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Molecular and morphological characterization of the willow rust fungus, *Melampsora epitea*, from arctic and temperate hosts in North America

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Abstract: Current taxonomy places all rust fungi that occur on willow (*Salix* spp.) in North America in one species complex, *Melampsora epitea* Thüm. Characteristics of *M. epitea* isolates from the Canadian arctic were compared to *M. epitea* isolates from temperate regions of North America. Sequences from internal transcribed spacer (ITS) regions of rDNA were obtained from urediniospores from rust-infected *Salix* leaves collected in the Canadian arctic and in Minnesota and compared. Phylogenetic analysis of nuclear ribosomal ITS regions indicated that arctic *M. epitea* samples were divergent from temperate *M. epitea* isolates, perhaps in part because all rusts examined diverged according to host species. Four urediniospore characteristics were examined: area, circularity (shape factor), major axis length and spine density. Statistically significant ($P < 0.05$) differences were observed for spine density among all host species except *S. nigra* and *S. bebbiana*. However major axis length differed between these species. These results represent the first evidence that arctic and temperate *Melampsora* species on *Salix* hosts in North America have evolved distinct molecular and morphological characters.

Key words: arctic fungal diversity, fungal phylogenetics, fungal taxonomy, Melampsoraceae, Uredinales, willow diseases

INTRODUCTION

Biotrophic rust fungi in the genus *Melampsora* (Basidiomycetes, order Uredinales) are common parasites of arctic willows (*Salix* spp.) with at least 12 willow species reported as hosts in arctic Canada alone (Parmelee 1989). At one location, seven species of willow have been reported to be severely affected by rust (Savile 1953). Many species of *Salix* in the European and Asian arctic also have been reported to be infected with *Melampsora* rust (Beerling 1998). It is likely that none of the 400 or 500 species of *Salix* of the world are entirely free of rust infection. In temperate regions, *Melampsora* spp. are macrocyclic and heteroecious with *Tsuga, Abies, Larix* and *Ribes* spp. being described as aecial hosts (Pei et al 1993, Sinclair 1987). However in the arctic none of these aecial hosts are found. Although *Saxifraga* species have been suggested as another possible aecial host of *Melampsora* in the arctic (Savile 1953), it still is unknown how frequently the life cycle is completed in this region.

Although Ziller (1974) recognized five species of *Melampsora* on *Salix* in western North America, he noted that "overlapping dimensions of spores and spore walls in Canadian willow rusts make their specific identification difficult or impossible, unless the aecial state is known." He thus considered all five to belong to a species complex, termed *Melampsora epitea* (Cummins 1962, Savile 1953, Ziller 1974). However, the cryptic species within the *M. epitea* complex potentially are distinguishable; in Europe at least six species of rust on willow have been identified based on urediniospore morphology and inoculations of aecial hosts (Pei et al 1993). It has been suggested that the *Melampsora* rusts of arctic *Salix* are a particularly diverse complex (Savile 1953, Parmelee 1989). *Melampsora epitea* from the arctic (Ellesmere Island, Nunavut, Canada) and temperate North America (Minnesota) was the focus of molecular and morphological analyses in this study. The objective of this study was to examine geographic and host specialization and speciation of arctic and temperate *M. epitea* from *Salix* hosts.

MATERIALS AND METHODS

Study sites and sampling.—Isolates of *Melampsora* rust on *Salix* species were collected from northern Ellesmere Island, Nunavut, Canada, and from Minnesota during the summers of 2001 and 2002 (FIG. 1 and TABLE I). Rust samples were taken from *S. arctica* at four locations on Ellesmere Island (approximately 82° N): Fort Conger, Air Force Glacier, Lake Hazen and Tanquary Fiord. At Lake Hazen a survey was conducted along an approximately 400 m tran-

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FIG. 1. Map of arctic field collection sites of *Melampsora epitea* on *Salix arctica* on northern Ellesmere Island, Nunavut, Canada. Inset shows the four locations where collections were made.

