

Species Delimitation and Host Specialization of *Ceratocystis laricicola* and *C. polonica* to Larch and Spruce

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ABSTRACT

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Ceratocystis laricicola and *C. polonica* are fungal symbionts of bark beetle species of the genus *Ips* that attack species of *Larix* and *Picea*, respectively, across Eurasia. Earlier studies found that these fungal species were morphologically identical, had similar isozymes patterns, and had identical internal transcribed spacer (ITS) sequences of the rDNA operon. We analyzed 27 isolates from Europe, southwestern Siberia (Russia) and Japan, representing the known geographic ranges of the two species. Phylogenetic analysis of the DNA sequences of a portion of the *MAT-2* idiomorph showed these species to be distinct, with the Japanese isolates of *C. laricicola* having a sequence slightly different (5 bp) from those of the Russian and European isolates of *C. laricicola*. Sexual compatibility tests showed full interfertility among isolates of *C. polonica* from Europe, Russia and Japan, but isolates of *C. polonica* were not fully interfertile with isolates of *C. laricicola*. A Russian and a European isolate of *C. laricicola* mated with each other but not with the Japanese isolates of *C. laricicola*. Mature *L. sibirica* and *P. obovata* were inoculated with isolates of *C. laricicola* and *C. polonica* from Europe, Russia, and Japan, and measurement of lesions in the inner bark/cambium region demonstrated strong host specialization. The data suggest that the two fungal species are very closely related and are distinguished primarily by their physiological specialization to the hosts of their bark beetle vectors.

Additional keywords: biological species, *Ips cembrae*, *Ips typographus*

Bark beetles (Coleoptera: Scolytidae) are among the most important killers of trees in the family Pinaceae, especially on *Pinus*, *Picea* and *Larix* (15). Bark beetles are intimately associated with a variety of fungi, most notably, members of the genus *Ophiostoma* and their associated anamorphic species of *Leptographium* (5–7). Although species of *Ceratocystis sensu stricto* are generally associated with other insects, there are three species of *Ceratocystis* associated with tree-killing bark beetles (12,21). Two of these *Ceratocystis* species, *C. laricicola* Redfern & Minter and *C. polonica* Siemaszko, are associated with closely related species of *Ips*, *I. cembrae* and *I. polonica*, which attack *Larix* and *Picea* species, respectively, across Eurasia, from northern Europe to Japan (12–14,16–18,22,23,25,26). When inoculated into healthy trees, these *Ceratocystis* species induce extensive lesions (2,13,14,16,18,25,26), and it is thought that the

respective bark beetles kill their hosts through the actions of these plant pathogenic fungal species (2,7,13,14,16,18,25,26). Of the fungi associated with tree killing bark beetles, these two species may be the most aggressive pathogens to trees (15), and thus, these species are important in the development of conceptual models of the interactions among fungi, bark beetles and their plant hosts.

It is questionable if *C. laricicola* and *C. polonica* are distinct species. Siemaszko (17) mistakenly connected a *Leptographium* anamorph to *C. polonica*, and because of this confusion, Redfern et al. (16) did not compare their new species, *C. laricicola*, to the earlier described *C. polonica* (20). Harrington and Wingfield (12) recognized the two fungal species as distinct, though no morphological feature appeared to separate them. Analyses of the internal transcribed spacer region (ITS) of the rDNA operon demonstrated no difference between the species (24), though there appeared to be some differences in the DNA sequence of a portion of the *MAT-2* mating type idiomorph of the two species (22). Also, isolates of *C. polonica* had a unique electromorph for the isozyme diaphorase (11).

