1995	HM131273	—	—	—
PUR N1389	V	no	*Rhamnus cathartica*	USA	1995	HM131294	—	—	—
K(M): 127750	V	no	*Schedonorus gigantea*	England	1988	HM131288	—	HM147424	—
BPI 871669	V	yes	*Schedonorus phoenix*	USA	2004	HM131275	HM147305	HM147431	—
DAOM 240723	V	no	*Schedonorus phoenix*	Canada	2006	**HM131287**	HM147290	—	—
PUR N1008	V	no	*Schedonorus phoenix*	USA	1995	**HM131291**	HM147333	—	—
PUR N1405	V	no	*Schedonorus phoenix*	USA	1995	**HM131295**	HM147325	HM147389	—
PUR N1015	V	yes	*Schedonorus phoenix*	USA	1995	HM131301	HM147341	HM147402	HM147348
BP 86576	V	no	*Schedonorus pratensis*	Hungary	1985	HM131262	—	—	—
PUR N1977	V	no	*Schedonorus pratensis*	USA	1998	**HM131297**	HM147324	HM147388	—
P. coronata var. *avenae* f. sp. *graminicola* (Clade IV): on *Arrhenatherum*, *Glyceria* and *Holcus*; North America, South America, Europe									
BP 91761	IV	no	*Arrhenatherum elatius*	Hungary	1982	**HM131240**	—	HM147409	—
PUR F16064	IV	no	*Arrhenatherum elatius*	Great Britain	1958	—	—	HM147394	HM147380
PUR N5407	IV	no	*Arrhenatherum elatius*	USA	1991	**HM131253**	HM147329	—	—
PUR N5409	IV	no	*Arrhenatherum elatius*	USA	1990	**HM131254**	HM147330	—	—
K(M): 104797	IV	yes	*Glyceria maxima*	England	2002	HM131244	HM147298	HM147423	—
B 70 0007213	IV	no	*Holcus lanatus*	Austria	1994	HM131239	—	—	—
BPI 871068	IV	yes	*Holcus lanatus*	USA	2005	HM131243	HM147306	HM147432	HM147376
K(M): 70209	IV	no	*Holcus lanatus*	England	2000	HM131248	—	—	—
PUR F19479	IV	yes	*Holcus lanatus*	Argentina	1970	HM131249	HM147332	HM147392	—
PUR N1251	IV	yes	*Holcus lanatus*	USA	1992	**HM131250**	HM147342	HM147403	HM147351
PUR N1252	IV	no	*Holcus lanatus*	USA	1992	HM131251	—	—	—
PUR N5376	IV	no	*Holcus lanatus*	USA	1992	**HM131252**	—	—	—
PUR N95	IV	no	*Holcus lanatus*	Spain	1978	HM131255	—	—	—
K(M): 115658	IV	yes	*Holcus sp.*	England	2003	HM131245	HM147301	HM147426	HM147373
BPI 58842	IV	no	*Rhamnus cathartica*	Germany	1962	HM131241	—	—	—
K(M): 139492	IV	no	*Rhamnus cathartica*	England	2006	HM131246	—	—	—
K(M): 139520	IV	no	*Rhamnus cathartica*	England	2006	HM131247	HM147299	—	—
BPI 60120	IV	no	*Unknown grass*	Columbia	1971	HM131242	—	—	—

Table 1 (continued)

Species Name and Voucher Number[a]	Clade	PCA[b]	Host	Country	Year	ITS[c]	BT	COI	RPB2
P. coronata var *coronata* (Clade VII): mainly on *Calamagrostis*; Europe									
BR 3933-53	VII	no	*Agrostis stolonifera*	Belgium	1988	HM131315	—	—	—
PRM 155608 (ET)	VII	no	*Calamagrostis arundinacea*	Bohemia	n/a	HM131309	—	—	—
BP 89076	VII	yes	*Calamagrostis epigeios*	Hungary	1991	**HM057141**	HM068003	HM147414	HM147362
BP 89848	VII	yes	*Calamagrostis epigeios*	Hungary	1989	HM131314	HM147291	HM147413	HM147361
K(M): 107200	VII	no	*Frangula almus*	England	1989	HM131316	—	—	—
(S)reg.nr. F46266	VII	yes	*Frangula almus*	Sweden	2005	**HM131310**	HM147296	HM147420	HM147367
B 70 0006597	VII	yes	*Frangula almus*	Germany	2003	HM131312	HM147302	HM147427	—
BP 89353	VII	no	*Frangula almus*	Hungary	1991	**HM131313**	—	—	HM147360
B 70 0012416	VII	no	*Rhamnus saxatilis*	Austria	1995	HM131311	—	—	—
P. coronati-agrostis Liu & Hambleton sp. nov. (Clade IX): on *Agrostis* and *Phalaris*; Europe, North America									
K(M): 82517	IX	yes	*Agrostis cf tenuis*	England	2000	HM131323	HM147300	—	HM147371
PUR 54591	IX	no	*Agrostis gigantea*	USA	1955	HM131324	—	—	—
PUR 55024	IX	no	*Agrostis gigantea*	USA	1955	**HM131325**	HM147328	—	—
PUR 59741	IX	no	*Agrostis gigantea*	Canada	1960	HM131326		—	—
PUR N1029	IX	no	*Agrostis sp.*	Canada	1994	HM131327	HM147287	HM147406	—
BR 3928 48	IX	no	*Agrostis stolonifera*	Belgium	1988	HM131320	—	—	—
PUR N114 (T)	IX	yes	*Agrostis stolonifera*	Finland	1977	**HM131319**	—	HM147393	HM147379
PUR N5372	IX	yes	*Agrostis stolonifera*	USA	1992	HM131329	—	HM147407	HM147355
K(M) 60529	IX	yes	*Agrostis stolonifera*	Austria	1994	HM131322	—	HM147425	HM147372
PUR N1268	IX	no	*Phalaris arundinacea*	Canada	1994	HM131328	—		—
DAOM 220642	IX	yes	*Rhamnus* sp.	Canada	1995	HM131321	—	HM147397	HM147345
DAOM 240722	IX	no	*Rhamnus cathartica*	Canada	2006	—	HM147340	HM147401	—
P. coronati-brevispora Liu & Hambleton sp. nov. (Clade III): on *Bromus inermis*; North America									
DAOM 240063	III	yes	*Bromus inermis*	Canada	2006	HM057139	HM068001	HM147404	HM147353
DAOM 240183	III	yes	*Bromus inermis*	Canada	2006	HM131237	HM147289	HM147412	HM147359
PUR N1371	III	yes	*Bromus inermis*	USA	1997	**HM131238**	HM147322	HM147387	HM147377
PUR N652 (T)	III	yes	*Bromus inermis*	USA	1999	**HM131235**	HM147321	—	—
DAOM 235159	III	yes	*Rhamnus cathartica*	Canada	2005	HM131236	HM147315	HM147382	—
P. coronati-calamagrostidis Liu & Hambleton nom. et stat nov. (Clade VI): mainly on *Calamagrostis canadensis;* North America									
PUR N1973	VI	yes	*Berchemia scandens*	USA	1993	HM131343	HM147318	HM147383	—

Table 1 (continued)

Species Name and Voucher Number[a]	Clade	PCA[b]	Host	Country	Year	ITS[c]	BT	COI	RPB2
BPI 59821	VI	no	*Bromus anomalus*	USA	1962	HM131341	—	—	—
PUR 57282	VI	no	*Bromus anomalus*	USA	1960	**HM131351**	—	—	—
DAOM 204923	VI	yes	*Bromus ciliatus*	Canada	1918	HM131305	HM147339	—	—
DAOM 186149	VI	no	*Bromus pumpellianus*	Canada	1980	HM131348	—	—	—
BPI 747648	VI	yes	*Calamagrostis canadensis*	USA	1995	HM131303	—	HM147430	HM147375
DAOM 107653	VI	yes	*Calamagrostis canadensis*	USA	1964	HM131304	HM147337	HM147400	HM147347
PUR 22155 (LT)	VI	no	*Calamagrostis canadensis*	Canada	1917	HM131302	—	—	—
PUR 22167	VI	no	*Calamagrostis canadensis*	Canada	1917	HM131306	—	—	—
PUR 22185	VI	no	*Calamagrostis canadensis*	USA	1915	HM131307	—	—	—
PUR N2268	VI	yes	*Calamagrostis canadensis*	USA	2000	HM131308	HM147320	HM147385	—
PUR 66551	VI	no	*Calamagrostis rubescens*	USA	1982	HM131352	HM147335	—	—
DAOM 195783	VI	no	*Elaeagnus commutata*	Canada	1960	HM131349	—	—	—
DAOM 220889	VI	yes	*Elymus sp.*	Canada	1996	**HM131350**	HM147336	HM147398	—
BPI 1100292	VI	no	*Leymus innovatus*	Canada	1953	HM131346	—	—	—
DAOM 130314	VI	no	*Shepherdia canadensis*	Canada	1969	HM131347	—	HM147396	—
PUR 54152	VI	no	*Trisetum canescens*	USA	1934	**HM131342**	—	—	—
P. coronati-hordei Liu & Hambleton nom. et stat. nov. (Clade I): on *Elymus* and *Hordeum*; North America									
PUR N1396	I	no	*Elymus hystrix*	USA	1996	HM131227	—	—	—
DAOM 183691	I	yes	*Elymus repens* (= *Agropyron repens)*	Canada	1982	HM057138	HM068000	HM147399	HM147346
PUR N1406	I	no	*Elymus repens* (= *Agropyron repens)*	USA	1995	HM131228	HM147327	—	—
PUR N1413	I	no	*Elymus repens* (= *Agropyron repens)*	USA	1992	**HM131229**	—	—	—
PUR N1426	I	yes	*Elymus repens* (= *Agropyron repens)*	USA	1995	HM131230	HM147334	—	—
PUR N1358	I	no	*Elymus virginicus*	USA	1995	HM131226	HM147326	—	—
PUR N1539	I	no	*Hordeum jubatum*	USA	1992	HM131231	—	—	—
PUR 89857 (T)	I	yes	*Hordeum vulgare*	USA	1992	HM131225	—	—	—
P. coronati-japonica Liu & Hambleton sp. nov. (Clade VIII): on *Calamagrostis arundinacea*; Japan									
PUR N1055	VIII	yes	*Berchemia pauciflora*	Japan	1990	HM131318	HM147319	HM147384	—
PUR F16131 (T)	VIII	yes	*Calamagrostis arundinacea*	Japan	1958	**HM131317**	HM147331	HM147391	HM147378

Table 1 (continued)

Species Name and Voucher Number[a]	Clade	PCA[b]	Host	Country	Year	ITS[c]	BT	COI	RPB2
P. coronati-longispora Liu & Hambleton sp. nov. (Clade II): on *Bromus erectus*; Europe									
PRC 194	II	yes	*Bromus erectus*	Slovakia	2002	HM131233	HM147338	—	—
PRC 196 (T)	II	no	*Bromus erectus*	Czech Republic	2002	HM131232	—	—	—
PRC 194	II	no	*Rhamnus saxatilis*	Slovakia	2002	HM131234	—	—	—
PRC 247	II	yes	*Rhamnus saxatilis*	Slovakia	2002	HM057142	HM068004	—	—
P. coronata s.l. var. *gibberosa* (Lagerh.) Joerst. (Type seen but not sequenced)									
BRNM 111952 (T of var. *intermedia*)	XI	no	*Calamagrostis epigejos*	Czech Republic	1923	HM131332	—	—	—
PUR F17485	XI	no	*Festuca altissima*	Slovakia	1960	HM131333	—	—	—
P. coronata s.l. var. *himalensis* Barclay (Type not seen)									
PUR F15640 (ST of *P. brevicornis*)	X	no	*Calamagrostis canadensis*	Japan	1907	HM131330	—	—	—
PUR F15641 (ST of *P. brevicornis*)	X	yes	*Calamagrostis canadensis*	Japan	1907	HM131331	—	—	—
P. coronata s.l.									
BPI 746001	XII	no	*Rhamnus schneideri*	China	1985	HM131334	—	HM147435	—
HMAS 65723	XIII	yes	*Pennisetum flaucidum?*	China	1990	**HM131335**	HM147288	HM147408	HM147356
BPI 718354 (basal to III)	XIV	no	*Bromus erectus*	Germany	1949	HM131345	—	—	—
PUR 25955 (T of *P. subdigitata*)	XV	no	*Brachypodium mexicanum*	Guatemala	1915	HM131336	—	—	—
PUR N5403	XVI	no	*Rhamnus japonica*	Japan	1973	HM131337	—	—	—
PUR 59504	XVII	no	*Brachypodium mexicanum*	Mexico	1961	HM131338	—	—	—
PUR N1057	XVIII	yes	*Helictotrichon virescens*	Nepal	1986	HM131339	—	HM147395	HM147381
PUR F4486 (T of *P. melicae*)	XIX	no	*Melica nutans*	Sweden	1894	HM131340	—	—	—
PUR N1983	XX	no	*Rhamnus alnifolia*	USA	1994	HM131344	—	—	—
Outgroup Taxa									
P. brachypodii DAOM 185200		no	*Brachypodium silvaticum*	Hungary	1980	HM131353	—	—	—
P. bromina BPI 70086		no	*Bromus rubens*	USA	1941	HM131354	—	—	—
P. cesatii DAOM 214752		no	*Andropogon ischaemum*	Hungary	1991	HM131355	—	—	—
P. durangensis DAOM 163064		no	*Stipa pringlei*	USA	1975	HM131356	—	—	—
		yes	*Elymus repens*	Belgium	2001	**HM131357**	HM147308	—	HM147350