sect to determine the proportion of plants that were infected and to observe severity and tissues infected. Samples from Ramsey County, Minnesota, were taken from infected leaves of a mixed stand (approximately 2 acres) of *S. bebbiana, S. interior* and *S. nigra*. Collections of *Melampsora* occurring on *S. arctica* were obtained from infected leaves, stems and infected catkins. Many collections sometimes were made from the same plant, but different tissues are indicated by the lowercase letter in the sample names (i.e., AFG2001-1d). Infected tissues with urediniospores present were placed in sterile 15 mL collection tubes with desiccant. The samples were marked carefully to record the location and sex of the infected plant as well as what host tissues were infected. Samples were kept cool until returned to the laboratory. In addition, herbarium sample US0022745 (*M. epitea* from Abisko, Sweden, collected from *Salix* sp., Jun 1950 by C.W. Emmons) was obtained from BPI (U.S. National Fungal Herbarium, Beltsville, Maryland) and used for molecular comparison. Voucher specimens of all samples used in this study were deposited into BPI (U.S. National Fungal Herbarium, Beltsville, Maryland) and DAOM (Canadian National Mycological Herbarium/Herbier National de Mycologie, Ottawa).

DNA extractions, ITS-rDNA amplification and sequencing.—Upon return to the laboratory urediniospores were removed from the host tissue by disruption with a sterile glass rod. Spores were stored in 1.5 mL tubes with extraction buffer (Qiagen Inc.) and placed in the freezer at −80 C. For extraction of DNA, the spores were thawed, vortexed and placed in the heat block at 60 C. After 1 h, DNA extraction from the samples was performed using the Qiagen

TABLE I. Locations, host species, isolate codes and GenBank accession numbers for *Melampsora epitea* isolates used for molecular and morphological comparisons

Location	Host species	Isolate code	GenBank accession #
Fort Conger, Ellesmere Island	*Salix arctica*	FC2002-1	AY 471624
Fort Conger, Ellesmere Island	*Salix arctica*	FC2002-2	AY 471625
Fort Conger, Ellesmere Island	*Salix arctica*	FC2002-4	AY 471626
Fort Conger, Ellesmere Island	*Salix arctica*	FC2002-8	AY 471634
Lake Hazen, Ellesmere Island	*Salix arctica*	LH2001-2	AY 471635
Lake Hazen, Ellesmere Island	*Salix arctica*	LH2001-3	AY 471627
Lake Hazen, Ellesmere Island	*Salix arctica*	LH2001-4	AY 471628
Lake Hazen, Ellesmere Island	*Salix arctica*	LH2001-5c	AY 471629
Air Force Glacier, Ellesmere Island	*Salix arctica*	AFG2001-1d	AY 471630
Air Force Glacier, Ellesmere Island	*Salix arctica*	AFG2001-2e	AY 471631
Air Force Glacier, Ellesmere Island	*Salix arctica*	AFG2001-3e	AY 471632
Air Force Glacier, Ellesmere Island	*Salix arctica*	AFG2001-4	AY 471633
Tanquary Fiord, Ellesmere Island[1]	*Salix arctica*	TF2002-1	AY 471620
Tanquary Fiord, Ellesmere Island[1]	*Salix arctica*	TF2002-2	AY 471621
Tanquary Fiord, Ellesmere Island[1]	*Salix arctica*	TF2002-3	AY 471622
Tanquary Fiord, Ellesmere Island[1]	*Salix arctica*	TF2002-4	AY 471623
Abisko, Sweden (near Arctic circle)[1,2]	*Salix sp.*	SW1950-1	AY 471648
Ramsey County, Minnesota U.S.A.	*Salix nigra*	SN2002-1	AY 471640
Ramsey County, Minnesota U.S.A.	*Salix nigra*	SN2002-2	AY 471641
Ramsey County, Minnesota U.S.A.	*Salix nigra*	SN2002-3	AY 471642
Ramsey County, Minnesota U.S.A.	*Salix nigra*	SN2002-4	AY 471643
Ramsey County, Minnesota U.S.A.	*Salix interior*	SI2002-1	AY 471636
Ramsey County, Minnesota U.S.A.	*Salix interior*	SI2002-2	AY 471637
Ramsey County, Minnesota U.S.A.	*Salix interior*	SI2002-3	AY 471638
Ramsey County, Minnesota U.S.A.	*Salix interior*	SI2002-4	AY 471639
Ramsey County, Minnesota U.S.A.	*Salix bebbiana*	SB2002-1	AY 471644
Ramsey County, Minnesota U.S.A.	*Salix bebbiana*	SB2002-1	AY 471645
Ramsey County, Minnesota U.S.A.	*Salix bebbiana*	SB2002-1	AY 471646
Ramsey County, Minnesota U.S.A.	*Salix bebbiana*	SB2002-1	AY 471647

[1] Molecular comparisons only.
[2] Herbarium sample US0022745 from BPI, U.S. National Fungal Herbarium.

