ARTICLE IN PRESS FUNBIO672_proof = 4 February 2016 = 1/9

FUNGAL BIOLOGY XXX (2016) 1-9



Mating type markers reveal high levels of heterothallism in *Leptographium sensu lato*

Q6 Tuan A. DUONG^{a,*}, Z. Wilhelm DE BEER^b, Brenda D. WINGFIELD^a,
 Michael J. WINGFIELD^a

^aDepartment of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa ^bDepartment of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI),

University of Pretoria, Pretoria 0002, South Africa

Q3 ARTICLE INFO

Article history: Received 25 September 2015 Received in revised form 23 December 2015 Accepted 3 January 2016 Corresponding Editor: Pedro W Crous Keywords: Grosmannia Heterothallism Homothallism Leptographium Q4 Mating type markers

ABSTRACT

Species of Leptographium sensu lato (Ophiostomatales, Ascomycetes) are sap-stain fungi vectored by bark beetles (Coleoptera, Scolytinae) and some species cause or are associated with tree diseases. Sexual states have been reported for more than 30 species in this group and these have been treated in the sexual genus Grosmannia. No sexual state is known for at least 59 additional species and these reside in the genus Leptographium. The discovery of sexual states for species of Leptographium relies mainly on the presence of fruiting bodies on host tissue at the time of isolation and/or intensive laboratory mating studies, which commonly have a low levels of success. In this study, markers were developed to diagnose mating type and to study sexual compatibility of species in Leptographium sensu lato using these markers. To achieve this objective, available mating type sequences for species of Leptographium sensu lato and Ophiostoma were obtained, aligned and used to design primers to amplify MAT genes in Grosmannia and Leptographium species. Using these primers, it was possible to amplify portions of the mating type genes for 42 species and to determine thallism, in many species for the first time. Surprisingly, the results showed that heterothallic and putatively heterothallic species are abundant (39 out of 42 species) in Leptographium sensu lato, and only three species were confirmed to be homothallic. The mating type markers developed in this study will be useful for future studies concerning mating type and sexual compatibility of species in this genus.

© 2016 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.

Introduction

Leptographium sensu lato is an ascomycete genus that includes both sexual and asexual species (De Beer & Wingfield 2013). Species with known sexual states have been treated in the genus Grosmannia while those for which sexual states are unknown have been assigned names in *Leptographium* (Zipfel et al. 2006). There are currently 34 *Leptographium sensu* lato species with known sexual states. Of these, some have the ability to produce ascomata in cultures derived from single conidia or ascospores and are thus homothallic (Jacobs et al. 1998). Others are heterothallic and require crossing between isolates

E-mail address: tuan.duong@fabi.up.ac.za (T. A. Duong).

http://dx.doi.org/10.1016/j.funbio.2016.01.001

1878-6146/© 2016 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.

Please cite this article in press as: Duong TA, et al., Mating type markers reveal high levels of heterothallism in *Leptographium* sensu lato, Fungal Biology (2016), http://dx.doi.org/10.1016/j.funbio.2016.01.001

^{*} Corresponding author. Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa. Tel.: +27 (12) 420 3938; fax: +27 (12) 420 3960.

2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68

of opposite mating type to produce sexual structures (Jacobs et al. 1998; Masuya et al. 2005; Yamaoka et al. 2008; Duong et al. 2012). The remaining members of *Leptographium sensu* lato are known only by their asexual morphs.

Sexual compatibility in ascomycetes is determined by genes residing in the mating type (MAT) locus. The MAT locus has different alleles (idiomorphs) (Turgeon & Yoder 2000), containing mating type genes that encode transcription factors controlling mate recognition and sexual processes (Metzenberg & Glass 1990). In heterothallic ascomycetes, the mating types of isolates are determined by the presence of corresponding MAT idiomorphs in the haploid genome. Individuals of heterothallic ascomycetes have either the MAT1-1 or MAT1-2 idiomorph in their haploid genome and sexual reproduction occurs only when isolates of opposite mating type interact. In contrast, individual strains of homothallic ascomycetes contain both the MAT1-1 and MAT1-2 idiomorphs in their genomes and they are, therefore, self-fertile. While this is generally true, there are some exceptions, such as in the case of Ophiostoma quercus (Wilken et al. 2012).