The two species also appear to have an interfertility barrier, but sexual compatibil-

ity is difficult to test in the homothallic *Ceratocystis* species (9). Most *Ceratocystis* species are capable of unidirectional mating type switching, in which one of the mating types (*MAT-2*) is capable of selfing, producing progeny that are self-sterile (*MAT-1*) or self-fertile (*MAT-2*) in a 1:1 segregation pattern (8). The *MAT-1* progeny is always self-sterile but can cross with *MAT-2* strains, whereas *MAT-2* progeny is usually self-fertile but can also cross with *MAT-1* strains. Selfing in *MAT-2* strains of *Ceratocystis* species occurs via a deletion of the *MAT-2* idiomorph, which transforms the strain to the *MAT-1* mating type (23). Normal mating apparently occurs between the switched (*MAT-1*) and unswitched (*MAT-2*) nuclei (8). Testers of *MAT-1* strains are easily identified as self-sterile progeny derived from selfings. The *MAT-2* testers are more difficult to identify but can sometimes be found in self-sterile sectors of otherwise self-fertile *MAT-2* strains. These *MAT-2* testers are generally only able to form perithecia and ascospores when paired with *MAT-1* testers, though sometimes they can be induced to self when paired with either *MAT-1* or *MAT-2* strains (9). Using such *MAT-2* mutants, pairings between *C. polonica* and *C. laricicola* were only partially interfertile (9).

Because of the importance of these respective bark beetle species and the fact that the *Ceratocystis* species are important plant-pathogenic symbionts, we made a detailed comparison of isolates of the two species from Europe, southwestern Siberia (south-central Russia), and Japan. We employed phylogenetic analysis of a portion of the *MAT-2* idiomorph, conducted tests for sexual interfertility, and made morphological comparisons. Lastly, we inoculated mature larch and spruce trees to determine if the isolates were physiologically specialized to the respective tree hosts.

MATERIALS AND METHODS

Isolates. In all, 27 isolates of *C. polonica* and *C. laricicola* were examined. Most of the isolates studied are in the collection of the T. C. Harrington, (isolate numbers beginning with the letter C), while some of the Siberian isolates are maintained in the collection of the N. V. Pashenova. Some of the isolates are also maintained in the Centraalbureau voor

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Schimmelcultures (CBS), the American Type Culture Collection (ATCC), or the Norwegian Forest Research Institute (NISK): C181 = CBS 100207, C320 = CBS 228.83, C322 = CBS 133.38, C731 = ATCC 62335 = NISK 80-53-7, C746 = CBS 100208, C755 = CBS 100206, and C791 = CBS 100205 = NISK 90-120-181. Collection information for most of the isolates can be found in Harrington and Wingfield (12), while the Russian isolates of *C. laricicola* and *C. polonica* were collected in 1994 to 1996 from galleries of *Ips cembrae* or *I. typographus* in *Larix sibirica* Ledeb. or *Picea obovata* Ledeb., respectively, in southern Siberia, near Krasnoyarsk, Russia.

MAT-2 Sequences. Isolates were grown in liquid culture with 20 g/liter malt extract and 10 g/liter yeast extract for 10 to 14 days at room temperature (approximately 21°C) before DNA extraction. Mycelium was harvested, and the DNA was extracted using the method described by DeScenzo and Harrington (3). The primers used for *C. laricicola* and *C. polonica* were LMAT2-F (5'-CGGAAAGACAGACACC AAGAC-3') and LMAT2-R (5'-GGTTGT AACGGTAGTGGGATA-3') (22), except for the Japanese isolates of *C. laricicola*, which used LMAT2-F and the degenerate primer NeHMG-2 (1).

The 100 µl polymerase chain reaction (PCR) reactions included 200 µM of dNTPs, 0.5 µM of the degenerate primers or 0.25 µM of the specific primers, 4 mM MgCl₂, 1× PCR buffer (supplied with the enzyme), 2.5 units *Taq* polymerase (Promega, Madison, WI), and 10 to 50 ng of extracted DNA. The PCR products were generated using an initial denaturation of 60 s at 95°C, followed by 35 cycles of 95°C for 35 s, annealing at 60°C (52°C for the Japanese *C. laricicola* isolates) for 60 s, and elongation at 72°C for 60 s. Amplified products of the expected size were purified using the Concert Rapid PCR Purification Kit (Invitrogen Corporation, Carlsbad, CA) for direct sequencing with the primers LMAT2-F and LMAT2-R at Iowa State University DNA Sequencing and Synthesis Facility using an ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, CA). The PCR products for the Japanese *C. laricicola* isolates were gel purified (GeneClean II kit, BIO 101 Inc., Vista, CA) and cloned into pGEM-T easy vector (Promega Inc., Madison, WI). The plasmids were purified using the QIAprep Spin Miniprep plasmid DNA extraction kit (Qiagen Inc., Valencia, CA) and sequenced with the primers T7-2 and SP6.