Plant DNeasy Mini-kit (Qiagen Inc., Valencia, California) following manufacturer's instructions.

Polymerase chain reactions (PCRs) were used to amplify internal transcribed spacer region rDNA (ITS-rDNA) of the samples. Basidiomycete-specific primers ITS1-F and ITS4-B (Gardes and Bruns 1993) were used to amplify ITS-rDNA. PCR amplification was performed using Amplitaq Gold PCR Master-mix following manufacturer's instructions (Applied Biosystems). PCRs were performed in a MJ Research PTC Mini-cycler thermocycler. PCR conditions were: 94 C for 5 min; 35 cycles of 94 C for 1 min, 50 C for 1 min, 72 C for 1 min, followed by a final extension step of 72 C for 5 min.

Amplified products were purified and prepared for sequencing using EXO-SAP-IT PCR clean-up kits (USB Inc.) following manufacturer's instructions and checked on agarose gels. Some samples were re-amplified to obtain sufficient quantities of DNA. Sequencing reactions were performed using both primers with the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and an ABI Prism 377 automated DNA sequencer. DNA sequence data were assembled into contigs using Chromas software (Technelysium Ltd.), and the Emboss pairwise alignment algorithm (www.ebi.ac.uk/emboss/align/), and the sequences were deposited at GenBank (www.ncbi.nlm.nih.gov) (see TABLE I for accession numbers). Multiple sequence alignments were made with Clustal W (www.ebi.ac.uk/clustalw/) and hand edited using MacVector (Accelrys Inc.).

Phylogenetic analysis.—Phylogenetic analysis was performed with three samples from each host species/location using PAUP version 4.04b (Swofford 2002). Of the total of 810 aligned characters, 563 were used in the analysis after removing regions in which the alignment was ambiguous. Because all samples from the same host and geographical location had identical sequences, only three samples from each location/host were used in phylogenetic analysis. Parsimony analysis was performed using a strict heuristic search with 10 random stepwise additions. Branches were collapsed if lengths were zero. Bootstrap values were determined using 1000 replications, and only groups with frequencies greater than 50% were retained. Final alignments and trees were deposited at TreeBase (www.treebase.org/treebase) in

FIG. 2. A. Healthy, female *Salix arctica* at Lake Hazen, Ellesmere Island. B. Close-up of rust-infected leaves of *S. arctica* at Fort Conger. C. Heavy rust infection of *S. arctica* leaves at Lake Hazen. D. Infection of male catkins can be seen and yellow urediniospores are produced on the catkins of *S. arctica* at Fort Conger, Ellesmere Island. Also note uredinia on both abaxial and adaxial leaf surfaces.

Nexus format and a study accession number of S1092 and a matrix accession number of M1867 was assigned.

Morphological comparisons.—For morphological comparisons, 28 urediniospores from each sample were examined at 100× magnification using a Nikon E600 differential interference contrast and fluorescence microscope interfaced with a Nikon DXM1200F digital camera. Digital images were captured and analyzed using ScanPro software (SPSS Inc.). Morphological characteristics of urediniospores that have proven useful in taxonomic comparisons for other *Melampsora* species (Helfer 1992, Jennings et al 1990, Newcombe et al 2000) were measured and compared. Spore area (μm^2), major axis length (μm), spine density (spines/μm^2) and circularity (shape factor) of the spores were measured and compared for each collection. Mean values were calculated and statistical analyses were performed using one-way ANOVA and Waller-Duncan multiple means comparison using Statistical Analysis Software (SPSS Inc.). Subgroups were considered significantly different at the alpha = 0.05 level. Morphological characteristics were evaluated using discriminant analysis (SPSS Inc.).