Table 1 (continued)

Species Name and Voucher Number[a]	Clade	PCA[b]	Host	Country	Year	ITS[c]	BT	COI	RPB2
P. graminis BR 150069-10									
P. graminis PUR 66554		no	*Oryzopsis exigua*	USA	1982	**HM131358**	—	—	—
P. graminis BPI 803290		no	*Elytrigia repens*	China	1986	HM131359	—	—	—
P. poae-nemoralis DAOM 212041		no	*Poa pratensis*	Canada	1990	HM057152	—	—	—
P. poae-nemoralis DAOM 189681		no	*Arctagrostis latifolia*	Canada	1980	HM057153	—	—	—
P. poarum DAOM 193284		no	*Poa pratensis*	Canada	1983	HM057149	—	—	—
P. poarum DAOM 240188		no	*Tussilago farfara*	Canada	2006	**HM057150**	—	—	—
P. recondita DAOM 192559		no	*Triticum aestivum*	Australia	1982	HM057145	—	—	—
P. recondita DAOM 240185		no	*Agropyron repens*	Canada	2006	HM131360	HM147309	—	—
P. striiformis PUR N5378		no	*Dactylis glomerata*	USA	1992	HM057109	—	—	—
P. striiformis DAOM 240071		yes	*Triticum aestivum*	China	2006	HM057121	HM067991	—	HM147369
P. triticina BR 59352-85		no	*Elymus repens*	Belgium	1996	HM131361	HM147311	—	—
P. triticina B 70 0012410		no	*Elymus caninus*	Romania	1974	HM131362	HM147312	—	—
Uromyces alopercuri DAOM 234714		no	*Alopecurus aristulatus*	Canada	1917	HM131363	—	—	—
Uromyces clignyi DAOM 192217		no	*Andropogon sp.*	N. Rhodesia	1962	HM131364	—	—	—
Uromyces coronatus DAOM 32991		no	*Zizania latifolia*	Japan	1949	HM131365	—	—	—
Uromyces dactylis DAOM 216236		yes	*Dactylis glomerata*	Hungary	1992	HM057148	HM068010	—	—

[a] T=type, ET=epitype, LT=lectotype, ST=Syntype; **B**: Botanischer Garten und Botanisches Museum Berlin-Dahlem, Zentraleinrichtung der Freien Universität Berlin, BERLIN, Germany; **BP**: Hungarian Natural History Museum, BUDAPEST, Hungary; **BPI**: US National Fungus Collections, BELTSVILLE, Maryland, USA; **BR**: National Botanic Garden of Belgium, MEISE, Belgium; **BRNM**: Moravian Museum, BRNO, Czech Republic; **DAOM**: Agriculture and Agri-Food Canada, OTTAWA, Ontario, Canada; **HMAS**: Institute of Microbiology, Academia Sinica, BEIJING, People's Republic of China; **K(M)**: Royal Botanic Gardens, KEW, UK, England; **PRC**: Charles University in Prague, PRAHA, Czech Republic; **PRM**: National Museum, PRAHA, Czech Republic; **PUR**: Purdue University, WEST LAFAYETTE, Indiana, USA; **S**: Swedish Museum of Natural History, STOCKHOLM, Sweden.

[b] PCA=presence in combined analysis; yes=present, no=absent.

[c] Sequences in normal font include ITS2 region only, in bold font include complete ITS region.

Bayesian analysis was conducted for the combined data set to estimate the posterior probability for the branches using MrBayes 3.1 (Huelsenbeck and Ronquist 2001). Each gene partition was defined with different DNA substitution models, estimated with Modeltest 3.6 (Posada and Crandall 1998). Four chains of 5,000,000 Markov chain Monte Carlo generations were run. The first 5000 trees were discarded (burn-in), and the remaining trees were spooled to PAUP* to obtain posterior probabilities based on the 50 % majority-rule consensus.

Morphological examination

Dried herbarium specimens were softened in a moist chamber for at least 30 min. Entire fungal sori or clumps of spores were removed using tweezers and mounted on microscope slides in lactic acid cotton blue media. To observe germ pores, clumps of urediniospores were mounted in Congo Red (Urban 1963). All cross-sections were cut using a MicroTome Crystat, model HM500 OMs (MICROM Laborgeräte GmbH, Walldorf, Germany). Slide mounts were examined using an Olympus BX51 Differential Interference Contrast Light Microscope (Olympus Canada Inc., Markham, Canada). Macroscopic morphology was examined using a Zeiss Discovery v12 Stereomicrosope (Carl Zeiss MicroImaging GmbH, Germany). Colors of sori were recorded using Kornerup and Wanscher (1967). Digital micrographs were taken with an Olympus DP 70 camera and analyzed by Image-Pro Plus ver. 6.0 Image Processing and Analysis Software (MediaCybernetics, Inc. Bethesda, USA) to obtain measurements.

The morphological characters examined in this study were mainly from uredinial and telial stages because specimens with aecial sori were not well-represented for each clade in our analyses. With the goal of finding synapomorphies supporting the main lineages, the following characters were examined: telia/uredinia — location of sori (abaxial/adaxial), color, size, openness (exposed/covered), and degree of loculation; telial paraphyses — presence/absence and shape (cylindrical/fused); teliospores — shape (clavate/obvoid, constricted at septum or not), size (length of spores, length of lower cell, width of widest spot of upper cell), apical wall color and thickness, digitations (number and length of the longest digitation), pedicles (length of the remnant and width of hilum); urediniospores — size, surface ornamentation (distance between warts), germ pores (number and invagination). Selected quantitative characters, including teliospore length/width, width of teliospore hilum, length of longest digitations per teliospore, urediniospore length and width, were analyzed using Student's *t*-test. Pair-wise comparisons of means for each character, calculated for taxa within each clade, were performed to assess whether they were statistically different among clades.

Fig. 1 Phylogenetic relationships of 154 OTUs inferred from parsimony analyses based on the ITS region. L=720, CI=0.524, RI=0.836, RC=0.438, HI=0.476, and G-fit=-138.126. Bootstrap values higher than 50 % are shown on the corresponding branches. Bold font indicates an OTU included in the combined analysis (Fig. 3). The bars corresponding to Clades I-XI are colour-coded to match the taxonomic groups shown in Fig. 3. Sequence labels include specimen accession number, species name (P. = *Puccinia*, Pc = *Puccinia coronata*), host name, source. Those ending in clade designations XII-XIX (red font) indicate de novo lineages, † indicates a new type designated in this study, ♦ indicates the seven previously designated types listed in the Phylogentic Analyses section, Results and discussion

Results and discussion

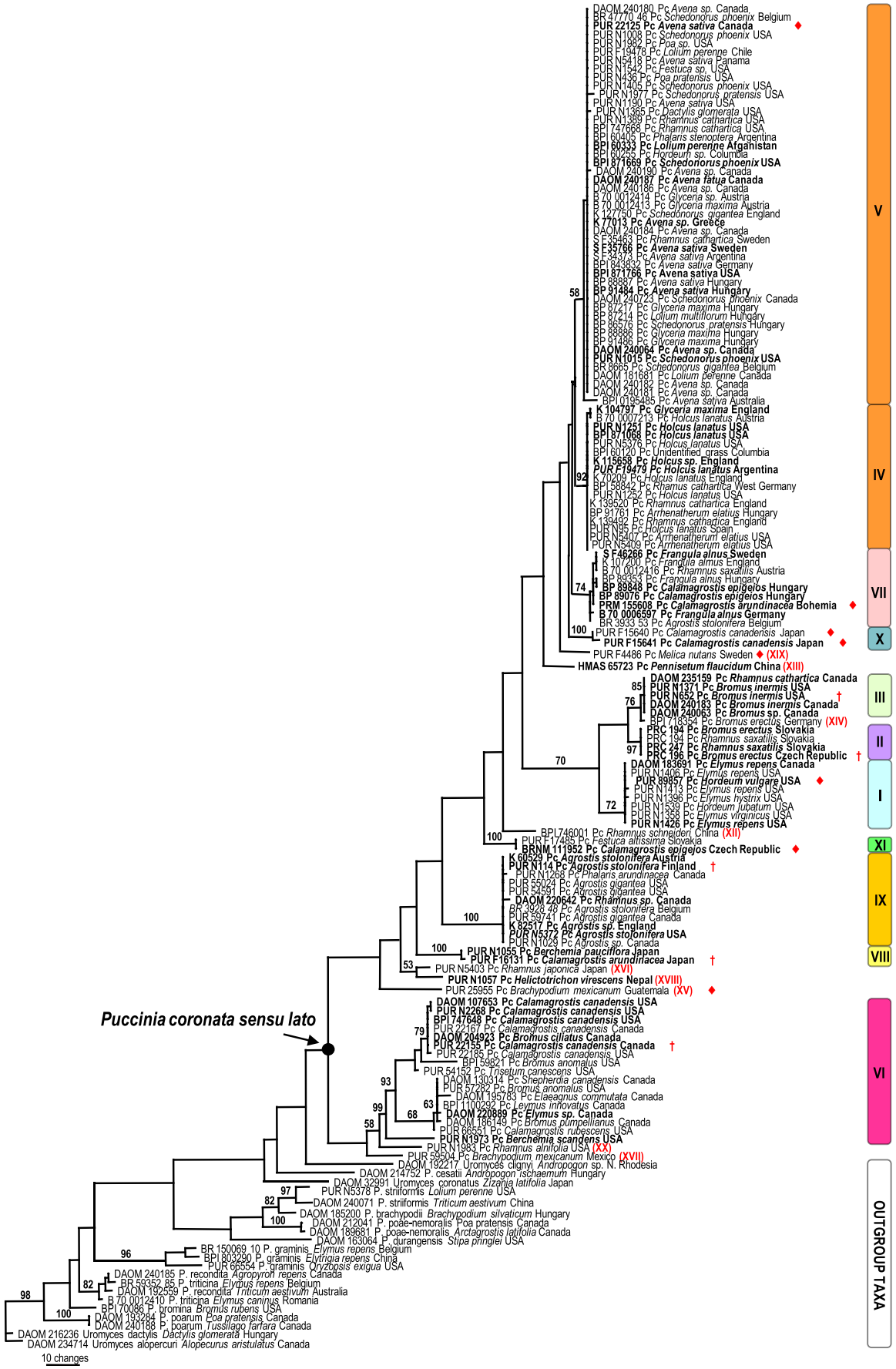
Genomic DNA extraction, PCR amplification and sequencing

Herbarium specimens of *P. coronata sensu lato* (s.l.) were successfully amplified and sequenced for the ITS region more often than for the protein-coding gene regions. For recently collected specimens, i.e. after the 1990s, the amplification success rate was 74 % while for those collected earlier than 1980, it decreased by about half to 34 % (Table 2). In total, 147 of 291 (50.5 %) processed specimens were successfully sequenced for the complete ITS region or for ITS2 alone (340-500 nt), of which 133 were selected for the analyses.

