
First report of rust on *Alnus* in New Zealand is *Melampsorium betulinum*, not *M. hiratsukanum*

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A rust disease was first observed in New Zealand on leaves of alder (*Alnus viridis*) in North Canterbury in 1980, then on *Alnus* spp. in Nelson in the 1990s and Auckland in 2012. The causal agent was determined by molecular techniques to be *Melampsorium betulinum* and not *M. hiratsukanum*, a species that is currently spreading throughout the northern hemisphere. A rust on *Betula nigra* and *B. populifolia*, and earlier specimens from *B. pendula*, were also confirmed as *M. betulinum*.

Key words – *Melampsorium alni* – pucciniales – rust fungi

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Introduction

Three species of *Melampsorium* infect alders (*Alnus* spp.): *M. alni* (Thüm.) Dietel, *M. betulinum* (Pers.) Kleb., and *M. hiratsukanum* S. Ito ex Hirats. f. (Hiratsuka et al. 1992). *M. alni* is restricted to East Asia and Siberia (Kaneko & Hiratsuka 1981, Hantula et al. 2009). *M. betulinum* is widespread and occurs throughout New Zealand, but appears most common in the South Island. *M. hiratsukanum*, which probably originated in East Asia, is currently spreading throughout Europe.

Morphologically the three rusts are quite similar. However, urediniospores of *M. alni* have a single polar germ pore at either end, which distinguishes them from both *M. betulinum* and *M. hiratsukanum*, which have 2–3 germ pores near each end of the spore (Kaneko & Hiratsuka 1981, Kurkela et al.

1999). There are small reported differences in the size of urediniospores of *M. betulinum* and *M. hiratsukanum* (Kurkela et al. 1999, Hantula et al. 2009), but these differences are, perhaps, insufficient to unequivocally distinguish the two species.

In June 2012 trees of *Alnus cordata* (Italian alder) growing on the Tamaki Campus, Auckland University, were noticed with abundant leaf spots (Fig. 1). Upon closer examination the spots were seen to contain uredinia of a rust fungus. Six weeks later another tree (*Alnus* sp.) in Mt Albert, Auckland was found with leaves covered in telia (Fig. 2); no uredinia were seen. Examination of leaves on the Tamaki trees one day later found a few telia scattered among the remaining uredinia. The heavy infection on the alders was suspected to be *M. hiratsukanum*.

However, further investigation revealed that rust had been found earlier on *Alnus* spp. in New Zealand. In May 1980 rust had been observed on leaves of *A. viridis* (green alder) at the then Forest Research Institute nursery in North Canterbury (NZFRI specimen). Light infection of the rust was seen subsequently on other species of *Alnus* in the nursery. Information was later obtained about rust on *Alnus* spp. in Appletons Tree Nursery, Wakefield, Nelson (Eric Appleton, pers. comm.), where rust had been present on *Alnus* spp. for several years. In April 2013, seedlings of *A. cordata* in Appletons Nursery were heavily infected and their leaves were starting to fall whereas normally they remained attached until mid to late June depending on intensity of frosts; a 20-year old shelter belt of *A. cordata* was showing rust infection; seedlings of *A. glutinosa* (black alder, European alder) were almost bare due to heavy rust infection; the top leaves of *A. glutinosa* subsp. *barbata* (bearded alder) were infected but not falling; and *A. rubra* (red alder) had a small amount of rust on the lower leaves. Interestingly, plants of *A. cremastogyna*, *A. firma*, *A. ?formosana*, *A. jorullensis*, *A. nitida* and *A. subcordata* were rust-free, although light infection had appeared by mid May 2013 on *A. ?formosana*, *A. nitida* and *A. subcordata*.

Melampsorium betulinum was known only on silver birch (*Betula pendula*) in New Zealand and was first recorded in the country by Robinson (1919). During an Auckland BioBlitz (late March 2012), a severe rust infection was noticed on leaves of *Betula nigra* (river birch, black birch) growing in the Auckland Botanic Gardens (Fig. 3). In Appletons Nursery, *B. nigra*, *B. pendula* and *B. populifolia* were all infected with rust in April 2013; the latter had shown infection for at least 10 years (Eric Appleton, pers. comm.). Some species appear to have remained rust-free including several Asiatic *Betula* spp. and a 35-year-old *Alnus nepalensis* tree.