In addition to comparisons using light microscopy, images were obtained of the spores using a Hitachi S3500 scanning electron microscope. Spores were coated in gold and placed in the low-vacuum, variable-pressure chamber of the SEM and photographed with a digital camera at 3500× magnification.

RESULTS

Field observations.—*Salix arctica* plants (FIG. 2A) were slightly-to-severely affected by rust at all study sites (FIG. 2B and 2C). At Lake Hazen, 26 infected plants were observed within 2 m of a 400 m long transect. Of these 26 with infection, 19 were female, five were male and two had not flowered. All plants had infected leaves with uredinia present on both abaxial and adaxial surfaces (FIG. 2B). In addition, two female plants had infected catkins (FIG. 2D) and six plants had infection of the current year's stem tissue. Some of the severely affected plants had one or more dead stems, likely due to rust infection. Catkin infection was common around Lake Hazen on both male and female floral tissues. Infected catkins also were observed on both sexes at Fort Conger and on female floral tissues at Air Force Glacier.

Amplification and sequencing of ITS-rDNA.—ITS-rDNA was successfully obtained and amplified for all samples. PCR products were approximately 820 base pairs in length. Successful sequences were obtained for all samples with less than 1% of the sequence appearing as "N" (undetermined nucleotide). Sequences obtained were about 810 base pairs long.

Phylogenetic analysis.—Final alignments used in phylogenetic analysis included 563 of the original 810 characters used in MacVector alignments. Of these 563 characters, 470 were constant, 91 were parsimony informative and two were parsimony uninformative. Parsimony analysis generated three most parsimonious trees, from which one was chosen for publication. Parsimony analysis supported four distinct clades (grouping among host species) with bootstrap values greater than 90% (FIG. 3). Parsimony analysis provided evidence that arctic samples from North America are more closely related to the arctic sample from Europe (SW 1950-1) than temperate samples from North America.

Morphological comparisons.—Urediniospores in collections of *Melampsora* from *S. arctica* had significantly greater spine density than those of collections from any of the three *Salix* species from Minnesota (TABLE II; FIG. 4). Collections from *S. bebbiana* had significantly ($P < 0.05$) longer urediniospores with less circularity than collections from *S. arctica, S. interior* or *S. nigra*. Urediniospores in collections from *S. interior* were significantly shorter, had significantly less cross-sectional area and significantly greater spine density than those in collections from *S. bebbiana* or *S. nigra,* which also came from Minnesota. Collections from *S. nigra* had urediniospores that were significantly longer than those from *S. interior* and significantly shorter than those from *S. bebbiana*. In addition, the collections from *S. nigra* had urediniospores that had significantly lower spine density than those from *S. interior* or *S. arctica*.

Spine density was the most informative character for classifying groups based on discriminant analyses. Wilks' lambda equaled .377 with an *F*-statistic of 59.45 and $P < 0.00001$. This supports Waller-Duncan analyses that significant differences between host species for this characteristic. Discriminant analysis of spine density resulted in 55.4% of all original cases being classified correctly. Discriminant analysis resulted in 81.3% of the original grouped cases being correctly classified when all four urediniospore characteristics were considered in analysis.

DISCUSSION

During field collections of arctic willow rust, we discovered that *Melampsora* rust infects *Salix arctica* catkins and both abaxial and adaxial leaf surfaces (FIG. 2). This has not been reported previously on *S. arctica* and might represent a special adaptation to the arctic. The ability to infect the floral tissue might aid in dispersion of urediniospores via wind currents (because the catkins are above associated vegetation) or by insect pollinators (the bright yellow rust spores might attract insects that are needed for willow pollination) that normally would visit *Salix* catkins and carry rust spores to other *Salix* plants that they visit subsequently. The presence of uredinia on the adaxial leaf surface also might aid in wind dispersion of urediniospores, but this needs further investigation.

Although the impact is not fully known, it appears that *Melampsora* rusts are a common cause of mortality of willows in the arctic. Certain species can be very damaging to *Salix* in temperate regions, causing defoliation and stem cankers that can result in decline and eventual death (Ostry and Anderson 2001, Pei and Ruiz 1999). This appears to be occurring because the rust infects arctic willow systemically, and severe disease was observed associated with mortality at Lake Hazen. More work is needed to determine what role these rust pathogens play in ecological succession in the arctic.