For the protein-coding genes, samples successfully sequenced were 54 of 64 attempted for COI (330 nt), 55 of 257 for BT (500–800 nt) and 32 of 257 for RPB2 (270–500 nt). Among 73 DNA samples attempted for PreCR repair, at least 30 were effectively amplified for at least one gene region. Overall older specimens were more difficult to amplify successfully. PrePCR treatment helped in some cases but success was unpredictable. For instance, the treatment was successful for the type specimen of *P. coronata* collected in 1894 but also for some recently collected specimens and not other older

Table 2 Number of DNA extractions and rDNA sequences obtained from *P. coronata* herbarium specimens

Year specimen collected	>2005	2004-2000	1999-1995	1994-1990	1989-1985	1984-1980	1979-1975	1974-1970	1969-1965	1964-1960	1959-1955	1954-1950	<1949	Total
Number of DNA extractions	38	24	29	31	20	25	12	15	10	29	20	10	28	291
Number of obtained ITS sequences	28	18	19	24	11	10	3	5	1	9	4	1	14	147
Rates (%)	73.7	75	65.5	77.4	55	40	25	33.3	10	31.0	20	10	50	50.5


Puccinia coronata sensu lato
V
IV
VII
X
III
II
I
XI
IX
VIII
VI
OUTGROUP TAXA
10 changes

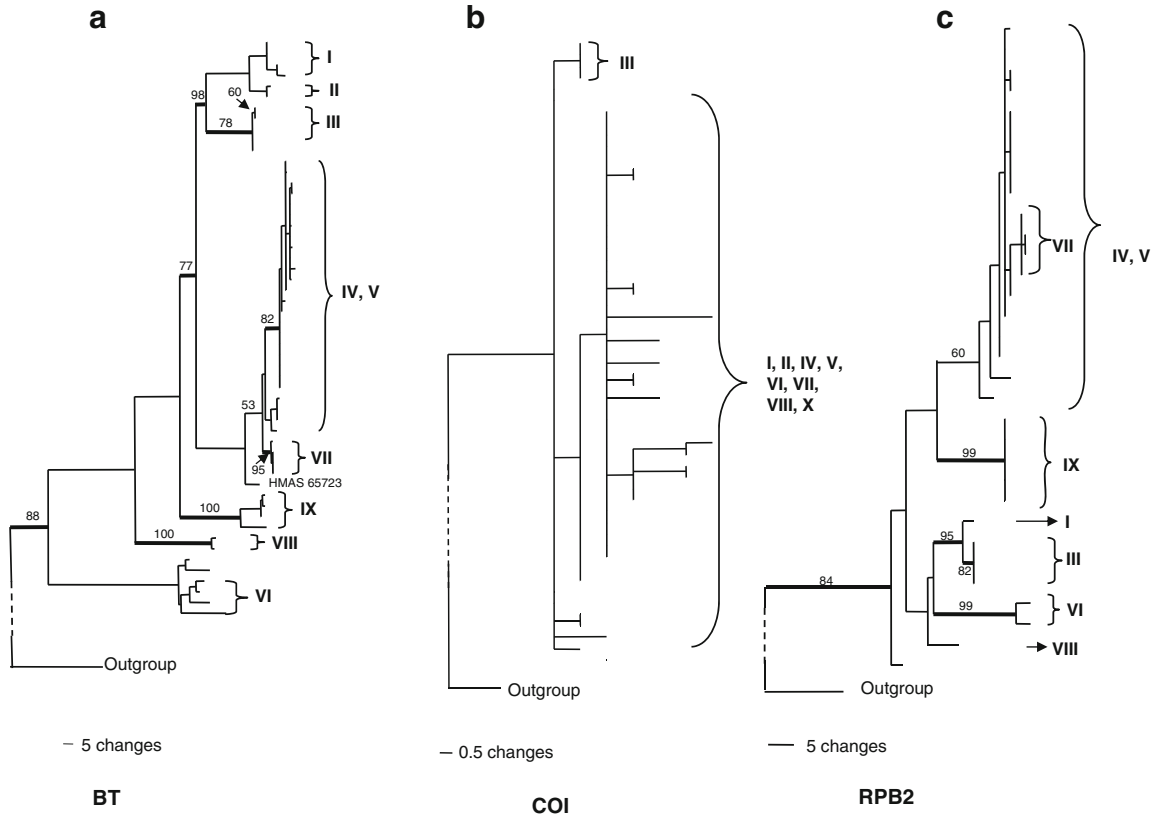


Fig. 2 Phylogenetic lineages of *Puccinia coronata* s.l. recovered by protein coding genes. **a**. BT, 1 of 65975 most parsimonious trees (MPTs), parsimony informative characters=250, L=736, CI=0.662, RI=0.856, RC=0.567, HI=0.338, and G-fit=-196.036; **b**: COI, 1 of 182,815 MPTs, parsimony informative characters=23, L=52, CI=0.962, RI=0.974, RC=0.936, HI=0.038, G-fit=- 22.500; **c**: RPB2, 1 of 14476 MPTs, parsimony informative characters=145, L=371, CI=0.728, RI=0.796, RC=0.579, HI=0.272, G-fit=- 123.357

specimens. The oldest specimen amplified and sequenced without PreCR treatment was collected in 1907.

Phylogenetic analyses

Partitioned analyses were conducted for each gene individually because of the disparity in taxon sets. Clade designations were applied as consistently as possible in the descriptions of the phylogenetic hypotheses resulting from each analysis.

ITS The data matrix comprised 133 *P. coronata,* 21 outgroup taxa and 734 characters (with insertions and deletions) of which 190 were parsimony informative. Parsimony analysis recovered 18,231 most parsimonious trees with length (L)=720, CI=0.524, RI=0.836, RC=0.438, HI=0.476, and G-fit=-138.126. Eleven major clades with moderate to high bootstrap support were recognized as shown on Fig. 1 (I–XI) plus nine additional single taxon clades (XII–XX). Six of these (Clade I–VI) were congruent with results from the previous study by Szabo (2006). For convenience, these clades were similarly labelled as Clades I–VI on Fig. 1. Consequently, the additional five new major clades were labelled as VII–XI.

The nine single taxon clades potentially represented de novo lineages within the *P. coronata* complex: BPI 746001 (XII) and HMAS 65723 (XIII) from China, BPI 718354 (XIV) from Germany (sister to the North American Clade III, see Discussion), PUR 25955 (XV) from Guatemala (type of *P. subdigitata*), PUR N5403 (XVI) from Japan, PUR 59504 (XVII) from Mexico, PUR N1057 (XVIII) from Nepal, PUR F4486 (XIX) from Sweden (type of *P. melicae*), and PUR N1983 (XX) from USA. ITS sequences were lacking for only two collections sampled for this study: DAOM 240722 from Canada which groups with Clade IX based on BT data only (not included in the final analyses) and PUR F16064 from Great Britain which groups with Clade IV/V based on RPB2 data but Clade IV based on host association (data not shown).

Seven previously designated types were included in the ITS analysis. The phylogenetic placement of these reference specimens in the ITS phylogram (Fig. 1; indicated by the symbol ♦ in red font) was as follows. Clade V: type of *P. coronata* var. *avenae* Fraser & Ledingham (PUR 22125, lectotypified by Cummins [1971]); Clade VII: type of *P. coronata* var. *coronata* Corda (PRM 155608); Clade X: *P. brevicornis* Ito (PUR F15640 and PUR F15641, syntypes), synonym of *P. coronata* var *himalensis* (Barcl.) Cummins 1971; Clade I: type of *P. coronata* var. *hordei* Jin and

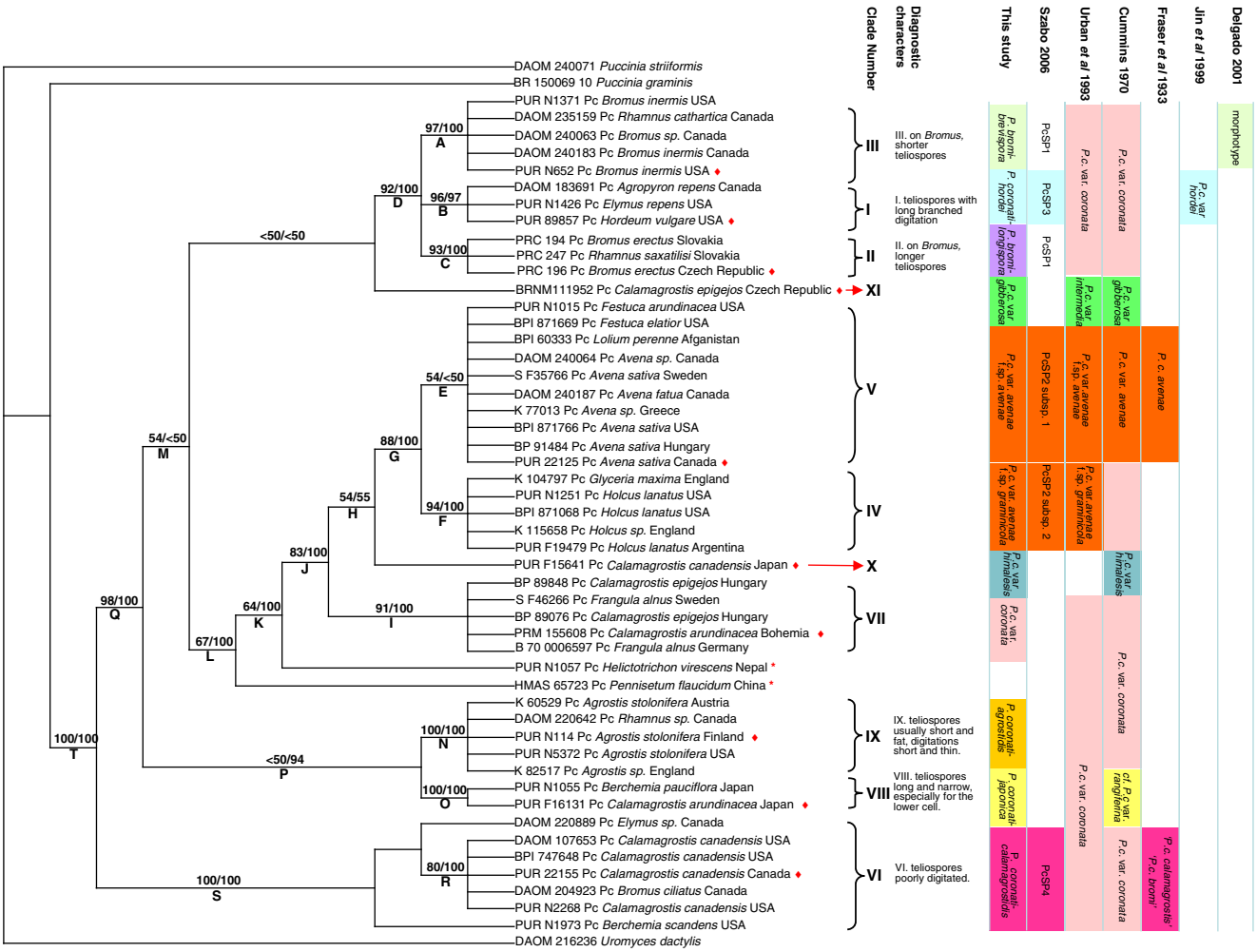


Fig. 3 Strict consensus of 60,916 most parsimonious trees based on ITS, BT, COI and RPB2 combined data set. Parsimony informative characters=370, L=1149, CI=0.746, RI=0.828, RC=0.617, HI=0.254, G-fit=- 308.314. Bootstrap values/posterior probability values are shown on the corresponding branches. Major nodes labeled as **A**–**T** were analyzed for Partitioned Bremer Support (see Table 3). In columns to the right of the phylogram, the taxon names accepted here for Clades I-XI are listed in the same colour-coded boxes as in Fig. 1, and compared with previous names or concepts accepted by the researchers indicated at the top of each column. Diagnostic characters are listed for selected taxa (Clades I, II, III, VI, VIII and IX). ♦ indicates a type specimen, * indicates a de novo lineage (Clades XIII and XVIII in Fig. 1)

Steffenson 1999 (PUR 89857); Clade XI: type of *P. coronata* var. *intermedia* Urban 1967 (BRNM 111952), accepted as a synonym of *P. coronata* var *gibberosa* (see Taxonomy section); and two single taxon lineages: type of *P. melicae* Sydow (PUR F4486, Clade XIX, sister to Clade IV+V+VII+X) and type of *P. subdigitata* Arth. & Holw. (PUR 25955, Clade XV). Types were designated in this study for Clades II, III, VI, VIII and IX (Fig. 1; indicated by the symbol † in red font).

BT Fifty four *P. coronata* and nine outgroup specimens were included in an alignment of 837 characters, among which 250 characters are parsimony-informative. Parsimony analysis recovered 65975 most parsimonious trees with L=736, CI=0.662, RI=0.856, RC=0.567, HI=0.338, and G-fit=-196.036. Clades congruent with the ITS tree include I–III, VI, VII, VIII, and IX, while an unresolved group comprised Clades IV and V (Fig. 2). DNA sequences for taxa grouping in Clades X and XI of the ITS tree were not obtained for this locus.

COI Fifty four *P. coronata* and nine outgroup specimens were included in an alignment of 373 characters, among which 23 characters are parsimony informative. Parsimony analysis recovered 182815 most parsimonious trees with L=52, CI=0.962, RI=0.974, RC=0.936, HI=0.038, and G-fit=-22.500. The analysis resulted in an unresolved phylogram except for Clade III (Fig. 2).