The structure and gene content of the MAT loci have been used to gain insights into the sexual compatibility of many species originally believed to be asexual. Typically, most of these purported asexual species have been found to have fully-functional heterothallic mating systems (Kück & Pöggeler 2009). Thus, mating type markers have been developed for numerous important fungi and these have been used to determine whether sexual recombination might occur in natural populations of, for example, plant pathogens (Linde et al. 2003; Paoletti et al. 2005b; Groenewald et al. 2006; Wada et al. 2012). Mating type markers have also been useful to determine the mating type of individual isolates, thus replacing the traditionally tedious approach of crossing isolates in culture with tester strains of known mating type (Santos et al. 2010). Importantly, application of the growing knowledge regarding the MAT locus in fungi has facilitated the discovery of sexual cycles in many fungi of clinical or industrial relevance that were thought to be asexual (Horn et al. 2009; Seidl et al. 2009).

Mating type gene sequences and the structure of the MAT locus are known for ten species of Leptographium sensu lato (including Grosmannia). These include Grosmannia clavigera and its closely related species (Tsui et al. 2013), Leptographium procerum and Leptographium profanum (Duong et al. 2013). The MAT loci of these species have structures typical of those of heterothallic ascomycetes with both of the MAT idiomorphs present in an individual haploid genome. The MAT1-1 idiomorphs have three mating type genes namely MAT1-1-1, MAT1-1-2, and MAT1-1-3. But notably, besides the MAT1-2-1 gene, the MAT1-2 idiomorphs, all of these species have a truncated version of MAT1-1-1, lacking the functional alpha domain (Duong et al. 2013; Tsui et al. 2013). The presence of the truncated MAT1-1-1 on the MAT1-2 idiomorph has also been noted in species of Ophiostoma (Tsui et al. 2013; Comeau et al. 2015), a sister genus of Leptographium sensu lato, suggesting that the truncation event might share an evolutionary history among these two genera and perhaps also with other genera in the Ophiostomatales.

Most species in Leptographium sensu lato are known as only mitosporic fungi. Based on the results of Duong et al. (2013) and Tsui et al. (2013), we hypothesized that many of these species might actually have heterothallic mating systems. This would explain the low level of incidence of sexual states encountered for these fungi in nature or in culture. The aims of this study were thus to develop mating type markers in order to diagnose mating type and to consider the possible role that sexual reproduction might play in a relatively large collection of *Leptographium sensu* lato species.

Material and methods

Cultures, growth conditions and DNA extraction

Fungal isolates used in this study (Table 1) were obtained from the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Single hyphal tip or single conidium cultures were grown in YM broth (2 % malt extract and 0.2 % yeast extract) for 3–5 d. Mycelium was harvested by centrifugation and DNA was extracted using PrepMan[™] Ultra reagent (Applied Biosystems, California, USA) following the methods described in Duong et al. (2012).

MAT primer design

The sequences of MAT1-2-1 locus in Grosmannia clavigera and its relatives (Tsui et al. 2013), Leptographium procerum, Leptographium profanum (Duong et al. 2013), Ophiostoma ulmi, and Ophiostoma novo-ulmi subsp. novo-ulmi (Paoletti et al. 2005a) were aligned and used to design primers to detect the MAT1-2 idiomorph. The primers, Oph-HMG1 (5'-CGYAAGGAYMAY-CACAAGGC-3') and Oph-HMG2 (5'-GGRTGAAGMMKCT-CAACCTG-3'), were designed to amplify part of the HMG domain from the MAT1-2-1 gene.