The species complex of *M. epitea* in North America might represent one of the most diverse and confusing groups of rusts. The species complex historically has been divided into separate species (sensu stricto) based apparently on host and geographical ranges rather than distinct morphological characteristics. A description of *M. arctica* Rostr. (syn. *M. alpina* Juel) on *Salix arctica* in North America, was provided by Arthur (1934). Arthur reported *Melampsora bigelowii* Thüm. (syn. *M. paradoxa* Diet. & Holw.) as the rust fungus found on *S. amygdaloides* and 28 other species of *Salix* across the continent. But, in the view of Anderson (1952), *M. bigelowii* was designated (along with *M. arctica*) as a form infecting *S. arctica*. The only difference appears to be that *Saxifraga* spp. serve as aecial hosts for *M. arctica* and *Larix* spp. serve as hosts for *M. bigelowii*. At least for *M. arctica,* the aecial host (or etiology in general) has not been substantiated by research (Ziller 1974). Collections of rust on *S. arctica* from the arctic likely have been designated as *M. arctica* and from the subarctic as *M. bigelowii,* simply due to aecial host distributions. The situation fortunately seems slightly less complicated for temperate willow rusts in North America (Savile 1953). Rust found on *S. interior* and *S. nigra* have been loosely grouped by Ziller as *M. epitea* sensu lato Arthur (1934), on the other hand, had argued that *S. interior* and *S. nigra* are parasitized by *M. abieticapraearum* Tubeuf. The rust found on *S. bebbiana*