To determine the species of rust present on alders and birches in New Zealand, samples from New Zealand and from Europe were examined by both morphological and molecular methods.

Methods

Isolates and morphology

For examination of morphological features the rusts were mounted in lactophenol and examined with an Olympus BH-2 microscope. Voucher materials were deposited in the PDD fungarium. Specimens were also borrowed from the Finnish Forest Research Institute, Finnish Museum of Natural History.

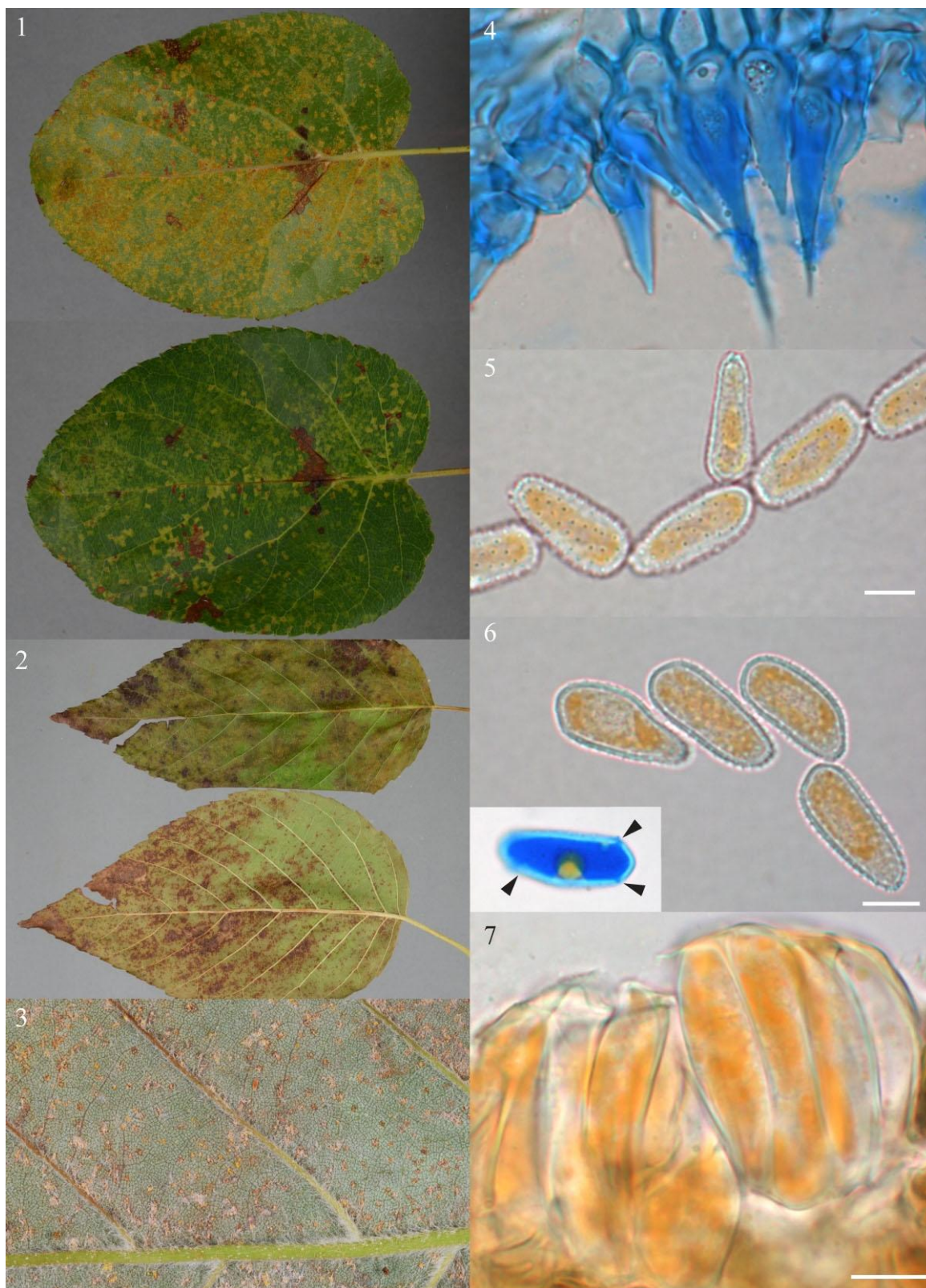
Phylogenetic analyses

The rust fungi were also examined by molecular methods. Sori were excised and DNA was extracted using an X-tractor Gene System (Corbett Life Science, NSW, Australia). The nuclear ribosomal large subunit (LSU) locus was amplified with a rust-specific primer Rust2inv (Aime 2006) and LR6 (Vilgalys & Hester 1990). The nuclear ribosomal internal spacer region (ITS) locus was amplified with ITS1F and ITS 4B (Gardes & Bruns 1993). ITS sequences generated were aligned with GenBank sequences of *M. alni*, *M. betulinum*, and *M. hiratsukanum* generated for two previous studies (Kurkela et al. 1999, Hantula et al. 2009). Phylogenies resulting from analyses of the LSU and ITS datasets were examined to identify any regions of conflict before concatenating the datasets in Geneious Pro v6.0.5 (Biomatters, <http://www.geneious.com/>). The concatenated dataset was analysed in PhyML 3.0 (Guindon & Gascuel 2003) using the GTR+ Γ +I model of evolution and 1,000 bootstrap support values were generated.

Results

Morphological examination

On *Alnus* spp. symptoms consisted of numerous orange uredinia on the lower leaf surface with a corresponding yellow-brown spot on the upper surface. The uredinia were covered by the epidermis, which eventually ruptured at a central pore. The sori were < 0.5 mm diam., irregular in shape due to being restricted by the veins, scattered or in groups, covering large portions of the leaf. Uredinia possess a peridium with ovate-conical ostiolar



Figs 1–7 – *Melampsorium betulinum* **1** Symptoms of uredinial infection on leaves of *Alnus cordata*. **2** Symptoms of telial infection on leaves of *Alnus* sp. **3** Symptoms of uredinial infection on leaves of *Betula nigra*. **4** Ostiolar cells in uredinia, tapering to a long acute apex. **5–6** Urediniospores; some are in a chain. Insert shows germ pores (arrowed). **7** Teliospores.