Because all the known MAT loci in Leptographium sensu lato have a truncated version of MAT1-1-1 in the MAT1-2 idiomorphs, we refrained from designing MAT1-1 primers from the MAT1-1-1 gene sequence. Primers Oph-MAT1F1 (5'-ATGKCCRATGARGAYTGCT-3') and (5'-Oph-MAT1R2 GGCGKTKGCRTTGTAYTTGTA-3') (Duong et al. 2015) were previously designed from the MAT1-1-3 gene, which appears to be commonly present in Leptographium sensu lato (Duong et al. 2013; Tsui et al. 2013) and other Ophiostoma spp. for which the full MAT locus has been characterized (Tsui et al. 2013; Comeau et al. 2015), for detection of the MAT1-1 idiomorph. In some species, where amplification with this primer combination failed, the primer Oph-MAT1F1 was used in combination with Oph-MAT1R1 (5'-GGCYYTRTGAAGYTTCTGTGC-3'), although this combination resulted in slightly shorter fragments.

PCR amplification, sequencing, and mating type assignment

A PCR reaction mixture of 25 μ l consisted of 2.5 μ l 10 \times PCR reaction buffer, 2.5 mM MgCl₂, 200 μ M each dNTP, 0.8 μ M of each primer (forward and reverse), 1 U FastStart Taq DNA Polymerase (Roche) and 20–50 ng of genomic DNA. The cycling conditions were an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 55 °C annealing for 30 s, and 72 °C

3 <mark>Q1</mark>

Table 1 – Fungal isolates used in this study, including GenBank accession numbers of partial sequences of the MAT1-2-1 and MAT1-1-3 genes. Mating types and thallism of isolates are also indicated. Abbreviations and symbols used in the table are explained in the footnote. Accession numbers of MAT sequences used in the phylogenetic analyses are printed in italic. Sequences generated in this study are printed in bold type.