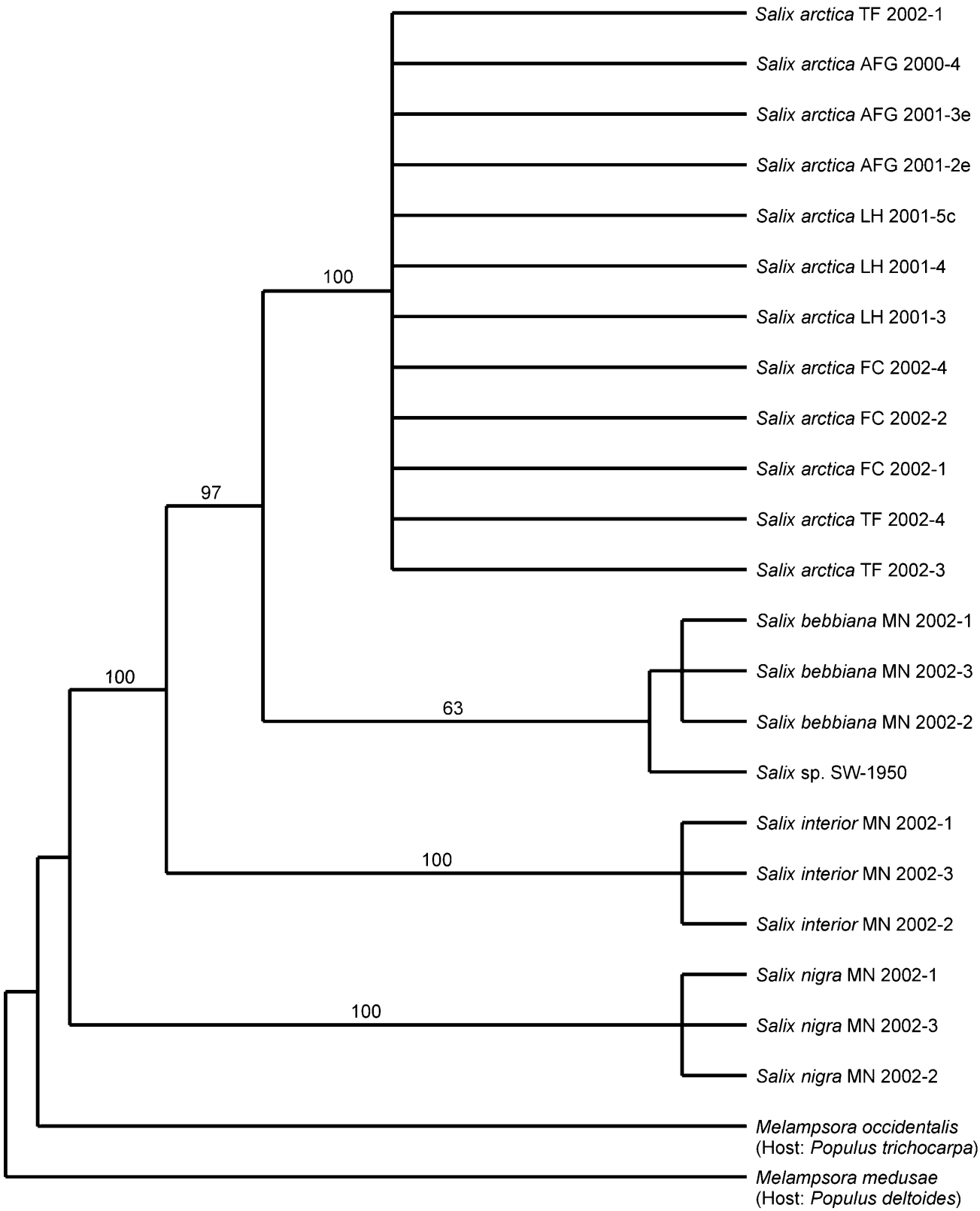

FIG. 3. Parsimony tree (of a total of three most parsimonious trees) from PAUP analysis using heuristic search and stepwise addition methods. Bootstrap values >50% (after 10^3 replications) are given at branching points. *M. medusae* and *M. occidentalis* (sequences obtained from GenBank, accession numbers AF087711 and AF087710, respectively) were defined as outgroups. Consistency index = 0.923, rescaled consistency index = 0.898, homoplasy index = 0.077, retention index = 0.973.

TABLE II. Mean values for spine density, area, length and shape factor of urediniospores (at × 100 magnification) from arctic (*Salix arctica* host) and temperate willow rusts (*S. bebbiana, S. interior* and *S. nigra* hosts) in North America

Host	Spine density (spines/μm²)[z]	Area (μm²)	Length (μm)	Shape factor (circularity)
S. arctica	0.44 (± 0.12) **c**	260.47 (± 66.12) **a**	19.65 (± 2.73) **ab**	0.86 (± 0.02) **b**
S. bebbiana	0.22 (± 0.04) **a**	319.76 (± 31.98) **b**	25.13 (± 2.62) **c**	0.79 (± 0.05) **a**
S. interior	0.39 (± 0.09) **b**	238.82 (± 68.86) **a**	18.79 (± 2.51) **a**	0.86 (± 0.02) **b**
S. nigra	0.20 (± 0.04) **a**	301.55 (± 68.70) **b**	20.71 (± 0.12) **b**	0.87 (± 0.02) **b**

[z] Standard deviations are given in parentheses and lower case letters in bold within columns denote statistically different homogenous subsets (at alpha = 0.05 level) from Waller-Duncan test.

also is lumped with *M. epitea* sensu lato, but in western North America Ziller (1974) nevertheless retains *M. ribesii-purpureae* Kleb.

Many researchers have determined that without detailed studies, there is no justification for providing species status for the different forms in North America. Parmelee (1989) said "*Melampsora epitea* . . . certainly contains more than one species, but until abundant cross-inoculations can be made and combined with detailed measurements, realistic treatment is impossible." Even in 1953, Savile recognized the diversity among arctic *Melampsora* on willows. Savile (1953) said: "The disposition of even many southern collections is difficult; but the numerous northern forms completely obliterate the presumed distinctions, and the situation is complicated by the fact that in the north, aecia are rarely produced. At present it seems advisable to follow Jörstad (1940) and call the whole complex *M. epitea,* regardless of aecial host. As our knowledge increases it will probably be possible to set up several varieties on the basis of host relationship and small morphological distinctions. The only alternative seems to be to make species of the forms on every willow." This statement was made before the development of molecular techniques now commonly used in fungal phylogenetic analyses. Our results from ITS sequence comparisons show that *M. epitea* from arctic willow represent a distinct clade and are easily distinguished from *M. epitea* from temperate hosts in North America. In addition our studies have revealed substantial molecular divergence of *M. epitea* at the host-species level. The four host species tested were infected with distinct forms of *M. epitea*; even when the three temperate hosts comingled in the same forest, the rust fungi were distinguishable. This is not the first report of differentiation of seemingly similar forms of rust on willow (Jennings et al 1990, Pei and Ruiz 2000). In fact molecular data has worked well to separate different forms of rust on the same *Salix* host. For example, the form of rust that causes lethal stem cankers on *S. viminalis* in the British Isles recently was separated from the foliar-infecting form by molecular comparisons (Pei and Ruiz 2000). This information indicates that further studies of the relationships between the catkin-infecting form, stem infecting form (Ostry and Anderson 2001) and other infection types of *M. epitea* in North America are needed.