The MAT-2 HMG box nucleotide sequences were manually aligned, with 10 characters from the intron region missing in the *C. polonica* and *C. laricicola* sequences when compared to the outgroup taxon (*C. virescens*). Including gaps, there were 197 characters in the analysis. Parsimony analysis (PAUP version 4.0b5, [19])

used the heuristic search option (optimality criterion = maximum parsimony, gaps treated as a newstate or fifth character, simple taxon addition, with the branch-swapping algorithm = tree-bisection re-connection). Confidence levels were determined using 1,000 bootstrap replicates (4).

Interfertility. The MAT-2 isolates of these species are usually self-fertile, but we identified MAT-2 testers of *C. laricicola* and *C. polonica* that were no longer self-fertile, rarely produced perithecia and ascospores through selfing, or in the case of C1226-3, produced perithecia without necks. All but one of the MAT-2 isolates (C757) used in interfertility tests were proven to be MAT-2 by amplifying a portion of the MAT-2 idiomorph using the above described PCR primers. Self-sterile, MAT-1 progeny were selected from selfings of C178 of *C. laricicola* and C791 and C1226 of *C. polonica*. Self-sterile field isolates lrc02/94, C745, and C755 were presumed to be MAT-1.

Cultures were grown and pairings were conducted as described by Harrington and McNew (9). The MAT-1 testers were used as recipients (females) and the MAT-2 testers were donors (spermatizing strains or males). Recipient cultures were grown for 8 days on 1.5% malt extract with 2.0% agar (MEA) in 95-mm-diameter plastic petri dishes; an autoclaved 3-cm-long twig of *Pinus strobus* with bark removed was placed in the recipient plate before addition of molten MEA. Spermatizing cultures were grown on MYEA (2.0% malt extract, 0.2% yeast extract, and 1.5% agar), which is more suited than MEA for conidium production. The spermatizing cultures were flooded with sterile, deionized water. Conidia, conidiophores, and mycelial fragments were loosened by lightly scraping with a spatula, and 1 or 2 ml of this suspension was added to each recipient culture. The recipient plate was swirled slightly to disperse the inoculum. Plates were incubated at room temperature (20 to 23°C) and lighting, and the recipient plates were inspected periodically for perithecia and ascospore production, which was generally evident within 2 weeks, but plates were observed periodically for up to 5 weeks. The MAT-2 testers were also paired with themselves, but no perithecia were noted in these self-pairings, except that self-pairings of C1226-3 produced abundant perithecia without necks.

Single-ascospore progeny was recovered as described by Harrington and McNew (8,9) after first dispersing the ascospores in a light oil (Isopar M). Germination of ascospores was assessed by streaking the dispersed ascospores across a MEA-coated microscope slide, incubating at room temperature for 24 h, and examining the spores at ×400.

Morphology. Because of the reported difference in size of the perithecial bases in

C. laricicola and *C. polonica* isolates from Japan (25), we measured perithecial bases on MEA and other media, and measurements were made at ×200 or ×400.

Inoculations. Inoculations were performed in the Krasnoyarsk Territory and Khakassya (southern Siberia, Russia). Six trees of *Picea obovata* (Siberian spruce) and 12 trees of *Larix sibirica* (Siberian larch) of 25 to 30 cm diameter at breast height (dbh) were selected for inoculation. The *L. sibirica* trees were inoculated on 24 June 1998, and the *P. obovata* trees were inoculated on 29 June 1998. Three of the *P. obovata* trees were on a well-drained site, while three others were in a poorly drained area with excessive soil moisture. The *L. sibirica* trees had been subjected to natural defoliation by the Siberian moth (*Dendrolimus superans sibiricus* Tschetvr.). Three of the *L. sibirica* trees were not apparently defoliated, three of the trees had an estimated 50% foliage missing, three trees were 75% defoliated, and three trees were completely (100%) defoliated at the time of inoculation.