anigmatica CMW219 ips typographus japonicus Japan NA alacris CMW231 Pirus pinaster Portugal MATI CMW621 Pirus pinaster Portugal MATI CMW2305 Larix sp. USA MATI CMW2306 Larix sp. USA MATI CMW2306 Larix sp. USA MATI aurea CB5438.69 Pirus contorta var. latifolia Canada MATI CMW2306 Pirus contorta var. latifolia Canada MATI cMW2306 Pirus contorta var. latifolia Canada MATI CMW2306 Pirus contorta var. latifolia Canada MATI CMW2306 Pirus contorta var. latifolia Canada MATI CMW2306 Pirus contorta Canada MATI CMW2306 Pirus contorta Canada MATI CMW2306 Pirus contorta Canada MATI CMW1407 Pirus contorta Canada MATI CMW1407 Pirus contorta Canada MATI CMW1406 Hylurgus ligniperda New Zealand MATI CMW1406 Hylurgus ligniperda New Zealand MATI CMW1200 Pirus densifora Korea MATI cMW14201 Pirus ginea Italy MATI cMW245 Pirus pinea Italy MATI cMW246 Pirus pinea Italy MATI cMW249 Pirus pinea Italy MATI cMW229 Pirus pinea Italy MATI cMW229 Pirus pinea Italy MATI cMW229 Pirus pinea Italy MATI cMW230 Pirus substris Italy MATI cMW230 Pirus pinea Italy MATI cMW230 Pirus substris Italy MATI cMW230 Pirus substris Italy MATI cMW346 Pirus substris Italy MATI cMW230 Pirus substris Italy MATI cMW352 Pirus substris Italy MATI cMW352 Pirus substris Italy MATI cMW230 Pirus substris Italy MATI cMW352 Pirus substris Italy MATI cMW230 Pirus substris Italy MATI cMW230 Pirus substris Italy MATI cMW230 Pirus substris Italy MATI cMW230 Pirus substris It	ng type MAT1-2-1	MAT1-1-3	Thallism
lacris CMW621 Pinus pinus prime Portugal MAT1 CMV136 Pinus pinaster Portugal MAT1 CMV136 Pinus pinaster Portugal MAT1 CMV136 Pinus pinaster South Africa MAT1 CMV2980 Larix sp. USA MAT1 americana CMV2980 Larix sp. USA MAT1 cMV2980 Pinus contort aur. latifolia Canada MAT1 cMV2980 Pinus contort aur. latifolia Canada MAT1 cMV2980 Pinus contorta Canada MAT1 cMV2920 Pinus contorta Canada MAT1 cMV2020 Pinus contorta Canada MAT1 cMV1007 Pinus contorta Canada MAT1 cMV1065 Pinus ponderosa Canada MAT1 cMV1006 Hylurgus ligniperda New Zealand MAT1 cMV1010 Unknown New Zealand MAT1 cMV1200 Pinus densifora Korea MAT1 cMV1200 Pinus densifora Korea MAT1 cMV1201 Pinus densifora Korea MAT1 cMV1202 Pinus densifora Korea MAT1 cMV1202 Pinus densifora Korea MAT1 cMV1201 Pinus densifora Korea MAT1 cMV1202 Pinus densifora Korea MAT1 cMV1204 Pinus densifora Korea MAT1 cMV1205 Pinus pinea Italy MAT1 cMV280 Pinus subseris China MAT1 dibetinum CMV2817 Pice angemanti USA MAT1 cMV2820 Pinus subseris China MAT1 cMV2821 Pinus wanthensis China MAT1 dibetinum CMV2817 Pice angemanti USA MAT1 cMV2820 Pinus wanthensis China MAT1 cMV2821 Pinus wanthensis China MAT1 cMV2821 Pinus wanthensis China MAT1 cMV2821 Pinus wanthensis China MAT1 cMV2822 Pinus wanthensis China MAT1 cMV2821 Pinus wanthensis China MAT1 cMV2821 Pinus condentalis Dominican Rep. MAT1 cMV2821 Pinus senonensis China MAT1 cMV282	KT779243	KT779220	НО