cells, 30–53 μm long, tapering to a long acute apex (Fig. 4). Urediniospores (Fig. 5, 6) were ellipsoidal to ovoid, usually broadly rounded at

apex, obtusely rounded or angular at base, sometimes adhering in chains. They measured (23–)25–30(–33) \times (9–)11–13(–16) μm (av.

27.7 × 12.1 µm), with orange contents. The wall was thin, (0.5–)1–1.5 µm, hyaline, and echinulate. The broad end of the spore was often smooth (tonsure). Germ pores were obscure, but when stained and heated with aniline blue two pores were visible at either end of the spore (Fig. 6). Uredinia were eventually replaced by yellowish brown, subepidermal telia, which later became darker in colour. Telia were confined to the lower leaf surface, with a brown spot on the corresponding upper surface. They were < 0.5 mm diam., irregular in shape due to restriction by the veins, scattered or in groups, covering large portions of leaf. Teliospores (Fig. 7) were compacted laterally in a single layer beneath the epidermis, prismatic, cylindrical or elongate-clavate, sometimes inequilateral in shape, obtusely rounded at each end, sessile, and non-septate. They measured (27.5–)33–45(–55) × (9–)11–15.5(–17.5) µm (av. 40.5 × 12.7 µm), with pale yellow-brown contents. The wall was smooth, pale yellow-brown, 0.5–1.25 µm thick at the sides and 1.5–3 µm at apex.

Urediniospores were measured from 14 New Zealand specimens. Those from several *Alnus* spp. averaged 27.7 × 12.1 µm, while those from *Betula* spp. averaged 29.1 × 12.0 µm (Table 1).

Phylogenetic analyses

The DNA sequences generated for this study have been deposited in GenBank under accession numbers KF031533—KF031565. Molecular analyses (Fig. 8) confirmed the New Zealand rust on both *Alnus* spp. and *Betula* spp. is *M. betulinum* and not *M. hiratsukanum* (TreeBASE accession number 14290, (<http://purl.org/phylo/treebase/phyloids/study/TB2:S14290>)).

Specimens examined – II indicates the presence of uredinia and III the presence of telia.

***Melampsorium alni* — Russia**, Khabarovskii krai, Ulchskii region, on *A. mandshurica*, 7 September 1976, Pentti Alanko 31971 (H 7019539–II).