RPB2 Thirty two *P. coronata* and seven outgroup specimens were included in an alignment of 501 characters,

Table 3 Partitioned Bremer Support for selected nodes on combined gene tree

Node	ITS	β-tubulin	COI	RPB2	Total
A	1.6	2.4	-0.1	0.2	4.0
B	1.3	-0.3	0.1	-0.1	1.0
C	1.8	0.1	0.2	-0.1	2.0
D	5.5	0.2	-0.5	-0.2	5.0
E	3.9	-2.9	0.2	-0.1	1.0
F	1.3	-0.1	0.0	-0.2	1.0
G	4.2	-3.5	0.2	0.1	1.0
H	1.6	-0.7	0.2	-0.1	1.0
I	0.2	2.2	0.2	0.5	3.0
J	0.3	0.6	0.2	0.0	1.0
K	0.0	-2.3	0.3	4.1	2.0
L	0.1	-2.1	0.2	3.9	2.0
M	2.0	0.6	-0.3	-0.3	2.0
N	6.6	-0.4	0.1	3.8	10.0
O	0.0	5.6	1.2	0.2	7.0
P	Not present in strict consensus tree				
Q	5.6	4.5	-0.3	0.2	10.0
R	1.6	0.3	0.0	0.2	2.0
S	11.6	0.6	-1.4	0.2	12.0
T	14.5	-1.3	5.6	13.2	32.0

among which 145 characters are parsimony informative. Parsimony analysis recovered 14476 most parsimonious trees with L=371, CI=0.728, RI=0.796, RC=0.579, HI=0.272, and G-fit=-123.357. Clades III, VI, VII and IX were recovered while Clades IV and V presented as a single unresolved paraphyletic group (Fig. 2). Clades I, VIII and XI were single-taxon lineages, due to the reduced number of specimens sampled for this gene.

Although there were no conflicts in terms of the groupings of taxa into clades among three loci, ITS, BT and RPB2, relationships among the clades were inconsistent and multiple internal branches received low bootstrap support (BP), likely caused by the limited number of characters. In this case a holistic analysis is more appropriate to maximize the phylogenetic signal (Smith 2000). Partitioned Bremer Support (PBS) was used to measure the degree of support provided by each locus. For the combined data set, we included taxa for which DNA sequences were obtained for at least three loci (with the additional of some type specimens for which only ITS was available). The unavailable loci for some taxa were treated as missing data. Fifty-two taxa were compiled resulting in a matrix with 2,447 characters, among which 370 characters are parsimony informative. Parsimony analysis recovered 4901 most parsimonious trees with L=1149, CI=0.746, RI=0.828, RC=0.617, HI=0.254, and G-fit=-308.493 (Fig. 3). Nine clades received high BP support, except Clade V (branch E, BP=54 %) and some internal branches (H, K, L, and M). The low support for branch E was predicted by the incongruence between the ITS and BT data sets, as shown by PBS values (ITS=3.9, BT=-2.9, Table 3), as well as the low or no support from the other two loci (COI=0.2 and RPB2=-0.1). The low support values for branches K and L were likely similarly due to the incongruence between BT and RPB2 (Table 3). In the other cases, signals were generally low while branch P was not retained in strict consensus tree and thus no PBS was available.

The models and priors for each gene partition estimated using Modeltest were as follows: for ITS, selected model F81+I+G, base frequency (A, C, G, T) 0.3511 0.1889, 0.1106, 0.3494, all substitution rates equal, proportion of invariable sites (I)=0.4873, gamma distribution shape parameter=0.5811; for BT, HKY+G model, base frequency 0.2338 0.2785 0.2104 0.2774, ti/tv ratio=1.8865, I=0, gamma distribution shape parameter=0.2436; for COI, F81+G model, base frequency 0.2507 0.1596 0.2545 0.3351, all substitution rates equal, I=0, gamma distribution shape parameter=0.5346; for RPB2, TrNef+I+G, base frequency equal, substitution rates are 1.0000 4.7382, 1.0000 1.0000 7.9439 1.0000, I=0.3910, gamma distribution shape parameter=0.3803. The Bayesian analyses generally resulted in higher posterior probability (PP) support for most branches (Fig. 3), although branches E, H and M still had low support (both low BP and PP). The tree topology estimated from the combined data set recovered Clade VI as the sister lineage to the one including all other taxa in the *P. coronata* complex. Phylogenetic trees based on ITS, BT, COI, RPB2 and the concatenated matrix can be accessed through TreeBASE at http://purl.org/phylo/treebase/phylows/study/TB2:S12078?x-access-code=cda847cd1fe4f973c6106a06f0912e25&format=html.

Morphology

Generally speaking, the morphological features useful for characterizing rust fungi are variable within lineages of this species complex and overlapping among them. Student's *t*-test analysis of selected quantitative characters, including teliospore length/width, width of teliospore hilum, length of longest digitations per teliospores, urediniospore length and width, showed that although the ranges overlapped, the means of these measurements were significantly different (Table 4) among some clades and as a result, selected taxa can be recognized using a combination of morphological characters, host association and geographic origin. Groupings of clades based on our statistical tests are summarized in Table 5. A synoptic key is presented to facilitate the identification of the nine accepted taxa (corresponding to Clades I–IX).

Recognition of taxa

Based on our molecular evidence, in conjunction with morphology, telial host range and geographic distribution, and comparison with the previous classifications, seven species were recognized (Table 1). The type species, *P. coronata* was further divided into two varieties, of which one comprises two *formae speciales*. All crown rust specimens from oats were resolved as a phylogenetically distinct group within the *P. coronata* complex (for more discussion see *P. coronata* var. *avenae*, Taxonomy section). Considering the strong support for *P. coronata* s.l. as a monophyletic group (100 %), we erected a new Series *Puccinia* Series *Coronata* to define this phylogenetically cohesive lineage. As argued in Liu and Hambleton (2010) for the Series *Striiformis*, the incorporation of additional subgeneric ranks will contribute to a refined classification within the genus *Puccinia*.

For each taxon accepted, the host ranges provided are based only on the provenance of the specimens included in the phylogenetic analyses presented here and do not include other hosts documented elsewhere in the literature. Therefore, alternate host associations are listed only if specimens on the aecial host were sampled. Several taxa correspond to previously published concepts for varieties or f. spp. (Fig. 3): two named by Fraser and Ledingham (1933), four by Cummins (1971), four by Urban and Marková (1993), six clades recognized by Szabo (2006); and also the varieties recognized by Jin and Steffenson (1999) and Delgado et al. (2001).

Among members of Series *Coronata* there is a large degree of genetic diversity, and morphological variation within and between taxa. Multiple orphan taxa were present in our ITS tree, which might represent discrete species, and each one deserves a detailed study based on multiple representatives. Far from being a complete monograph, our review serves as a framework and stepping-stone for further investigation. Although individual clades corresponding to the recognized species were strongly supported by statistical measures, most of the relationships among clades remained unresolved. It is essential to obtain sequences from additional loci and specimens to resolve the uncertain relationships.

Taxonomy

Puccinia Series Coronata Liu and Hambleton **ser. nov**. (MycoBank #: MB563540)

Uredosporis globosis vel ellipsoideis, tenuiter echinulatis, poris 4–15, sparsis, obscurissimis; teleosporis, bicellularis, vel interdum unicellularis aut tricellularis, clavatis, ad apicum coronatis digitiformis.

Type species: *Puccinia coronata* A.C.J. Corda

A synoptic key to treated taxa in Puccinia Series Coronata

Each taxon is referenced in the key by its number assigned as follows:

1. *P. coronata* var. *avenae*
   1a. *P. coronata* var. *avenae* f. sp. *avenae*
   1b. *P. coronata* var. *avenae* f. sp. *graminicola*
2. *P. coronata* var. *coronata*
3. *P. coronati-agrostidis*
4. *P. coronati-brevispora*
5. *P. coronati-calamagrostidis*
6. *P. coronati-hordei*
7. *P. coronati-japonica*
8. *P. coronati-longispora*

Twenty-seven host genera/species were summarized based on examined specimens. For each morphological character there are multiple states. Each character state and host genus/species is connected to numbers, which represent correlated taxa. Underlined numbers indicate taxa exhibiting multiple possible character states.

1. Host

Agrostis 2, 3
Arrhenatherum 1b
Avena 1a
Berchemia scandens 5
Bromus anomalus 5
Bromus ciliatus 5
Bromus erectus 8
Bromus inermis 4
Calamagrostis arundinacea 2, 7
Calamagrostis canadensis 5
Calamagrostis epigeios 2
Calamagrostis rubescens 5
Dactylis glomerata 1a
Elymus 5, 6
Festuca 1a
Frangula alnus 2
Glyceria 1a, 1b
Holcus 1b
Hordeum 1a, 6
Leymus innovatus 5
Lolium 1a
Phalaris 1a, 3
Poa 1a
Rhamnus cathartica 1a, 1b, 4, 3
Rhamnus saxatilis 2, 8
Schedonorus 1a
Shepherdia 5
Trisetum 5

2. Telium

2.1 Position
2.1.1 Predominantly abaxial, almost no infection on adaxial ...5, 7
2.1.2 Infection on abaxial surface heavier ...2, 3
2.1.3 Amphigenuos ...1, 4, 6, 8

2.2 Loculation
2.2.1 Not loculate ...1b, 4, 6, 7, 8
2.2.2 Slightly loculate ...1b, 3, 4,, 6, 8
2.2.3 Clearly loculate ... 1a, 3, 5

2.3 Presence of paraphyses
2.3.1 Abundant ...5
2.3.2 Present, a few ...1a, 1b, 2
2.3.3 Rarely observed ...3, 4, 6, 7
2.3.4 Not observed ...8

2.4 Shape of papraphyses
2.4.1 Fused sheet-like ...1a, 2, 3, 5
2.4.2 Clavate or cylindrical ...1a, 1b, 3, 4, 6, 7

3. Teliospore

3.1 Shape of spores
3.1.1 Clavate ...1a, 1b, 2, 3, 4, 6, 7, 8
3.1.2 Short clavate ...1a, 1b, 3, 4, 5
3.1.3 Obovoid ...1a, 1b, 3, 5, 6

3.2 Presence of 3-celled spore
3.2.1 Common ...8
3.2.2 Occasionally observed ...2
3.2.3 Not observed ...1a, 1b, 3, 4, 5, 6, 7

3.3 Presence of mesospore
3.3.1 Present ...1a, 2, 3, 4, 5, 6, 8
3.3.2 Not observed ...1b, 7

3.4 Mean length excluding digitations
3.4.1 Shorter than 45 μm ...3, 4,
3.4.2 Medium 45–55 μm ...1a, 1b, 5, 6, 7, 8
3.4.3 Long, over 55 μm ...2

3.5 Mean width
3.5.1 Narrow 13–15 μm ...2, 4, 8
3.5.2 Medium 15–16 μm ...1b, 3, 5, 6, 7
3.5.3 Wide, over 16 μm ...1a

3.6 Mean width of hilum
3.6.1 Narrow 5–6 μm ...3, 4, 5, 8
3.6.2 Medium 6–6.5 μm ...2, 6
3.6.3 Wide, over 6.5 μm ...1a, 1b

3.7 Apical digitation
3.7.1 Poorly digitated, digitation toe-like; or mean length of the longest digitation shorter than 5 μm ...5
3.7.2 Mean length of the longest digitation 6–8 μm ...2, 3, 7
3.7.3 Mean length of the longest digitation 8–12 μm ...1a, 1b, 4, 8
3.7.4 Mean length of the longest digitation over 12 μm ...6

3.8 Septum
3.8.1 Spore constrict at septum ...1a, 1b, 2, 3, 4, 5, 6, 7, 8
3.8.2 Constriction not obvious ...1a, 1b, 2, 4, 5, 6, 8

4. Uredinium (taxa 2 and 8 were not available for this state)

4.1 Size
4.1.1 Regular size 0.2–1.0×0.1–0.2 mm ...1a, 1b, 4, 5, 6, 8
4.1.2 Look smaller than regular size, 0.1–0.5×0.05–0.1 mm ...3

4.2 Position
4.2.1 Predominantly adaxial ...1b, 3, 8
4.2.2 Amphigenous ...1a, 4, 5, 6

5. Urediniospore (taxa 2 and 8 were not available for this state)

5.1 Mean length
5.1.1 Shorter than 20 μm ...4
5.1.2 Medium 20–22 μm ...1b, 3, 6, 8
5.1.3 Longer than 22 μm ...1a, 5

5.2 Mean width
5.2.1 Narrower than 18 μm ...1b, 3, 4
5.2.2 Medium 18–19 μm ...1a, 6, 8
5.2.3 Wider than 19 μm ...5

1. P. coronata var. avenae Fraser & Ledingham, Scientific Agriculture (1933) 13:313-323. (Figs. 4a–z; Clades IV and V)

Uredinia amphigenous or predominantly on adaxial leaf surface, 0.2–0.8×0.1–0.2 mm, covered or ruptured (Fig. 4a), paraphyses cylindrical; spores 15–29 (32)×(11) 15–25 μm, germ pores 5–15, scattered, vague, some show invagination or cuticular caps in congo-red stain. Telia often covered on abaxial leaf surface, ruptured on adaxial, 0.1–0.4×0.1–0.2 mm, ovoid to spherical, merged into long strips or irregular shapes, loculated or slightly loculated; teliospores variable, obvoid, short-clavate and clavate, not

constricted or constricted at septa, some with a bump close to septum in lower cell, (28) 30–75×(9) 11–21 (24) μm; digitations of various shapes, either long and sinuous or branched, medium length and sturdy, short and bump-like, or otherwise irregularly formed; the longest digitations per spore (3) 6–16 (20) μm; pedicel remnants hyaline to brownish, hila (3) 5–10 μm.