CMW0633 Pinus pinaster Portugal MAT1 CMW2144 Pinus pinaster South Africa americana CMW2280 Iarix sp. USA MAT1 aurea CBS438.69 Pinus contorta Canada MAT1 CMW22866 Pinus contorta Canada MAT1 CMW22866 Pinus contorta Canada MAT1 CMW22867 Pinus contorta Canada MAT1 CMW22867 Pinus contorta Canada MAT1 CMW02868 Pinus contorta Canada MAT1 CMW0287 Pinus contorta Canada MAT1 CMW061 Hylurgus ligniperda New Zealand MAT1 CMW0106 Hylurgus ligniperda CMW0106 Pinus sp. USA MAT1 CMW0107 Pinus sp. USA MAT1 CMW10287 Pinus sp. USA MAT1 CMW12804 Pinus sp. USA MAT1 CMW12804 Pinus sp. USA MAT1 CMW12814 Pinus densifora Korea MAT1 CMW12814 Pinus densifora Korea MAT1 CMW12814 Pinus densifora Korea MAT1 CMW12814 Pinus densifora Korea MAT1 CMW2814 Pinus pinea Italy MAT1 CMW2819 Pinus pinea Italy MAT1 CMW290 Pinus subnamensis China MAT1 Matanense CMW18650 Pinus wallichiana Bhutan MAT1 astellanum CMW2651 Pinus wallichiana Bhutan MAT1 Astellanum CMW2651 Pinus wallichiana Bhutan MAT1 Astellanum CMW2651 Pinus wallichiana Bhutan MAT1 Astellanum CMW2667 Pinus wallichiana Bhutan MAT1 Amt1 Amt2000 Pinus cocidentalis Dominican Rep. MAT1 Amt2000 Pinus cocidentalis Dominican Rep. MAT1 Amt2000 Pinus cocidentalis Dominican Rep. MAT1 Amt2000 Pinus contorta Canada MAT1 Amt2000 Pinus densifora Japan MAT1 Amt2000 Pinus densifora Japan MAT1 Amt2000 Pinus densifora Japan MAT1 Amt2000 Pinus densifora Japan MAT1 Amt2000 Pinus densifora J	T1-2 KP171183		HE
CMW136 Pirus tarda USA MAT1 CMV2980 Larix sp. USA MAT1 aurea CMV2980 Larix sp. USA MAT1 aurea CBS438.69 Pirus contorta var. latifolia Canada MAT1 CMV2980 Pirus contorta Canada MAT1 CMV2980 Larix sp. USA MAT1 cMV10280 Pirus contorta Canada MAT1 CMV1298 Pirus contorta Canada MAT1 CMV1029 Pirus contorta Canada MAT1 CMV1010 Pirus contorta Canada MAT1 CMV1010 Pirus contorta Canada MAT1 CMV1010 Unknown New Zealand MAT1 CMV1010 Unknown New Zealand MAT1 CMV1020 Pirus densifora Korea MAT1 CMV1201 Pirus densifora Korea MAT1 cMV1201 Pirus densifora Korea MAT1 cMV1201 Pirus densifora Korea MAT1 cMV2811 Pica rubens USA NA riceiperda B* CMV452 Pseudotsuga menziesii USA NA riceiperda C* CMW446 Pica abies Norway NA robusta CMV710 Unknown Unknown MAT1 CMV281 Pirus spinea Italy MAT1 CMV282 Pirus pirea Italy MAT1 CMV282 Pirus sylversis Italy MAT1 CMV281 Pirus sylversis Italy MAT1 CMV281 Pirus sylversis Italy MAT1 CMV282 Pirus sylversis Italy MAT1 CMV282 Pirus sylversis Italy MAT1 CMV281 Pirus sylversis Italy MAT1 CMV282 Pirus sylversis Italy MAT1 CMV281 Pirus sylversis Italy MAT1 CMV282 Pirus sylversis Italy MAT1 CMV280 Pirus syl	.T1-1	KP171181	HE