Wounds were made in the stems in seven rings spaced at 80 to 200 cm height above the ground. Three wounds were evenly spaced around the circumference at each height, and the three wounds at each height were staggered with the wounds of adjacent heights. The wounds were made by a cork borer (8 mm diameter) to the depth of the cambium/xylem area. Inoculum consisted of mycelium and spores at the advancing margins of MEA grown isolates taken with the cork borer. On each tree, the three wounds of the top-most ring were not inoculated (controls), the next three lower rings were inoculated with *C. polonica* isolates pln24/96, C708, and C787, respectively, and the lowest three rings were inoculated with *C. laricicola* isolates lr05/94, C746, and C178, respectively. The bark was removed 5 weeks after inoculation and the vertical extent (above and below the inoculation point) of necrosis in the cambium region was measured.

A three-way analysis of variance (ANOVA) was performed with host, fungal species, and stress as the variables (SAS Institute Inc., Cary, NC). For stress, the lesion size in the three *P. obovata* trees on the well-drained site was compared with that in the three trees on the poorly drained site, and the lesion size in the six *L. sibirica* trees with little defoliation (0 or 50% defoliation categories) was compared with the lesion size of the six *L. sibirica* trees with 75 or 100% defoliation.

RESULTS

MAT-2 Sequences. Parsimony analysis of the aligned 197 characters from the DNA sequence of a portion of the MAT-2 HMG box determined that 48 characters were informative, 47 were parsimony uninformative, and 102 characters were constant. Two most parsimonious trees of 128

steps were found, and the two trees differed only in the grouping of the three Japanese isolates of *C. laricicola*, which grouped as a single clade in the tree shown (Fig. 1), but the Japanese branch collapsed in polytomy with the other *C. laricicola* sequences in the other tree (not shown). The consistency index (CI) was 0.8359 (excluding uninformative characters = 0.7237), the rescaled consistency index (RC) was 0.7503, and the retention index (RI) was 0.8976. All *C. polonica* isolates had identical sequences, and there was strong bootstrap support for the clade grouping the *C. polonica* isolates (Fig. 1). The clade that included all the *C. laricicola* isolates also had strong support, though the Japanese isolates of *C. laricicola* differed slightly (5 bp) from the *C. laricicola* isolates from Europe and Russia (Fig. 1).

Interfertility. The isolates of *C. laricicola* from Scotland (MAT-1 isolate C178-

38) and Russia (MAT-2 isolate C1285) were fully interfertile with each other (Table 1), producing perithecia with an abundance of normal ascospores with a high germination rate. Similarly, the *C. polonica* MAT-1 testers from Norway and Russia were fully compatible with MAT-2 isolates of *C. polonica* from Norway, Russia, and Japan. The progeny recovered from fully interfertile crosses between isolates of *C. laricicola* or between isolates of *C. polonica* segregated for colony morphology or, in the case of C1226-3 × C791-23, the presence/absence of perithecial necks.

In 12 of 18 pairings between isolates of *C. laricicola* and *C. polonica*, no perithecia or only sterile perithecia (without ascospores) were found (Table 1). In the six other pairings between these species, perithecia with only small ascospore masses were seen, the droplet of spores was watery rather than the pink-opaque

spore mass formed from interfertile crosses, the ascospores were misshapen, and the ascospores did not germinate. This type of sexual incompatibility has been found in other interspecific pairings of *Ceratocystis* species (9).

Surprisingly, typical hybrid perithecia with misshapen ascospores of low germination rate were found in the intra-specific pairing of the Japanese MAT-2 isolate of *C. laricicola* and the Scottish MAT-1 isolate of *C. laricicola*. A few progeny were recovered from this latter cross, some of these isolates grew slowly, also typical of an interspecific hybrid (9), and the colony morphology of these progeny differed from that of either parent.