Lectotype: on *Avena sativa*, leg. Fraser, Canada, Saskatchewan, Saskatoon, July 25, 1923 (PUR 22125). Lectotype designated by Cummins (1971). Type seen!

1a. **P. coronata var. avenae f. sp. avenae** Urban & Marková, Acta Universitatis Carolinae (1993) 37:93-147 (Figs. 4a–o; Clade V)

Uredinia amphigenous, spores 15–29 (32)×13–25 μm, most cases with 9–15 germ pores, usually show clear invagination or culticular caps in congo-red stain (Fig. 4j), occasionally 5–8 obscure pores. Telia often clearly loculated (Fig. 4d, i); teliospores (28) 30–67 (72)×12–21 (24) μm, 1-celled spores observed.

Host: on *Avena*, *Dactylis*, *Hordeum*, *Festuca*, *Glyceria*, *Lolium*, *Phalaris*, *Poa* and *Schedonorus*.

Distribution: cosmopolitan.

Other specimens examined: **Afghanistan**, intercepted in New York?, on *Lolium perenne*, 1976 (BPI 060333) — **Austria**, Bergenland , Gussing, 'S der Strabe nach Sulz am N-Ufer des Fischteiches', on *Glyceria maxima*, 31 Aug. 1981, J. Haifellner & J. Poelt (B 70 0012413). Styria, south-east of Stubenberg, 'beim SchloB Schielleiten, Ufer des groBen Badeteiches (Gelande der Bundessportschule), on *Glyceria* sp. , 23 Aug. 1983, J. Poelt & H. Pittoni (B 70 0012414) — **Canada**, Ontario, Niagara fall, Vineland station, bank of the lake Ontario, 20 Aug. 2006, M. Liu (DAOM 240723) — **Columbia**, Intercepted in Miami Florida #10464, on *Hordeum* sp., 15 Jan 1960 (BPI 060255) — **Greece**, Isle of thasos, Skala Potamia, on *Avena* sp., 21 May 2000, A. Andrews (K(M): 77013) — **Hungary**, 'comit. Vas, ad/haud procul opp. Kőszeg', on *Glyceria maxima*, 21 Oct. 1986 S. Tóth (BP 91486). 'Comit. Pest, pr. Pag. Cinkota ad lacum "Naplás-tó" in Phragmitetum', on *Glyceria maxima*, 10 Oct 1993, E. I. Simay (BP 88886). 'Comit. Vas, in agris pr. Pag. Torony haud procul opp. Szombathely', on *Lolium multiflorum*, 21 Oct. 1982, S. Tóth (BP 87214). 'Comit. Pest, in pratis udis ad rivulum Rákospatak, immediate infra opp. Gödöllő', on *Glyceria maxima*, 24 Sept. 1980, S. Tóth (BP 87217). 'Comit. Veszprém, pr. Rivulum Bakony-ér: haud procul pagi Pápakovácsi', on *Avena sativa*, 3 July 1986 S. Tóth & A. Horánszky (BP 91484). 'Comit. Pest, pr. Pag. Tápiószele in agris.', on *Avena sativa*, 5 Oct. 1988, E. I. Simay (BP 88887) — **Sweden**, Gastrikland, valbo, on *Avena sativa*, 7 Sept. 2003, G. Odelvik & B. Hellstrom ((S)reg.nr. F 35766) — **United States**, Minnesota, Rock Creek State Park, west of Grinnell, Jasper Co., on *Schedonorus phoenix*, 21 Sept. 1995, A. P. Roelfs & J. W. McCain (PUR N1015). North Dakota, Cass Co., Fargo, on *Avena sativa*, 29 Sept. 2004, R. W. Stack (BPI 871776). Virginia, Montgomery Co., Blacksburg 607 Lucas Dr., alt. 2175 ft, on *Schedonorus phoenix*, 04 Oct. 2004, C. W. Roane (BPI 871669).

1b. **P. coronata var. avenae f. sp. graminicola** Urban & Marková, Acta Universitatis Carolinae (1993) 37**:**93-147 (Figs. 4p–z; Clade IV)

Uredinia predominantly adaxial, spores 16–28 (31)×(11) 15–22 μm, germ pore 6–11, invagination not clear and no culticular caps in congo-red (Fig. 4w, x). Telia slightly to no loculated, teliospores (30) 34–75×(9) 11–19 (21) μmm, 1-celled spores not observed.

Host: *Arrhenatherum elatius*, *Glyceria* and *Holcus.*

Distribution: North America, South America, Europe.

Specimens examined: **England**, Middlesex, Ealing, Perivale Wood, on *Glyceria maxima*, 05 Oct. 2002, A. Henrici (K(M): 104797). Berkshire, Windsor Great Park; Bishopsgate entrance, on *Holcus lanatus*, 18 Aug. 2000, N. W. Legon (K(M): 70209). South Somerset, Staple Common (Blackdown Hills); near Castle Neroche, on *Holcus* sp., 15 July 2003, N. W. Legon (K(M): 115658) — **Hungary**, 'montes Bükk-hegység, ad margines silvarum "Bolhás" pr. Fontem Jávor-kut', on *Arrhenatherum elatius*, 09 Sept. 1982, S. Tóth (BP 91761) — **United States**, Hawaii, Big island, Mauna Loa Road above Hawaii Volcanoes National Park, alt. 1000 m, 19°26′281″N, 155°18′334″W, on *Holcus lanatus*, 05 Aug. 2005, M. Schöller MCA2958 (BPI 871068). California, Redwood National Park, Bald Hill Prairies, on *Arrhenatherum elatius*, 01 Oct. 1991 (PUR N5407). Virginia, Montgomery, Blacksburg, on *Arrhenatherum elatius*, 27 Jun. 1990, C. W. Roane (PUR N5409).

Discussion: *Puccinia coronata* var. *avenae* was first designated by Fraser and Ledingham (1933), and accepted by Cummins (1971) and Urban and Marková (1993). We examined and sequenced the type specimen (PUR 22125), which grouped in Clade V of our phylogenetic hypotheses. Urban and Marková (1993) further separated *P. coronata* var. *avenae* into two *formae speciales*, noting that f. sp. *avenae* was narrowly specialized on cultivated and other oat species, while f. sp. *graminicola* was specialized on *Ar. elatius* and *R. carthatica.* Both our ITS tree (with extensive sampling) and combined tree provide support for this partition (samples on *Avena* grouped in Clade V and those on *Ar. elatius* in Clade IV). Although Clades IV and V were strongly supported as sister clades in our combined gene tree (Fig. 2), Clade IV received strong individual support but not Clade V. Therefore, the combination of these two groups is recognized as *P. coronata* var. *avenae*. However, the statement by Urban and Marková (1993) that oat crown rust was

Table 4 Unbalanced two tail *T*-test of selected quantitative characters on pair-wise comparison of clades

clade	Mean (μm)	n	clade II	clade III	clade IV	clade V	clade VI	clade VII	clade VIII	clade IX
Teliospore length										
clade I	45.6	128.0	<0.01	<0.01	<0.01	<0.01	**0.153**	<0.01	**0.229**	<0.01
clade II	51.9	89.0		<0.01	**0.161**	<0.01	<0.01	<0.01	<0.01	<0.01
clade III	42.2	81.0			<0.01	<0.01	<0.01	<0.01	<0.01	<0.05
clade IV	50.5	145.0				**0.180**	<0.01	<0.01	<0.01	<0.01
clade V	49.0	177.0					**0.076**	<0.01	<0.05	<0.01
clade VI	47.0	133.0						<0.01	**0.825**	<0.01
clade VII	59.0	107.0							<0.01	<0.01
clade VIII	46.7	46.0								<0.01
clade IX	40.0	162.0								
Teliospore width										
clade I	15.4	144	<0.01	<0.01	**0.948**	<0.01	**0.416**	<0.01	**0.142**	**0.788**
clade II	13.7	81		**0.294**	<0.01	<0.01	<0.01	**0.079**	<0.01	<0.01
clade III	13.3	79			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
clade IV	15.4	158				<0.01	**0.461**	<0.01	**0.137**	**0.849**
clade V	16.7	179					<0.01	<0.01	<0.01	<0.01
clade VI	15.7	108						<0.01	**0.044**	**0.538**
clade VII	14.1	106							<0.01	<0.01
clade VIII	15.0	46								**0.079**
clade IX	15.5	158								
Teliospore hilum width										
clade I	6.4	119	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.01	<0.01
clade II	5.4	79		**0.741**	<0.01	<0.01	**0.138**	<0.01	<0.05	**0.126**
clade III	5.4	58			<0.01	<0.01	**0.398**	<0.01	<0.05	**0.372**
clade IV	7.0	185				**0.256**	<0.01	<0.01	<0.01	<0.01
clade V	6.8	227					<0.01	<0.01	<0.01	<0.01
clade VI	5.5	193						<0.01	<0.01	**0.938**
clade VII	6.1	97							<0.01	<0.01
clade VIII	5.0	31								<0.01
clade IX	5.5	154								
Teliospore longest digitation										
clade I	12.6	108	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
clade II	8.5	79		<0.01	**0.194**	**0.441**	<0.01	<0.01	<0.01	<0.01
clade III	10.9	83			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
clade IV	8.9	172				**0.525**	<0.01	<0.01	<0.01	<0.01
clade V	8.7	180					<0.01	<0.01	<0.01	<0.01
clade VI	4.7	154						<0.01	<0.01	<0.01
clade VII	7.2	125							**0.621**	**0.058**
clade VIII	7.0	111								**0.299**
clade IX	6.7	210								
Urediniospore length [a]										
clade	mean	n	clade II	clade III	clade IV	clade V	clade VI	clade IX		
clade I	20.5	143.0	<0.01	<0.01	<0.05	<0.01	<0.01	<0.05		
clade II	21.4	45.0		<0.01	**0.669**	<0.05	<0.01	**0.456**		
clade III	19.4	67.0			<0.01	<0.01	<0.01	<0.01		
clade IV	21.2	102.0				<0.01	<0.01	**0.796**		
clade V	22.2	197.0					**0.051**	<0.01		
clade VI	22.8	76.0						<0.01		
clade IX	21.1	103.0								

Table 4 (continued)

clade	Mean (μm)	n	clade II	clade III	clade IV	clade V	clade VI	clade VII	clade VIII	clade IX
Urediniospore width [a]										
clade I	18.3	143.0	**0.063**	<0.01	<0.01	**0.239**	<0.01	<0.01		
clade II	18.8	45.0		<0.01	<0.01	**0.446**	<0.01	<0.01		
clade III	17.3	67.0			**0.203**	<0.01	<0.01	**0.491**		
clade IV	17.7	102.0				<0.01	<0.01	**0.333**		
clade V	18.6	197.0					<0.01	<0.01		
clade VI	19.7	76.0						<0.01		
clade IX	17.5	103.0								

[a] Urediniospore specimens were not available for clade VII and VIII, thus only seven clades were compared

specialized only on oats was not supported. Based on our sampling and genes analysed, *P. coronata* var. *avenae* f. sp *avenae* has a wide host range, i.e. 7 genera in 3 tribes while f. sp *graminicola* occurs not only on *Ar. elatius*, but also on *Holcus* spp and *Glyceria* spp.

Both f. spp. are characterized by having relatively wide hila and variable teliospores. Telia of *P. coronata* var. *avenae* f. sp. *avenae* are more clearly loculated (Figs. 4d, i) than f. sp. *graminicola* (Figs. 4u, z). Urediniospore germ pore invaginations and cuticular caps are usually present in the former (Fig. 4j) but less often in the later (Figs. 4w, x).