Morphological differences in urediniospore spine characteristics found in this study mirror results from previous studies of other rust fungi (Jennings et al 1990, Helfer 1992, Newcombe et al 2000). For example, spine density worked well to separate *Puccinia allii* from two separate host species (leek and *Allium*) (Jennings et al 1990). Although these characteristics might not be useful in separating every species of *Melampsora* or reflect host species differences, it is clear that certain morphological characteristics (such as spine density) can be useful for differentiating certain forms or species.

Although *Saxifraga* species have been suggested as possible aecial hosts (Savile 1953), the most northerly collections of rust-infected *Saxifraga* are from 75°N and the most northerly collections of *Melampsora* from *Salix* are about 82°30′N (Savile 1953). Thus it is unlikely that the life cycle is completed in the far north and the rust may persist in these northern regions of the arctic as a systemic infection on willow (Savile 1953, 1963). New infections that occur on willow apparently result from urediniospore infection (Savile 1953). Nothing is known about the population genetics of rust in the arctic and how they generate pathogenic variability. Clearly there are adaptations in arctic *Melampsora* that are not present in temperate willow rusts. Given that molecular evidence reported here indicate that arctic rust from North America is more closely related to arctic rust from Europe than temperate rust from North America, it is evident that current taxonomy does not reflect ecological adaptation.

Our phylogenetic evidence broadly suggests that host specialization is a key factor in the evolution of *M. epitea* sensu lato. Host specialization in theory can result in sympatric speciation because populations that lack a common host will become isolated reproductively. Although this hypothesis can be simply ap-