***Melampsorium betulinum* — New Zealand**, Auckland, Auckland University,

Tamaki Campus, on *Alnus cordata*, 21 June 2012, E.H.C. McKenzie (PDD 102645–II); *ibid.*, 5 August 2012 (PDD 102945–II, III); Mt Albert, on *Alnus* sp., 4 August 2012, E.H.C. McKenzie & F.A. McKenzie (PDD 102647–III); Manurewa, Auckland Botanic Gardens, on *Betula nigra*, 30 March 2012, E.H.C. McKenzie (PDD 102445–II). Nelson, Wakefield, Appletons Tree Nursery, on *A. oblongata*, 24 May 1998, E. Appleton (NZFRI-M 3834–II); *ibid.*, on *A. glutinosa*, March 2013 (PDD 103306–II); *ibid.*, on *A. acuminata* × *glutinosa*, 23 April 2013 (PDD 103310–II); *ibid.*, on *A. cordata* (PDD 103307–II); *ibid.*, on *A. glutinosa* subsp. *barbata* (PDD 103309–II); *ibid.*, on *A. rubra* (PDD 103308–II); *ibid.*, on *B. nigra* (PDD 103312–II); *ibid.*, on *B. pendula* (PDD 103311–II); *ibid.*, on *B. populifolia*, 30 April 2013 (PDD 103316–II); *ibid.*, on *A. ?formosana* 22 May 2013 (PDD 103397–II); *ibid.*, on *A. nitida* (PDD 103399–II); *ibid.*, on *A. subcordata* (PDD 103398–II). North Canterbury, Rangiora, Forest Research Institute Nursery, on *A. viridis*, 21 May 1980, C.W. Barr (NZFRI-M 1893–II). Mid Canterbury, Christchurch, Addington, on *B. pendula*, 8 May 1995, A.J. Healy 95/60 (PDD 64927). **Austria**, Steiermark, Gurtaler Alpen, on *B. nana*, 6 September 2001, E. Teppner, H. Teppner (PDD 77196). **China**, Xinjiang, Gongliu, on *B. pendula*, 28 August 1986, Zhuang Jian-yun (PDD 59034). **Finland**, Helsinki, Kumpula, New Botanical Garden, on *B. pendula*, 9 October 2009, Pentti Alanko 143861 (H 6035396–II); Kuusamo, Oulanka, Biological Station, on *B. pubescens*, 7 August 2009, Pentti Alanko 142093 (H 6035417–II).

***Melampsorium hiratsukanum* — Austria**, Steiermark, Grazer, on *A. pubescens*, 19 September 2002, C. Scheuer, C.M. Denchev (PDD 77191); Steiermark, Schladminger Tauern, on *A. incana*, 5 August 2003, H. Riegler-Hager, Ch. Scheuer (PDD 78493). **Finland**, Kuhmo, Jauhovaara, on *A. incana*, 10 September 1998, Timo Kurkela (PDD 102745–II); Helsinki, Vuosaari, on *A. incana*, 2 September 1998, Veikko Hintikka (PDD 102747–II); Varsinais-Suomi, Lohja, Sammatti, Luskala, on *A. incana*, 23