Morphology. Although the perithecial sizes varied greatly from isolate to isolate and even within a single petri plate, we did not see consistent differences among the media tested. We found that *C. laricicola* isolate C746 from Japan when grown on MEA had perithecial bases ranging from 280 to 352 µm wide, similar to the range found in the *C. laricicola* isolate C179 from Scotland (272 to 380 µm wide) and within the range of isolates of *C. polonica* from Europe and Russia (216 to 372 µm wide). Mean width of perithecial bases for *C. laricicola* isolates C179 and C746 were 318 and 323 µm, respectively; while those from *C. polonica* isolates C708, C791, C732, C1226, and C1227 were 338, 302, 248, 326, and 290 µm, respectively.

Inoculations. Larger lesions were found in the *L. sibirica* trees inoculated with *C. laricicola* isolates than in the *L. sibirica* trees inoculated with *C. polonica* isolates, and the reverse was true in the *P. obovata* trees (Table 2). The ANOVA indicated that the host species, the source of the isolates (whether from Europe, Russia, or Japan), the fungal species, and the level of stress all contributed significantly to the variation (Table 3). In general, the *L. sibirica* trees with 75 or 100% defoliation had larger lesions than similarly inoculated trees with no or only 50% defoliation, and this trend was particularly evident with the trees inoculated with *C. laricicola* isolates (Table 2). Similarly, *P. obovata* trees on the poorly drained soil had larger lesions than

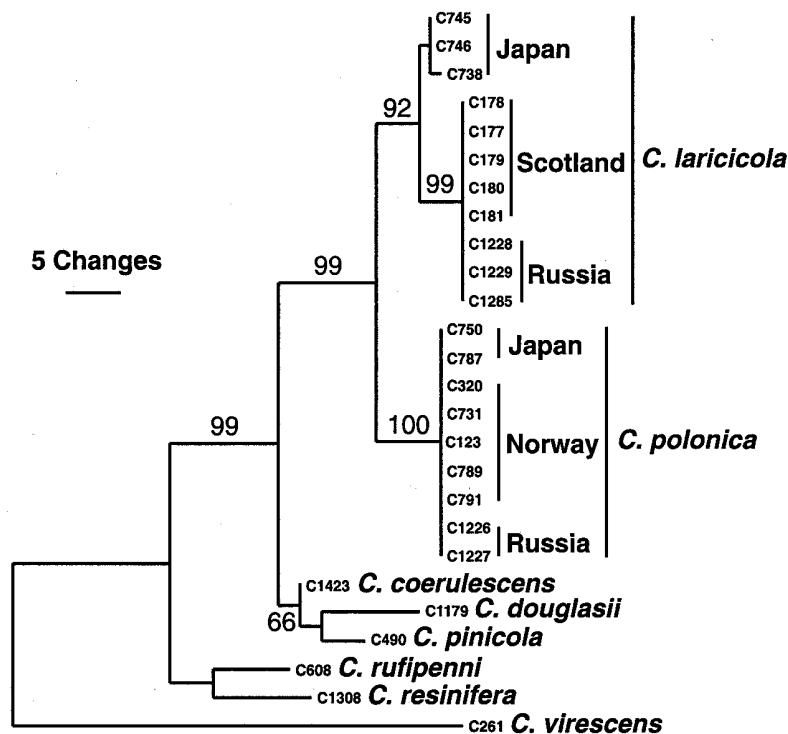


Fig. 1. One of two most parsimonious trees using a portion of the MAT-2 idiomorph of species in the *Ceratocystis coerulescens* complex. Bootstrap values greater than 50% are indicated.