CMW2244 Pinus pinaster South Africa MAT1 americana CMV2286 Larix sp. USA MAT1 aurea CB438.69 Pinus contora USA MAT1 cMV2286 Pinus contora Canada MAT1 cLavigera ATCC1808 Pinus ponderosa Canada MAT1 cLavigera ATCC1808 Pinus contora Canada MAT1 cLavigera ATCC1808 Pinus contora Canada MAT1 cLavigera ATCC1808 Pinus contora Canada MAT1 cMV107 Pinus contora Canada MAT1 cMV108 Pinus sontora Canada MAT1 cMV2824 Pinus sontora Canada MAT1 cMV2824 Pinus sontora Canada MAT1 cMV2824 Pinus sp. USA MAT1 cMV2824 Pinus sp. USA MAT1 cMV2824 Pinus sp. USA MAT1 cMV1200 Pinus densifora Korea MAT1 cMV1200 Pinus densifora Korea MAT1 inciepiperda B* CMV452 Pseudotsuga menziesii USA NA cMV2811 Picea rubens USA NA cMV2824 Pinus pinea Italy MAT1 cMV192 Pinus pinea Italy MAT1 cMV283 Pinus pinea Italy MAT1 cMV283 Pinus pinea Italy MAT1 cMV280 Pinus subetris Ita	T1-2 KI528492		HE
americana CMW2990 Larix sp. USA MATT aurea CFS438.69 Pinus contorta var. latijolia Canada MATT CMW29869 Pinus contorta var. latijolia Canada MATT the CMW29869 Pinus contorta Canada MATT KW1407 Pinus contorta Canada MATT KW1407 Pinus contorta Canada MATT CMW622 Pinus contorta Canada MATT CMW1400 Hylurgus ligniperda New Zealand MATT CMW1006 Hylurgus ligniperda New Zealand MATT CMW1015 Unknown New Zealand MATT CMW1200 Pinus densifora Korea MATT cMW1200 Pinus densifora Korea MATT cMW1200 Pinus densifora Korea MATT cMW1201 Pinus densifora Korea MATT cMW281 Pieca rubens USA NA robusta CMW710 Unknown Unknown MATT CMW281 Pinus pinea Italy MATT CMW292 Pinus pinea Italy MATT CMW293 Pinus pinea Italy MATT CMW280 Pinus pinea Italy MATT CMW280 Pinus spinea Italy MATT cMW281 Pinus spinea Italy MATT cMW281 Pinus spinea Italy MATT cMW281 Pinus spinea Italy MATT cMW281 Pinus spinea Italy MATT cMW282 Pinus spinea Italy MATT cMW283 Pinus spinea Italy MATT cMW284 Pinus sultichiana Bhutan MATT cMW285 Pinus sultichiana Bhutan MATT cMW286 Pinus sultichiana Bhutan MATT cMW286 Pinus wallichiana Bhutan MATT cMW1865 Pinus wallichiana Bhutan MATT cMW1865 Pinus wallichiana Bhutan MATT cMW220 Pinus occidentalis Dominican Rep. MATT cMW2190 Pinus occidentalis Dominican Rep. MATT cMW2190 Pinus contorta Canada MATT cMW2190 Pinus contorta Canada MATT cMW2100 Pinus contorta Canada MATT cMW2100 Pinus contorta Canada MATT cMW2100 Pinus sylvestris Norway MATT cMW2100 Pinus sylvestris Norway MATT cMW2101 P	T1-2 KP171184		HF
anierkania Carwaya Car	T1_2 KT779263		D_HF
AntenChW29869Finus contortaCanadaMAT1clavigeraATCC18066Finus contortaCanadaMAT1huntiiCMW622P. pinascertPortugalMAT1huntiiCMW622P. pinasterPortugalMAT1CMW624Pinus sp.USAMAT1CMW1006Hylurgus ligniperdaNew ZealandMAT1CMW1010UnknownNew ZealandMAT1CMW1202Pinus densifloraKoreaMAT1coreanaCMW14201Pinus densifloraKoreaMAT1ciceiperda B*CMW422Pseudotsuga menziesiUSANAciceiperda C*CMW446Picea rubensUSANAciceiperda C*CMW410UnknownUnknownMAT1coreanaCMW110UnknownUSAMAT1coreanaCMW111Pinus pineaItalyMAT1coreanaCMW112Pinus pineaItalyMAT1coreanaCMW112Pinus pineaItalyMAT1coreanaCMW111Pinus pineaItalyMAT1coreanaCMW112Pinus pineaItalyMAT1coreanaCMW1210Pinus pineaItalyMAT1coreanaCMW1210Pinus pineaItalyMAT1coreanaCMW1210Pinus pineaItalyMAT1coreanaCMW2866Abies balsameaUSAMAT1coreanaCMW2865Pinus subletsisUKAMAT1ubeitnumCMW2865 <td>T1 1</td> <td>17402051</td> <td>UP</td>	T1 1	17402051	UP