2. **P. coronata** Corda Icones fungorum hucusque cognitorum (1837) 1:6 **var coronata** (Figs. 5l, m; Clade VII)

Uredinia not observed. Telia amphigenous, but abaxial infection heavier, covered or with a longitude rupture, 0.2–0.4×0.1–0.2 mm, ovoid, merge to irregular shapes, slightly loculated; paraphyses fused; teliospores clavate to long clavate (Fig. 5m), (27) 32–80 (84)×11–19 μm, often with long lower cells, average around 32 μm, 3-celled spores present (Fig. 5l), digitations tuberculate, cylindrical, bifurcate, the longest per spore 4–13 (15) μm, pedicel hilum 4–8 μm.

Lectotypus (hic designatus): Icones fungorum hucusque cognitorum (1837) 1:6 Tab II fig 96.

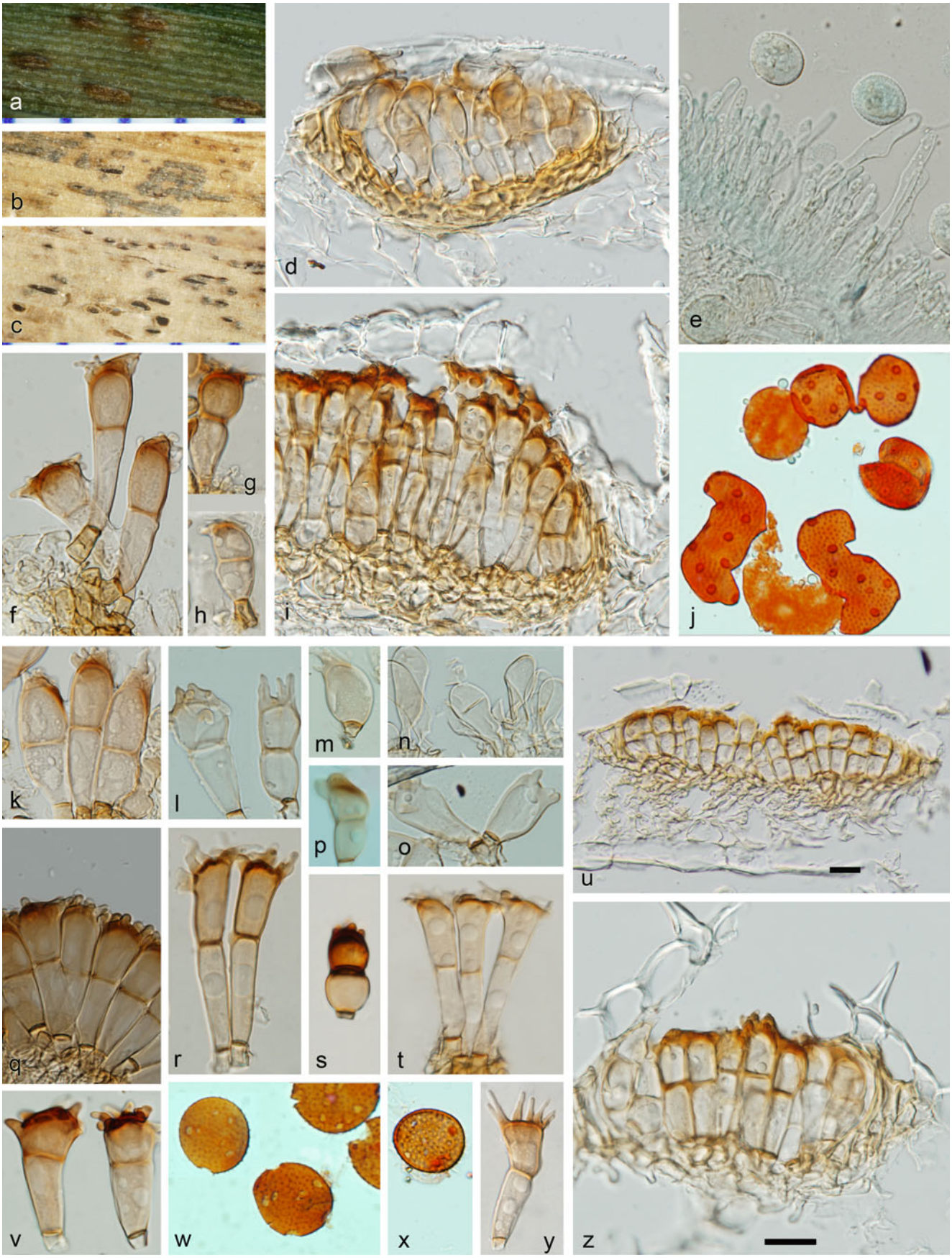
Epitypus (hic designatus): PRM 155608, reported to be 'in foliis Luzulae albidae prope Reichenberg' by Corda (1837). The host was redetermined to be *Calamagrostis arundinacea* or *Cal. villosa* by M. Deyl (II 1961, note with specimen), while previously it was suggested to be *Bromus erectus* (Mühlethaler 1911) which was contested by I. Jørstad (May 1949, note with specimen). 'Reichenberg' is probably Liberec, Bohemia according to the current label, leg. & det. Corda. Type seen!

Host: *Agrostis stolonifera, Calamagrostis arundinacea* seu *villosa*, and *Calamagrostis epigeios*.

Table 5 Grouping of the clades based on statistical tests on selected quantitative morphological characters

teliospore length (μm) [a]	VII > 59	I, II, IV, V, VI,VIII > 45<M<52	III > 42	IX 40	
teliospore width (μm)	V > 16.7	I, IV, VI, VIII, IX > 15<M <16	II, III, VII 13<M <15		
teliospore hilum width (μm)	IV, V > 6.5<M	I > 6.36	VII > 6.1	II, III, VI, IX > 5<M<6	VIII 5.0
teliospore longest digitations (μm)	I > 12.6	III > 10<M <12	II, IV, V > 8<M<10	VII, VIII, IX > 6<M<8	VI M<5
urediniospore length (μm)	V, VI > 22<M	II, IV, IX > 21<M<22	I > 20<M<21	III M<20	
urediniospore width (μm)	VI > 19.7	I, II, V > 18<M<19	III, IV, IX M<18		

[a] Roman letters stand for the clades; M: mean, >: significantly larger than the next category.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◀ **Fig. 4** Telial and uredinial states of ***P. c.* var. *avenae***. **a**–**o**: *P. c.* var. *avenae* f. sp. *avenae*; **p**–**z** : ***P. c.* var. *avenae*** f. sp. ***graminicola***. **a**. uredinia with longtitudinal rupture (BPI 871766). **b**. telia of abaxial leaf surface mostly covered (BP 91486); **c**. telia on adaxial leaf surface often ruptured (BP 91486); **d** and **i**. loculated telia (PUR N1015); **f**–**h**, **k**–**o**: teliospores of various shapes (**f**: B 70 0012413, G, **h**: BPI 871669); **e**. uredinospores and paraphyses (BPI 871766); **j**. urediniospores showing cuticular caps and invaginations in congo-red stain (K (M):77013) **p**–**t**, **v**, **y** showing variable shapes of teliospores (BP 91761, PUR N5409, K(M):104797, BP 91761, K(M):104797); **u**, **z** showing telia slightly loculated (K(M):115658, K(M):115658); **w** urediniospore germ pores with no invagination and cuticular caps in congo-red stain (K(M):115658); **x** urediniospore germ pores with slight invagination and cuticular caps in congo-red stain (K (M):70209). For **a**, **b** and **c**, 1 unit=1 mm, **d**–**z** (except **u**) share the same scale, scale bar=20 μm

Alternate host: *Frangula alnus*, *Rhamnus saxatilis*.

Distribution: Austria, **Belgium**, Bohemia, England, Germany, Hungary, Sweden.

Other specimens examined: Belgium, on *Agrostis stolonifera*, 13 Aug. 1988, De Meulder 1661 (BR 3933-53) — **Hungary**, 'montes Mátra-hegység, in mte Sombokor hegy pr. Tabernam recreandi Honvéd-üdülő', on *Calamagrostis epigeios*,
20 Sept 1989, S. Tóth (BP 89848). 'montes Mátra-hegység, in mte Hegyes-hegy ad locum otii Fallóskut haud [procul pagi Mátrakeresztes', 05 Sept 1991, S. Tóth (BP 89076).

Discussion: The epitype designated here has been considered to be the holotype by Urban (1966 note with specimen; 1967) and by Kaufman (1967). However the specimen lacks collection date data. Corda's (1837) illustration is the only reliable original material and it is hereby designated as lectotype with PRM 155608 designated as its epitype. The type variety is distinguished by having 3-celled teliospores, only observed otherwise in *P. coronati-longispora*. The name was previously considered as a catch-all for crown rust fungi excluded from other named varieties, and consequently credited with a wide host range, i.e. 48 grass genera (Cummins 1971; Urban and Marková 1993). Our molecular data do not support this scenario: the type specimen grouped in a well-supported and cohesive Clade VII. It may be endemic in Europe, with a telial host range mainly restricted to *Cal. epigeios* and *Cal. arundinacea.* One of four telial collections sampled here was collected on *Agrostis* from Belgium. DNA verification of the host identification was attempted but was not successful. The more restricted host range reported here may be partly due to sampling bias since many grass genera listed by Cummins (1971) and Urban and Marková (1993) were not sampled in our data, such as *Aegilops*, *Alopecurus*, *Ammophila*, *Anthoxanthum*, *Apera*, *Beckmannia*, *Catabrosa*, *Cinna*, *Corynephorus*, *Cynodon*, *Cynosurus*, *Deschampsia*, *Hierochloë*, *Koeleria*, *Lamarkia*, *Melica*, *Milium*, *Molinia*, *Parapholis*, *Phleum*, *Phragmites*, *Polypogon*, *Scolochloa*, *Sesleria*, *Setaria* and *Vulpia*. On the other hand, specimens on numerous hosts previously documented for this variety were excluded, grouping in other clades. For instance, *Agropyron repens* (current name: *Elymus repens*; GRIN), *Elymus* and *Hordeum* (Clade I), *Bromus* (Clade II, III and VI), *Arrhenatherum* and *Holcus* (Clade IV), *Festuca*, *Lolium*, *Poa* (Clade V), *Glyceria* (Clade IV and V), *Agrostis* spp. (Clade IX), *Cal. canadensis* (Clade VI). For some specimens on *Brachypodium*, *Helictrichon, Phalaris*, *Trisetum*, relationships to the varieties described here are uncertain. Additional sampling of hosts not represented here will help to clarify the host range and distribution of the type variety of the species.

3. **P. coronati-agrostidis** Liu & Hambleton **sp. nov**. (Figs. 5b, c, s; Clade IX; MycoBank #: MB563543)

Puccnia coronata affinis sed teliosporis brevisibus et obesis, (22) 27–56 (61)×11–20 (22) μm, coronatis digitiformis tenuis, septis constrictorum differt.

Holotypus: on *Agrostis stolonifera*, Finland, 'Regio aboensis (Ab/V). Västanfjärd: Södersundvik, Grundsund. Humous-sandy littoral meadow, abundant', 05 Aug. 1977, H. Roivainen (PUR N114).