Table 1. Intraspecific and interspecific pairings among MAT-1 and MAT-2 strains of *Ceratocystis laricicola* and *C. polonica*

	Male/female	<i>C. laricicola</i> , MAT-1			<i>C. polonica</i> , MAT-1		
		C178-38, Scotland	Irc02-94, Russia	C745, Japan	C791-23, Norway	C1226-5, Russia	C755, Japan
<i>C. laricicola</i> , MAT-2	C178, Scotland	Sterile	Hybrid ^w
	C1285, Russia	Perithecia ^x	Hybrid ^w	Hybrid ^w	...
	C738, Japan	Interfertile ^y	Hybrid ^w
<i>C. polonica</i> , MAT-2	C320, Norway	Sterile	Interfertile ^y	Interfertile ^y	...
	C1226-3, Russia	Perithecia ^x	Interfertile ^y	Interfertile ^y	...
	C757, Japan	Hybrid ^w	Interfertile ^y	Interfertile ^y	...

^w Few perithecia and ascospores misshapened and mixed with debris. No viable progeny recovered.

^x Perithecia but no ascospores produced.

^y Abundant perithecia and ascospores, and ascospores of high germination rate.

^z Few perithecia and some viable ascospore progeny with colony morphology differing from either parent.

the trees on the better drained soil, and the difference was more pronounced with the *C. polonica* isolates than with the *C. laricicola* isolates. The largest source of variation in the ANOVA was host specialization of the fungal species, i.e., the host species × fungus species interaction term (Table 3). Because of this strong interaction, no statistical comparison of means was made.

DISCUSSION

The comparisons of DNA sequences of a portion of the *MAT-2* idiomorph, intersterility, and physiological specialization to their respective hosts were consistent in distinguishing *C. laricicola* from *C. polonica*. The fixed difference in the electromorphs for diaphorase (11) also supported the distinction between these two species. The lack of difference in ITS sequences (24) and the morphological similarity of the two species suggest that they are very recently diverged, and host adaptation may have been the driving force in speciation.

In addition to the pairings reported here and in an earlier study (9), we have made many other attempts to cross isolates of *C. polonica* and *C. laricicola*, but these pairings have failed to produce perithecia and ascospores, or the ascospores produced were indicative of an incompatible cross between two species. In the earlier study (9), a small number of perithecia with small ascospore masses were seen in the pairing of isolates C123 (*C. polonica*, *MAT-2*) and C178-38 (*C. laricicola*, *MAT-1*), and the few viable progeny recovered had very slow growth and produced few perithecia. In the present study, we were unable to obtain viable progeny from the six interspecific hybrid pairings in which ascospores were produced.

Fully interfertile crosses were seen in intraspecific pairings between isolates of *C. laricicola* from Russian and European isolates, but not in intraspecific pairings of Japanese isolates of *C. laricicola* with European or Russian isolates. Our Japanese isolates of *C. polonica* and *C. laricicola* have lost their fertility (the *MAT-2* isolates no longer produce perithecia through selfings), and the *MAT-1* isolates from Japan failed to produce perithecia or ascospores when paired with *MAT-2* iso-

lates. No perithecia were formed in the *MAT-1* × *MAT-2* cross of the Japanese *C. laricicola* testers, perhaps because C755 is a self-sterile *MAT-2* isolate and not a *MAT-1* isolate, as presumed. Because of this ambiguity, it is questionable if the Japanese isolates of *C. laricicola* are truly incompatible with European and Russian isolates of this species. However, the Japanese isolates of *C. laricicola* had *MAT-2* sequences that differed slightly from those of *C. laricicola* isolates from Europe and Russia. Thus, there is some indication that *C. laricicola* from Japan is distinct from the *C. laricicola* found in Russia and Europe.

We have been unable to distinguish these two species morphologically, but Yamoaka et al. (25) reported that Japanese isolates of *C. laricicola* have perithecial bases that are 145 to 300 μm wide, while *C. polonica* isolates from Japan had perithecial bases 200 to 390 μm wide. However, under our conditions, *C. laricicola* isolate C746 from Japan had perithecial bases ranging from 280 to 352 μm wide, similar to those of other *C. laricicola* isolates and to isolates of *C. polonica*. Unfortunately, our *C. polonica* isolates from Japan no longer produce perithecia, but the measurements provided by Yamoaka et al. (25) are consistent with those of our European and Russian isolates of *C. polonica* and *C. laricicola*. In general, sizes of perithecial bases and necks vary greatly within and among *Ceratocystis* species (12), and it has been difficult to distinguish closely related species by such features. With respect to evolutionary pressure, the size of perithecia may not be significant in the respective ecological niches of these two species as their respec-

tive vectors, *Ips cembrae* and *I. typographus*, do not differ substantially in the types of galleries they construct nor in the size of the adult beetles, the stage at which ascospores are acquired.