Laivigera ATCC18065 Finus ponterosa Canada MAT1 huntii CMW622 P. pinaster Portugal MAT1 CMW622 P. pinaster Portugal MAT1 CMW654 Finus sp. USA MAT1 CMW065 Hylurgus ligniperda New Zealand MAT1 CMW1015 Unknown New Zealand MAT1 CMW1020 Finus densiflora Korea MAT1 CMW14201 Pinus densiflora Korea MAT1 inutia CMW4452 Pinus sp. USA MAT1 cMW14201 Pinus densiflora Korea MAT1 cMW1420 Pinus pinea USA NA cMW1420 Pinus pinea USA MAT1 cMW299 Pinus pinea Italy MAT1 CMW299 Pinus pinea Italy MAT1 cMW299 Pinus pinea Italy MAT1 cMW290 Pinus pinea Italy MAT1 cMW291 Pinus pinea Italy MAT1 cMW291 Pinus pinea Italy MAT1 cMW292 Pinus pinea Italy MAT1 cMW293 Pinus pinea Italy MAT1 cMW293 Pinus pinea Italy MAT1 cMW294 Pinus pinea Italy MAT1 cMW295 Pinus pinea Italy MAT1 cMW296 Pinus pinea Pinea MAT1	T1 0 VT7702F2)7402931	
Kuvigera A HCULBOB Finus ponderosa Ganada MAT1 Kuviao Finus contorta Canada MAT1 CMW100 Finus contorta Canada MAT1 CMW100 Finus contorta Canada MAT1 CMW100 Finus contorta Canada MAT1 CMW100 Unknown New Zealand MAT1 CMW1200 Finus densifora Korea MAT1 cMW1200 Finus densifora Korea MAT1 cMW1201 Pinus densifora Korea MAT1 cMW2812 Pseudotsuga menziesii USA NA cMW2811 Pica rubens USA NA cMW2811 Pica rubens USA NA cMW2811 Pica rubens USA NA cMW2811 Pica rubens USA MAT1 cMW2811 Pica rubens USA NA cMW2811 Pica rubens USA MAT1 cMW2811 Pica rubens USA MAT1 cMW34175 Finus ponderosa USA MAT1 cMW34175 Pinus pinea Italy MAT1 cMW289 Pinus pinea Italy MAT1 cMW289 Pinus pinea Italy MAT1 cMW289 Pinus pinea Italy MAT1 cMW289 Pinus pinea USA MAT1 cMW289 Pinus pinea USA MAT1 cMW289 Pinus pinea Italy MAT1 cMW280 Pinus guinea USA MAT1 ibieticlons CMW2867 Pinus sulticina Bhutan MAT1 ibietinam CMW2816 Pinus usineina Bhutan MAT1 ibietinam CMW2816 Pinus usilicina Bhutan MAT1 ibietinam CMW2817 Pinus usilicinan Bhutan MAT1 ibietinam CMW3852 Pinus wallichiana Bhutan MAT1 cMW1885 Pinus wallichiana Bhutan MAT1 ibietinam CMW3854 Pinus wallichiana Bhutan MAT1 idutanense CMW2864 Pinus salicitis Dominican Rep. MAT1 cMW1885 Hylaryus mickliki Spain MAT1 cMW1836 Hylaryus mickliki Spain MAT1 cMW1836 Hylaryus mickliki Spain MAT1 cMW230 Pinus ccidentalis Dominican Rep. MAT1 ini-densiflorae CMW2060 Pinus sondichiana Bhutan MAT1 astellanum CMW383 Hylatse ater UK MAT1 ini-densiflorae CMW2169 Pinus sendorus Japan MAT1 ini-densiflorae CMW2169 Pinus sendorus Japan MAT1 ini-densiflorae CMW2169 Pinus sendorus Japan MAT1 ini-densiflorae CMW3165 Pinus sendorus Japan MAT1 ini-densiflorae CMW3165 Pinus sendifora Japan MAT1 ini-densiflorae CMW3165 Pinus sendifora Japan MAT1 ini-densiflorae CMW3165 Pinus sendifora Japan MAT1 ini-densiflor	T1 1	17400040	HE