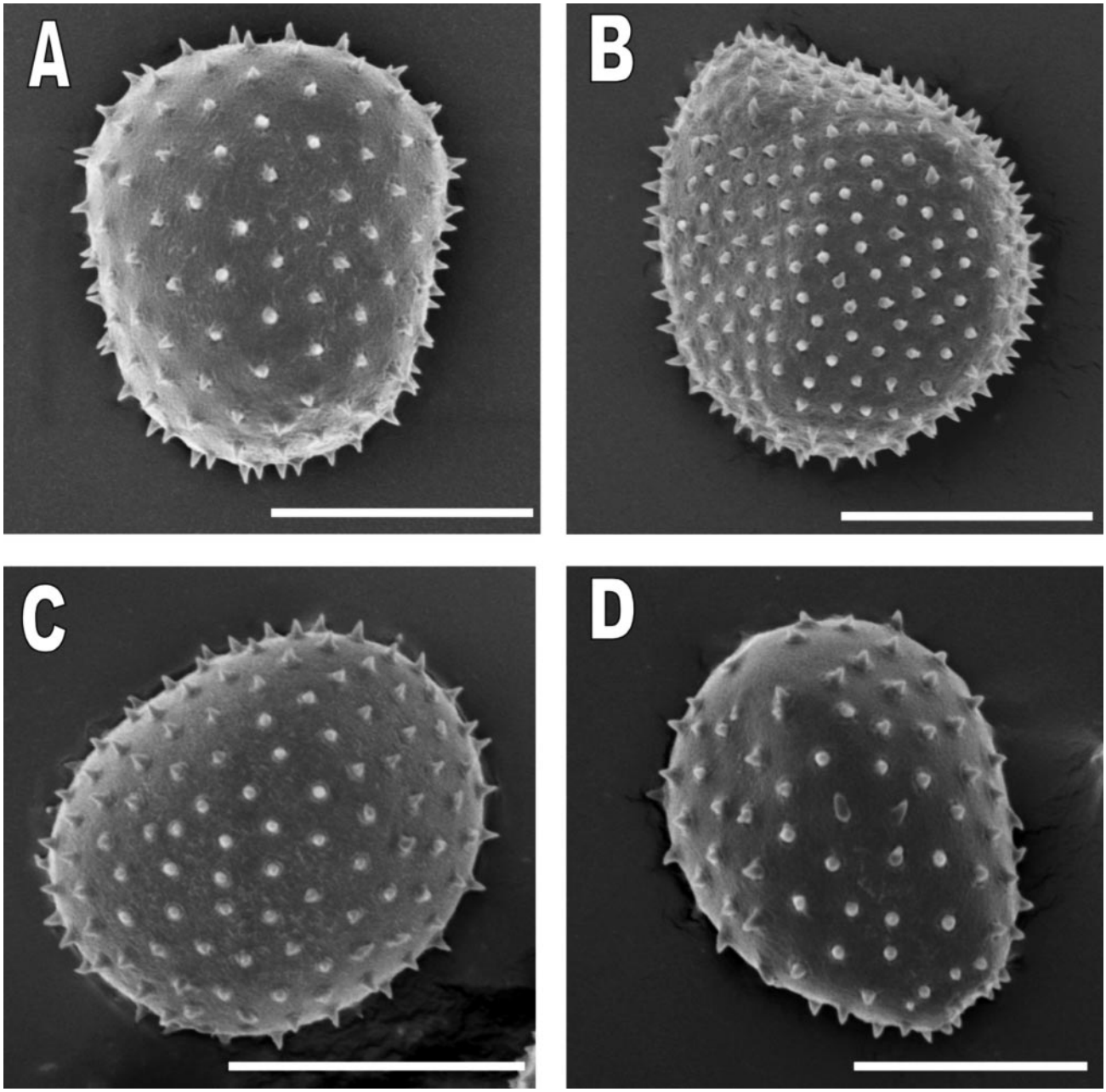

FIG. 4. Scanning electron micrographs of gold-coated urediniospores from *Melampsora epitea* urediniospores at 3500× magnification (bar = 10 μm). A. Urediniospore from rust collected from *Salix bebbiana* in Minnesota. B. Urediniospore from rust collected from *Salix arctica* at Fort Conger, Ellesmere Island, Nunavut, Canada. C. Urediniospore from rust collected from *Salix interior* in Minnesota. D. Urediniospore from rust collected from *Salix nigra* in Minnesota.

plied to speciation of autoecious rust fungi, heteroecious species are more complicated. Populations, for instance, may lack a common telial host but still possess a common aecial host. It is in this context that systemic infection of *S. arctica* by *M. epitea* sensu lato should be considered. On the one hand, systemic infection would appear to increase the likelihood of both winter survival and of successful re-infection of the telial host in the brief arctic summer (Savile 1953). But by successfully perennating, or cycling without aecial hosts, the reproductive isolation of arctic populations of *M. epitea* may be enhanced. Given infection of an aecial host that bridges between boreal or subarctic populations at lower latitudes and arctic populations, gene flow between the two might occur. Without infection of the aecial host, that gene flow would cease and with it the ability to infect boreal or subarctic congeners of *S. arctica*. Of course, a

tradeoff between adaptation to the arctic and host specialization remains conjectural without further research. Although our emphasis in this study was on arctic populations, temperate populations of *M. epitea* sensu lato also appear to be specialized on their telial hosts (*S. bebbiana, S. interior* and *S. nigra*) because differing forms of *Melampsora* were found in the same location on different host species. Phylogenetic evidence of specialization however is indirect. We thus are seeking to corroborate our findings with direct tests via inoculation.

These results represent the first evidence from temperate and arctic North America that specialization and speciation characterize the co-evolutionary history of *Melampsora* and *Salix*. The results reported here justify future research to determine the extent of diversity among rusts of other *Salix* hosts in North America and relationships to rust fungi found in Europe and Asia, including circumpolar *Salix* species such as *S. arctica, S. herbacea* and *S. reticulata*. Investigations to study the ecology and biology of these pathogens in the arctic also are warranted.

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