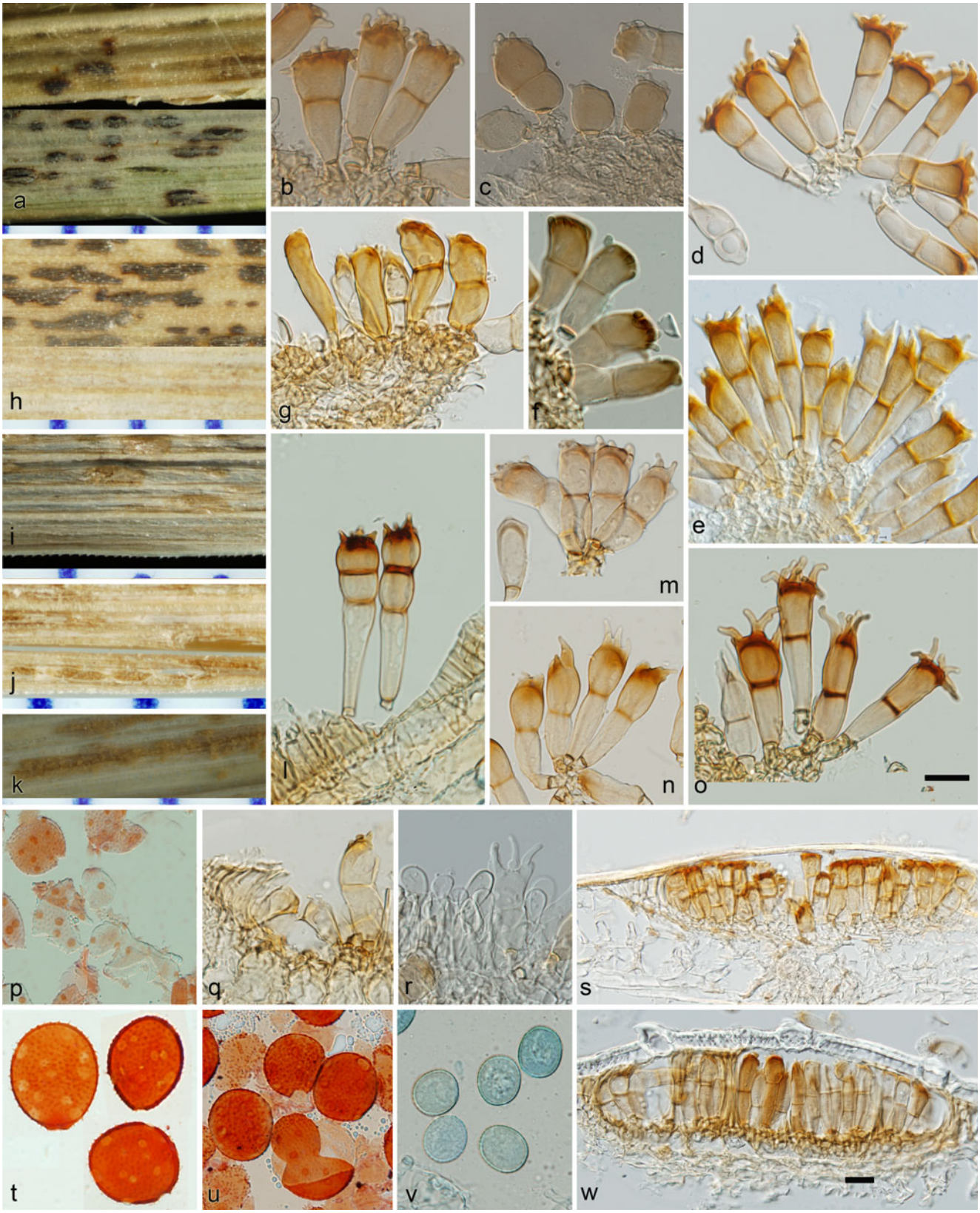
Etymology: Refers to the predominant telial host association for this variety.

Uredinia amphigenous but often adaxial, 0.1–0.5× 0.1 mm, small, fusiform, ruptured; spores 16–26 (28)×(12) 15–21 μm; germ pores (4) 5–10 (11), invagination and caps vague or clear in congo-red stain. Telia mostly abaxial, or on stem, 0.1–0.4×0.1–0.2 mm, ovoid to cylindrical, slightly rise or submerged, mostly covered, slight to medium loculated (Fig. 5s); telio-paraphyses rare, clavate, barely fused; teliospores obvoid to clavate, constrict at septa (Figs. 5b, c), (22) 27–56 (61)×11–20 (22) μm; digitations tuberculate to cylindrical, longest per spore (2) 3–13 μm; hila (3) 4–7 (8) μm.

Host: *Agrostis gigantea*, *Agrostis stolonifera*, *Phalaris arundinacea*.

Distribution: Austria, Belgium, Canada, England, Finland, United States.

Other specimens examined: **Austria**, Viennese Basin (Wiener Becken), Gramatneusiedl (at or near the train station), 8°02′ N, 16°29′ E, on *Agrostis stolonifera*, 23 Aug. 1994, Th. Barta (K(M): 60529) — **Canada**, British Columbia, [Victoria], Royal Oak Drive, roadside near Lochside Drive, on *Agrostis gigantean*, 09 Oct. 1960, G. B. Cummins (PUR 59741). Vancouver, University of British Columbia, near tennis court, on *Phalaris arundinacea*, 19 Aug. 1994, A. P. Roelfs (PUR 1268) — **England**, Berkshire, Windsor Great Park; Bishops gate entrance, on A*grostis tenuis*?, 18 Aug. 2000, N. W. Legon (K(M): 82517) — **United States**, Wisconsin, Dane, Madison, on *Agrostis gigantean*, 30 July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◀ **Fig. 5** Morphological features of species other than ***P. c.* var. *avenae***. ***P. c.* var *coronata***: variation of teliospores; **l**: 3-celled spores (BP 89848); **m**: 1-celled and normal spores (PRM155608 type). ***P. coronati-agrostis***: **b**, **c**: ovoid to clavate teliospores (PUR 55024, PUR N114 type); **s**: slightly loculated telia (K(M):82517). ***P. coronati-brevispora***: **d**: short spores (compare with **e**) (DAOM 240183); **i**: uredinia covered (PUR N1371); **p**: clear cuticular caps (DAOM 240183). ***P. coronati-calamagrostidis***: **f**, **g**: poor digitations (PUR 57282, BPI 747648); **h**: telia predominantly on abaxial (lower) not adaxial (upper) surface of the leaf (BPI 1100292); **j**: open uredinia (BPI 59821); **q**: fused telia paraphyses (PUR N2268); **t**: invagination not clear in congo-red stain (BPI 59821); **w**: well loculated telia (PUR N2268). ***P. coronati-hordei***: **k**: uredinia with spores piled on top (PUR 89857 type); **o**: teliospores with long digitations (PUR 89857 type); **r**: cylindrical paraphyses (PUR N1396); **u**: germ pores with clear invagination and cuticular caps visible in congo-red stain but not in cotton-blue (**v**) (PUR 89875 type). ***P. coronati-japonica***: **n**: teliospores contricted at septa (PUR F16131 type). ***P. coronati-longispora***: **a**: telia covered on abaxial (lower) and ruptured on adaxial (upper) leaf surface (PRC 194); **e**: longer teliospores (compare with **d**) (PRC 196 type). For **a**, **h**, **i**, **j**, and **k**, 1 unit=1 mm, **b**–**g**; **l**–**r**, **t**–**v** share the same scale, **s** and **w** share same scale, scale bar=20 μm

1955, H. C. Greene (PUR 54591). Wyoming, Albany, Wheatland Cutoff, west end of Wildlife Station, Laramie Mountains, alt. 6200 ft., on *Agrostis gigantea*, 01 Oct. 1955, W. G. Solheim & Mycology class (PUR 55024).

Discussion: Teliospores are short and plump, digitations short and thin. All but one telial specimen (9 of 10) examined were collected on *Agrostis* spp. suggesting a possible misdetermination of the host for the only specimen on *Phalaris*. Although *Phalaris* is easily distinguished from *Agrostis*, the specimen does not include diagnostic plant parts. In our experience sequencing of the host DNA has proved successful for confirming herbarium label identifications but in this case our attempts to sequence the *matK* gene were unsuccessful.

4. **P. coronati-brevispora** Liu & Hambleton **sp. nov**. (Figs. 5d, i, p; Clade III; MycoBank #: MB563541)

Teleosporis parvis, (29) 32–53 (56) x 10–17 (19) μm, in Bromus cognit.

Holotypus: on *Bromus inermis,* United States, Wisconsin, Columbia, breeding nurseries at the Univ. of Wisconsin Arlington Agricultural Research Station near Arlington, 01 Sept. 1999, C. R. Grau WPc-95 (PUR N652).

Etymology: Refers to the shorter size of teliospores compared with *P. coronati-longispora*.

Uredinia amphigenous, often produce light infection on both sides of the leaf, 0.3–1.0×0.1–0.2 mm, often covered (Fig. 5i), some ruptured longtitude; spores 14–24×(11) 13–22 μm, germ pores (7) 8–12 (14), scattered, cuticular caps clear in congo-red stain (Fig. 5p). Telia amphigenous, light infection on both sides, 0.1–0.4 (0.7)×0.1–0.2 mm, not to slightly loculated, telio-paraphyses rarely observed, clavate, to cylindrical; teliospores, short clavate to clavate (Fig. 5d) (29) 32–53 (56)×10–17 (19) μm, digitations cylindric wriggling, branched, slightly tapering, longest per spore (5) 7–17 (20) μm, pedicel hila 4.0–7.5 (9.0) μm.

Host: *Bromus inermis.*

Distribution: Canada, United States.

Other specimens examined: **Canada**, Ontario, Hastings, west of Trenton, on west side of Wooler Rd just north of Old Hwy 2, growing in oat field in production, on *Bro. inermis*, 12 July 2006 S. Hambleton, M. Liu, T. Fetch (DAOM 240063). Niagara Fall, Vineland Station, bank of the Lake Ontario, 20 Aug. 2006, M. Liu (DAOM 240183) — **United States**, Wisconsin, greenhouse generation of culture collected August 1996 at Univ. Wisconsin research station, Arlington, on *Bro. inermis*, 01 Mar. 1997, N. Delgado (PUR N1371).

Discussion: See below under *P. coronati-longispora.*

5. **P. coronati-calamagrostidis** Liu & Hambleton **nom. et stat. nov**. (Figs. 5f-h, j, q, t, w; Clade VI; MycoBank #: MB563544)

≡ *Puccinia coronata* Corda var. *calamagrostis* Fraser & Ledingham, Sci. Agric. 13:315 (1933), non *Puccinia calamagrostidis* P. Sydow Ured. Exsic. 13-15: no. 662 (1892)

Lectotypus (hic designatus): on *Calamagrostis canadensis*, Canada, Manitoba, Dauphin, Sept. 1917, W. P. Fraser (PUR 22155).

Uredinia often abaxial, opened with spores piled on top (Fig. 5j), fusiform or ellipsoid, 0.3–0.5×0.1–0.2 mm; spores 16–27×15–25 μm, germ pores 4–10, scattered, most cases invagination not clear and no caps shown in congo-red stain (Fig. 5t). Telia predominantly abaxial, almost no infection on adaxial surface (Fig. 5h), all covered, individual sorus look small, 0.1–0.3×0.1 mm, some merged into irregular stripes; loculated (Fig. 5w), telio-paraphyses fused (Fig. 5q), sheet-like, orange; teliospores obvoid to short clavate 31–72 (83)×10–21 μm, usually poorly digitated, digitations toe-like, some narrow cylindrical (Figs. 5f, g), longest per spore (1) 2–10 (12) μm, hila (2.5) 4.0–8.0 μm.

Host: *Bromus anomalus*, *Bromus ciliatus*, *Calamagrostis canadensis*, *Calamagrostis rubescens*, *Leymus innovatus*, *Trisetum canecens*

Distribution: Canada, United States

Other specimens examined: **Canada**, Alberta, Bow River, Banff National Park Valley near Mt. Eisenhower, on *Leymus innovatus*, 21 Aug. 1953, J. A. Calder and D. B. O. Savile (BPI 1100292). Manitoba, Brandon, on *Calamagrostis canadensis*, 25 Aug. 1917, W. P. Fraser (PUR 22167). Manitoba, on *Bromus ciliatus*, 01 Sept. 1918, V. W. Jackson (DAOM 204923). Saskatchewan, Foam Lake, on *Bromus ciliatus*, 13 July 1922, J. W. Scannell (PUR 22132). Saskatoon, on *Bromus ciliatus*, 06 Aug. 1922, W. P. Fraser (PUR 22133) — **United States**, Montana, Specimen Creek, Gallatin Canyon, Big Sky area, on

Calamagrostis rubescens, 11 Aug. 1982, G. B. Cummins (PUR 66551). Wisconsin, Sauk Co., near Leland, on *Calamagrostis canadensis*, 29 Aug. 1964, H. C. Greene (DAOM 107653). Burnett Co. Ca. 3 miles NW of Grantsburg. Ferry Road, edge of meadow, on *Calamagrostis canadensis*, 9 Sept. 1995 A. P. Roelfs (BPI 747648). Crex Meadows Wildlife Area N of Grantsburg, on *Calamagrostis canadensis*, 12 Aug. 2000, A. P. Roelfs, T. Crosby, J. W. McCain (PUR N2268). Wyoming, Gros Ventre Slide, near Jackson, on *Bromus anomalus*, 30 Aug. 1960, G. B. Cummins (PUR 57282).

Discussion: Both morphological and molecular evidence indicates that *P. coronata* collections on *Br. ciliatus* and *Cal. canadensis* are genetically alike and different from other taxa, thus we recognized the taxon at the species level and designated a lectotype from original material collected by Fraser in 1917. Host range is not restricted to these two species, but extends to other *Bromus* spp. and other grass genera. The species is distinctive for several characters: telia often predominantly present on the abaxial leaf surface; sori small and well-loculated, fused telial paraphyses, and teliospore digitations short and toe-like.