Perhaps the most important phenotypic character separating *C. laricicola* from *C. polonica* is the degree of aggressiveness to their respective plant hosts. Using standard inoculation methods and lesion size to assess aggressiveness (2,13,14,16,18,25,26), our inoculation data showed clearly that *C. laricicola* and *C. polonica* cause larger lesions sizes in their respective hosts than in their nonhosts. Trees stressed by insect defoliation or poorly drained soil showed greater lesion size than their unstressed counterparts. The host specialization found here further supports the contention that the pathogenicity of these fungi is ecologically significant, and that these fungi may be an important aid to their respective bark beetle vectors in killing trees (15).

Although the two fungal species are sympatric across Eurasia and are morphologically identical, the *MAT-2* sequences clearly indicate that they belong to separate lineages. Interfertility barriers appear to have maintained these separate lineages, lineages that were not recognized by ITS sequencing (23). In spite of our inability to distinguish these *MAT-2* lineages by morphology, the evidence presented here supports maintaining them as distinct species (12) and supports the assertion that physiological characters such as host specialization should be sufficient to delineate monophyletic lineages as species (10).

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Table 3. Three-way analysis of variance of lesion length in *Larix sibirica* and *Picea obovata* inoculated with *Ceratocystis laricicola* and *C. polonica*

Source of variation	df	Mean square	F value	P > F
<i>L. sibirica</i> vs. <i>P. obovata</i>	1	126,365	34.10	<0.0001
Stressed vs. healthy trees	1	193,891	52.33	<0.0001
<i>C. laricicola</i> vs. <i>C. polonica</i>	1	115,387	31.14	<0.0001
European vs. Russian vs. Japanese isolates	2	94,931	25.62	<0.0001
Host species × stress	1	120,077	32.41	<0.0001
Host species × stress × fungus species	2	207,169	55.91	<0.0001
Host species × fungus species	1	309,903	83.64	<0.0001
Error	314	3,705		

Table 2. Extent of lesions (mean ± standard error of 9 measurements, in mm) in trees of *Larix sibirica* and *Picea obovata* inoculated with *Ceratocystis laricicola* or *C. polonica*

Fungal species	Isolate no.	<i>Larix sibirica</i>				<i>Picea obovata</i>	
		No defoliation	50% defoliation	75% defoliation	100% defoliation	Healthy trees	Poorly drained site
<i>C. laricicola</i>	C178	26.8 ± 1.2	41.0 ± 3.8	41.4 ± 3.2	43.0 ± 2.0	21.1 ± 1.0	20.8 ± 2.3
	Irc05/94	40.0 ± 4.4	45.4 ± 3.4	210.2 ± 32.2	327.4 ± 65.0	20.1 ± 1.5	24.2 ± 1.2
	C746	39.3 ± 2.7	40.8 ± 6.0	252.0 ± 105.8	439.9 ± 19.9	19.6 ± 2.4	22.9 ± 3.5
<i>C. polonica</i>	C708	20.8 ± 1.3	19.1 ± 2.6	23.7 ± 2.7	24.3 ± 2.6	21.9 ± 3.5	42.8 ± 5.2
	pln24/96	18.3 ± 1.9	22.2 ± 7.4	26.4 ± 2.7	21.6 ± 1.9	42.9 ± 4.8	62.9 ± 5.5
	C787	22.1 ± 1.7	21.2 ± 3.1	27.2 ± 2.3	32.7 ± 1.9	46.7 ± 2.6	65.0 ± 10.8
Control		10.8 ± 1.1	17.3 ± 2.2	19.7 ± 1.6	17.3 ± 3.3	10.2 ± 1.0	10.2 ± 0.9

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