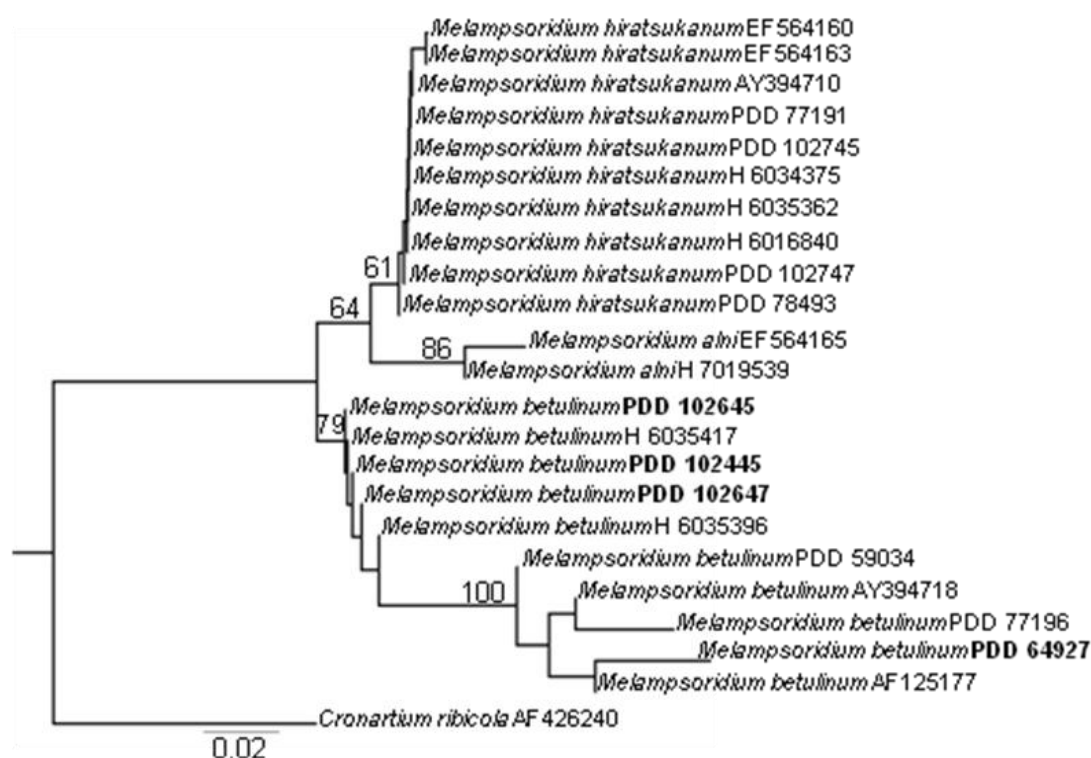


Fig. 8 – Phylogenetic tree based on maximum likelihood analyses of LSU and ITS sequences of multiple collections of *Melampsorium*, and rooted with *Cronartium ribicola*, identified by either GenBank or fungarium accession numbers. Origins of sequences are provided in the text; those in bold are from New Zealand material. Numbers above branches indicate maximum likelihood bootstrap support (1000 replicates). Branch lengths are ML estimates and are scaled in terms of number of nucleotide substitutions per site.

Table 1 Dimensions of urediniospores of New Zealand collections of *Melampsorium betulinum*

Host	PDD Nos	Urediniospores Length (μm)			Width (μm)		
		Minimum	Mean	Maximum	Minimum	Mean	Maximum
<i>A. rubra</i>	103308	23.8	26.5	31.0	10.7	12.9	16.0
<i>A. acuminata</i>	103310	23.0	27.2	32.9	10.3	12.0	13.6
× <i>glutinosa</i>							
<i>A. glutinosa</i>	103306	24.1	27.2	32.0	9.3	11.8	14.2
<i>A. viridis</i>	1893*	24.1	27.2	33.4	10.1	12.2	14.6
<i>B. nigra</i>	102445	23.3	27.5	32.0	9.6	11.7	13.3
<i>B. nigra</i>	103312	22.5	27.7	34.2	10.0	12.3	14.7
<i>B. pendula</i>	103311	24.1	27.8	30.4	8.2	10.7	13.4
<i>A. cordata</i>	102645	25.2	28.4	33.2	9.1	10.6	16.0
<i>A. cordata</i>	103307	22.7	28.6	32.1	10.7	12.5	14.7
<i>A. glutinosa</i>	103309	24.6	28.8	33.7	10.9	12.5	14.3
ssp. <i>barbata</i>							
<i>B. pendula</i>	64927	23.6	29.4	36.9	10.9	12.4	14.7
<i>B. populifolia</i>	103316	26.9	30.0	33.5	9.7	11.5	13.4
<i>B. pendula</i>	46735	25.9	30.6	34.2	10.0	11.9	13.5
<i>B. pendula</i>	45055	26.3	30.7	37.2	10.3	13.4	17.4
<i>Alnus</i> (av.)		23.9	27.7	32.6	10.2	12.1	14.8
<i>Betula</i> (av.)		24.7	29.1	34.1	9.8	12.0	14.3

*NZFRI-M

September 2009, Teuvo Ahti 69285 (H 6016840–II); Asikkala, Mustjärvi, Lyytikkä, on *A. incana*, 19 September 2012, Veli Haikonen 29109 (H 6034375–II); Sastamala, Suodenniemi, Torisevanmaa, on *A. incana*, 6 September 2009, Pentti Alanko 142823 (H 6035362–II).

Forest Health database record

Melampsorium betulinum — New Zealand, North Canterbury, Eyreton, on *A. rubra*, 19 May 1982.