6. **P. coronati-hordei** Liu & Hambleton **nom. et stat. nov**. (Figs. 5k, o, r, u, v; Clade I; MycoBank #: MB563545)

≡ *Puccinia coronata* Corda var. *hordei* Jin & Steffenson, Mycologia (1999) 91:878; non *Puccinia hordei* G. H. Otth Mitt.Naturf. Ges. Bern (1871)1870:114

Holotypus (hic designatus): on inoculated *Hordeum vulgare*, leg. Y. Jin JIN91-36, United States, North Dakota, Fargo, 46°52′30″N/96°47′30″W, Mar. 29, 1992 (PUR 89857).

Uredinia amphigeous, 0.2–0.7×0.1 mm, covered, ruptured longtitude or opened, fusiform with urediniospore piled on top (Fig. 5k); spores 16–25 (27)×15–22 (24) μm; germ pores (4) 6–11 (14), scattered or bizonate, wall invagination or culticular caps clear in congo-red stain (Fig. 5u). Telia amphigenous, some with heavier infection on adaxial side, 0.2–0.4×0.1 mm, ovoid to cylindrical, mostly covered, not loculated to slightly loculated, telio-paraphyses rarely observed (Fig. 5r); teliospores obvoid to clavate, 35–55 (57)×(10) 12–19 (21) μm, digitations long, various shapes (Fig. 5o): bump like, long tapering, irregular shape wriggling or bifurcate, longest per spore (6) 7–18 (21) μm, pedicel hilum 4.5–8 (10) μm.

Host: *Elymus* and *Hordeum*.

Distribution: North America.

Other specimens examined: **Canada**, Ontario, Ottawa, Western Parkway W of Maitland Ave., on *Elymus repens*, 09 July 1982, J. A. Parmelee and D. B. O. Savile (DAOM 183691) — **United States**, Minnesota, Hennepin, roadside across from Flying Cloud Airport, Eden Prairie, on *Elym. repens*, 28 Sept. 1995, A. P. Roelfs and J. W. McCain (PUR N1426). North Dakota, Cass, barley field, Casselton, on *Elym. repens*, 10 July 1992, A. P. Roelfs (PUR N1413). on *Hordeum jubatum*, 10 July 1992, A. P. Roelfs (PUR N1539). Wisconsin, Polk, St. Croix State Park, St. Croix Falls, on *Elym. virginicus*, 13 Sept. 1995, A. P. Roelfs (PUR N1358). Lion's Club Park, N edge of St. Croix Falls, on *Elym. repens*, 14 Sept. 1995, A. P. Roelfs (PUR N1406). Osceola, glen below Cascade Falls, on *Elym. hystrix*, 25 July 1996, J. W. McCain (PUR N1396).

Discussion: Barley crown rust was described by Jin and Steffenson (1999) and is distinguished by having long teliospore digitations and a restricted host association. The authors observed that the morphology of this fungus is very similar to the taxon Peturson (1954) described as *P. coronata* f. sp. *secalis*, which can heavily infect both rye and barley under artificial inoculation conditions. This relationship was not evaluated in our study as our sampling did not include any collections on *Secale*. DNA sequence data for the holotype specimen PUR 89857 grouped in Clade I, which included collections only from *Elymus* and *Hordeum*. Our results support the suggestion by Szabo (2006), that two samples from *Elyt. repens* (current name: *Elymus repens*; GRIN) were very similar to the taxon from *H. vulgare.* Fifteen host species were documented as susceptible by Jin and Steffenson (1999), including *Br. tectorum, Elym. canadensis*, *Elym. trachycaulus, Elym. virginicus*, *Elym. repens* subsp. *repens*, *Thinopyrum intermedium*, *H. jubatum*, *H. vulgare*, *Leymus angustus*, *Ley. cinereus*, *Ley. dahuricus, Ley. racemosus*, *Pascopyrum smithii*, *Psathyrostachys juncea* and *Secale cereale*.

7. **P. coronati-japonica** Liu & Hambleton **sp. nov**. (Fig. 5n; Clade VIII; MycoBank #: MB563546)

Puccinia coronata affinitis sed teleosporis hilis attenuatis et septis constrictis.

Holotypus: on *Calamagrostis arundinacea*, Japan, Yamanashi, Shoshenkyo, Prov. Kahi, 01 Nov. 1958, Y. Morimoto (PUR F16131).

Etymology: Refers to the known distribution of the species.

Uredinia not observed. Telia predominantly on abaxial, almost no infection on the adaxial surface, 0.3–0.5×0.1 mm, mostly cylindrical, not loculated; telio-paraphyses rarely observed, cylindrical; teliospores clavate, some with very long lower cells, severely constrict at septum (Fig. 5n), 36–58×12–18 μm; the longest digitations per spore (3) 4–13 (18) μm; pedicel hila narrow 3.5–6.5 μm.

Alternate host: *Berchemia pauciflora*.

Distribution: Japan.

Discussion: Telia occur mainly on the abaxial leaf surface, a striking feature shared only with one other species, *P. coronati-calamagrostidis*, which differs in having telia less frequently loculated, longer teliospores and longer

digitations. Teliospores are severely constricted at the septum as compared to most species in the Series, except for *P. coronati-agrostidis* and *P. coronata* s.str. Considering host and geographic distribution, this species correlates well with *P. coronata* var *rangiferina* (Cummins 1971), which is also found on *Cal. arundinacea* and distributed in Japan. Although we obtained the type specimen of *P. coronata* var. *rangiferina* from PUR, we were not successful in obtaining DNA sequence data so this relationship was not evaluated with molecular characters. But our comparative morphological study revealed obvious morphological discrepancies between the two, and therefore, *P. coronati-japonica* is recognized as distinct, having longer teliospores and shorter, unbranched digitations. Differences in urediniospore size could not be evaluated because the two specimens analysed in this study lacked uredinia.

8. **P. coronati-longispora** Liu & Hambleton **sp. nov**. (Figs. 5a, e; Clade II; MycoBank #: MB563542)

Teleosporis longis et attennuatis, 34–68 (–75)×10–17 (–19) μm, frequenter tricellularis, in Bromus congit.

Holotype: on *Bromus erectus*, Czech Republic, Hnojnice, 'České Středohoří', 31 Aug. 2002, J. Marková (PRC 196).

Etymology: Refers to the longer size of teliospores compared to *P. coronati-brevispora*.

Uredinia often generate light infections, adaxial, 0.3–0.4×0.1 mm, ruptured; spores 18–24 (26)×16–23 μm, germ pores 9–14 scattered, cuticular caps clear in congo-red stain. Telia amphigenous or adaxial, 0.2–0.8×0.1–0.2 mm, fusiform, the ones on abaxial side often covered (Fig. 5a), not to slight loculated; telio-paraphyses not observed; teliosopores clavate, some very long, slightly constrict to constrict at septum (Fig. 5e), 3- celled spores common, 34–68 (75)×10–17 (19) μm, digitations tuberculate, cylindrical or bifurcate, the longest per spore 5–12 (14) μm, pedicel hila 3.5–7 μm.

Host: *Bromus erectus.*

Alternate host: *Rhamnus saxatilis.*

Distribution: Slovakia, Czech Republic.

Other specimen examined: **Slovakia**, Bratislava, on *Bromus erectus*, 16, June 2002, K. Bacigalov PRC 194).

Discussion: The much longer mean length of the teliospores and the presence of 3-celled teliospores can be used to distinguish *P. coronati-longispora* from *P. coronati-brevispora*, the two species specialized on *Bromus* (see Tables 4, 5). However, teliospore and pedicel hilum widths are similar for both species but narrower than for the type species.

The variety "*P. coronata bromi"* was first recognized on *Br. ciliatus* in Canada by Fraser and Ledingham (1933). Later, Cummins (1971) combined it with *P. coronata* var. *coronata.* In Europe, multiple *Bromus* species including *Br. erectus* and *Br. inermis* (Mühlethaler 1911; Treboux 1912) were reported as hosts, leading Mühlethaler (1911) to designate a *forma specialis* for crown rust on *Bromus*. However, Urban and Marková (1993) disagreed and considered that the taxon on *Bromus* belonged to *P. coronata* var. *coronata* or to *P. coronata* var. *avenae* f. sp. *graminicola*.

A new morphotype (WPc-95A) on *Br. inermis* was discovered from Wisconsin, South Dakota and Minnesota, USA (Delgado et al. 2001; Anikster et al. 2003). Delgado et al. (2001) noted that WPc-95A was distinguishable from "*P. coronata bromi*" (discussed above) on the basis that the Canadian form did not infect *Br. inermis*. In addition, Anikster et al. (2003) observed differences between WPc-95A and three other varieties of Fraser and Ledingham (1933) and *P. coronata* var. *hordei* by Jin and Steffenson (1999). They also speculated that the new morphotype was different from *P. coronata* f. sp. *bromi sensu* Mühlethaler based on the evidence that WPc-95A has smaller teliospores. Results of our DNA analyses, in which samples on *Br. inermis* from North America grouped in a clade separate from those on *Br. erectus* from Slovakia and the Czech Republic were supported by differences in teliospore length (Table 4, 5). We recognize the North American taxon with shorter teliospores, *P. coronati-brevispora,* as a new species distinct from *P. coronati-longispora* from eastern Europe. Interestingly, a specimen from Germany on *Br. erectus* (BPI 718354) is more closely related to the North American variety in our ITS tree and statistical tests indicate that teliospore length is significantly shorter than that measured for the European taxon. This suggests that the species on *Br. erectus* may have diverged in Europe before the lineage migrated to North America. However, this hypothesis must be tested with comprehensive sampling (in term of genes and specimens) before drawing any conclusions.

In our study, a collection of crown rust on *Br. ciliatus* from Canada (DAOM 204923), presumably belonging to *Puccinia coronata* var. *bromi* Fraser and Ledingham, grouped in Clade VI, and not in either of the clades on *Bromus* discussed above. Clade VI comprises specimens mainly on *Cal. canadensis*, suggesting synonymy with *P. coronata* var. *calamagrostis* Fraser & Ledingham. In Szabo's molecular analysis (2006), a single specimen on *Cal. canadensis* formed a unique lineage considered to be a new species (PcSP4), while both Cummins (1971) and Urban and Marková (1993) lumped collections on this host in *P. coronata* var. *coronata*. Our data supports Fraser and Ledingham's concept of segregating *P. coronata* var. *calamagrostis*, but in combination with *P. coronata* var. *bromi* Fraser & Ledingham, from *P. coronata* var. *coronata*.

Unresolved lineages:

In addition to *P. coronata* var. *coronata*, *P. coronata* var. *avenae* and *P. coronata* var. *rangiferina*, Cummins (1971) accepted two more varieties, *P. coronata* var. *gibberosa* and *P. coronata* var. *himalensis.*

P. coronata Corda var. gibberosa (Lagerh.) Joerst. Avh. Norske Videnskaps-Akad. Oslo I. 1948:9. 1949 (Clade XI): on *Festuca altissim*a All., Europe, Type: Lagerheim, near Frieburg in Baden, German. Type seen!

Puccinia coronata var. *gibberosa* on *Fe. alitissima*, distributed in Europe, is reported to have teliospores with poorly defined digitations although Cummins (1971) cast doubt about the value of the "gibberose" character of the spore apex. In this study, we have shown that the shape and length of digitations combined with other characters can be useful for differentiating species. Within Series Coronata, few species are poorly digitated (*P. coronati-calamagrostidis*), or have short digitations (*P. coronati-agrostidis* and *P. coronati-japonica*). Unfortunately, we were unable to conduct DNA analysis for the type specimen of *P. coronata* var. *gibberosa* (S F30373) but based on our assessment, it is morphologically similar to the type specimen of *P. coronata* var. *intermedia* (BRNM 111952), a variety recognized by Urban (1967). The ITS sequence of the latter specimen grouped with a PUR specimen (PUR F17485) on *Fe. altissima* from Slovakia, suspected of belonging to *P. coronata* var. *gibberosa* because of the same host and origin. Based on this evidence, we hypothesize that the two taxa are synonymous.

P. coronata Corda var. himalensis Barclay Trans. Linn. Soc. Lodon 3:227. 1891 (Clade X): on *Calamagrostis canadensis*, Japan, Type: on *Brachypodium sylvaticum*, India, Simla (K), by Barclay. Type not seen.

Puccinia coronata var. *himalensis* was characterized by having small thin-walled urediniospores and exposed aparaphysate telia. Multiple previously accepted species were included as synonyms including *P. brevicornis*, *P. melicae*, and *P. subdigitata* (see Results and discussion section and Table 1 for citation and voucher information) by Cummins (1971). Based on analyses of ITS sequences, type specimens sampled for these synonyms did not form a monophyletic group indicating that they are not conspecific.

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