Discussion

When rust was observed on *Alnus* in New Zealand in 2012 it was suspected to be *M. hiratsukanum*. This species was described from Japan and is also known in other parts of Asia (China, Korea, Philippines). It also occurs in Central and South America (Argentina, Colombia, Costa Rica, Ecuador, Guatemala) (Farr & Rossman 2013). It was found in Europe (Estonia, Finland) in mid 1990s and later confirmed as the cause of an epidemic outbreak of rust on *Alnus* spp. in Europe (Kurkela et al. 1999, Hantula et al. 2009). The rust is continuing to spread throughout Europe (e.g., Sert & Sumbul 2005, Moricca & Maresi 2010), and most recently was reported from Wales, and also British Columbia, western Canada (Hantula et al. 2012). Based on analyses of ITS sequences, Hantula et al. (2009) confirmed that the epidemic in Europe had its origins in eastern Asia. They suggested that the introduction of *M. hiratsukanum* into Europe may be due to natural migration, however, Hantula & Scholler (2006) thought that introduction via contaminated plant material was most likely.

There are reported differences in the size of urediniospores of *M. betulinum* and *M. hiratsukanum*. Kurkela et al. (1999) found that in Finland and Estonia *M. betulinum*, collected from birch, had significantly longer urediniospores than *M. hiratsukanum* collected from alder (mean 31.6 μm on *B. pendula* and 33.6 μm on *B. incana*, versus 25.0 μm on *A. incana* and 26.6 μm on *A. glutinosa*). Similarly, Hantula et al. (2009) found that urediniospores of *M. hiratsukanum* averaged 26.0 \times 13.5 μm , whereas urediniospores determined as *M. betulinum* from *A. glutinosa*

and *A. incana* collected in Scotland were longer (30.9 \times 13.4 μm), as were those from *B. pendula* and *B. pubescens* (33.0 \times 14.7 μm) from Finland. The New Zealand specimens averaged 29.1 \times 12.0 μm (from *Betula* spp.), and 27.7 \times 12.1 μm (from *Alnus* spp.), both length measurements lying between those reported for *M. hiratsukanum* and *M. betulinum* (Kurkela et al. 1999, Hantula et al. 2009).

Two features seen in the New Zealand collections correspond with those described for *M. hiratsukanum* by Moricca & Maresi (2010). Firstly, the urediniospores were sometimes in chains. Secondly, Moricca & Maresi (2010) figured long tapering ostiolar cells, which were also noted in the New Zealand collections. However, the tapering ostiolar cells seem to be a feature of both *M. betulinum* and *M. hiratsukanum* (Wilson & Henderson 1966, Hiratsuka et al. 1992).

Maximum likelihood analysis resulted in a phylogeny that recovered the same arrangement of *Melampsorium* species as presented in Hantula et al. (2009) and confirmed that the rust on both *Alnus* and *Betula* in New Zealand is *M. betulinum*. There is a highly supported subclade (100% ML bootstrap) consisting of five collections from Austria, China, Finland, Scotland, and New Zealand within the *M. betulinum* clade (Fig. 8). The percentage of sequence identity between these and the sequence of *M. betulinum* from Finland (H 6035396) range between 95.6–99.8%. Further investigation using a different gene region is needed to determine if this subclade represents a distinct lineage. There were no obvious morphological differences between the urediniospores of the collections within the *M. betulinum* clade.

This is not the first recorded instance of *M. betulinum* on alders. In Scotland, an endemic rust indistinguishable from *M. betulinum* occurs on alders (*A. glutinosa*, *A. incana*), whereas alders in areas of Europe affected by the current epidemic are infected by *M. hiratsukanum* (Hantula et al. 2009). *Melampsorium hiratsukanum* infects a broad range of *Alnus* spp. and in Europe is common on *A. glutinosa* and *A. incana* (Hiratsuka et al. 1992, Hantula et al. 2009, Farr & Rossman 2013). Both *M. hiratsukanum* and *M.*

betulinum have an alternate host (*Larix* spp.) on which their aecial stages occur (Hiratsuka et al. 1992, Farr & Rossman 2013). *M. hiratsukanum* from naturally infected alder in Finland produced aecia on all *Larix* species tested but did not infect *Betula* (Hantula et al. 2009). Neither pycnia nor aecia are known for *M. betulinum* in New Zealand.

This is the first report of *M. betulinum* in New Zealand on *Alnus* spp. and on *Betula nigra* and *B. populifolia*.

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