kW 140/Prinus contortaCanadaMAT1NuntiiCMW622Pinus sp.USAMAT1CMW0105UnknownNew ZealandMAT1CMW1015UnknownNew ZealandMAT1CMW2824Pinus sp.USAMAT1cMW14200Pinus densiforaKoreaMAT1cMW14201Pinus densiforaKoreaMAT1cMW14200Pinus densiforaKoreaMAT1piceiperda B*CMW452Pseudotsuga menziesiiUSANAcMW14201Pinus densiforaKoreaMAT1comvayNACMW452Pseudotsuga menziesiiUSANAriceiperda C*CMW4452Picea abiesNorwayNArobustaCMW1510UnknownUnknownMAT1cMW3107Pinus pineaItalyMAT1cMW1920Pinus pineaItalyMAT1cMW1920Pinus pineaItalyMAT1cMW2904Pinus pineaItalyMAT1cMW2905Pinus pineaItalyMAT1cMW2905Pinus pineaItalyMAT1ibieticolensCMW2866Pinus svibestrisItalyMAT1ibieticolensCMW2867Pica engelmanniiUSAMAT1ibietinumCMW2867Pinus svibestrisUSAMAT1ibietinumCMW2867Pinus svibestrisUKMAT1ibietinumCMW2867Pinus svibestrisUKMAT1ibietinumCMW2669Pinus svibestrisUSA<	.II-I 	JX402948	HE
hunthi CMW622 P. pinaster Portugal MAT1 CMW654 Pinus sp. USA MAT1 CMW1006 Hylurgus ligniperda New Zealand MAT1 CMW10105 Unknown New Zealand MAT1 CMW1201 Vinus gensiflora Korea MAT1 CMW1201 Pinus densiflora Korea MAT1 piceiperda B* CMW452 Pseudotsuga menziesii USA NA cMW452 Pseudotsuga menziesii USA NA cMW452 Pseudotsuga menziesii USA NA cMW452 Pinus pinea USA NA cMW2811 Pica rubens USA NA piceiperda C* CMW446 Pica abies Norway NA robusta CMW710 Unknown Unknown MAT1 CMW34175 Pinus pinea Italy MAT1 CMW34175 Pinus pinea Italy MAT1 CMW290 Pinus pinea USA MAT1 libeitina CMW286 Abies balsamea USA MAT1 libeitina CMW286 Pinus strobus USA MAT1 libeitina CMW286 Pinus strobus USA MAT1 libeitina CMW2960 Pinus socidentalis Dominican Rep. MAT1 cMW1988 Hylurgus mickliki Spain MAT1 cMW2920 Pinus socidentalis Dominican Rep. MAT1 cMW2920 Pinus socidentalis Dominican Rep. MAT1 cMW2040 Pinus socidentalis Dominican Rep. MAT1 louglasii CMW2076 Pseudotsuga menziesii USA MAT1 indensifora CMW2066 Pica glauca Canada MAT1 cMW2060 Pinus soviestris Norway MAT1 anifestum CMW2060 Pinus soviestris Norway MAT1 anifestum CMW2061 Pinus soviestris Norway MAT1 anifestum CMW2060 Pinus soviestris Norway MAT1	ACXQ02000048	3	HE
CMW654 Pinus sp. USA MATT CMW1005 Hylurgus ligniperda New Zealand MATT CMW2824 Pinus sp. USA MATT Koreana CMW14200 Pinus densiflora Korea MATT piceiperda B* CMW420 Pinus densiflora Korea MATT piceiperda B* CMW442 Pseudotsuga menziesii USA NA siceiperda C* CMW281 Picea rubens USA NA robusta CMW210 Unknown Unknown MATT GMW3420 Pinus pinea Italy MATT CMW3420 Pinus pinea Italy MATT CMW3417 Pinus pinea Italy MATT CMW329 Pinus pinea Italy MATT CMW289 Pinus pinea Italy MATT CMW280 Pinus sylvestris Italy MATT CMW280 Pinus sylvestris Italy MATT piceiperida C* CMW2817 Picea rubens USA MATT CMW280 Pinus pinea Italy MATT CMW280 Pinus pinea Italy MATT CMW280 Pinus pinea Italy MATT CMW280 Pinus pinea Italy MATT CMW280 Pinus sylvestris Italy MATT punnanensis CMW2816 Pinus sylvestris Italy MATT punnanensis CMW2865 Pinus sunnanensis USA MATT bieticolens CMW2866 Pinus sunnanensis USA MATT bietinum CMW2817 Picea engelmannii USA MATT thutanense CMW3850 Pinus wallichiana Bhutan MATT astellanum CMW3767 Pinus wallichiana Bhutan MATT cMW3200 Pinus sunlichiana Bhutan MATT cMW2300 Pinus sundichiana Bhutan MATT cMW2300 Pinus sundichiana Bhutan MATT cMW2300 Pinus sundichiana Bhutan MATT cMW2300 Pinus sundichiana Bhutan MATT cMW2300 Pinus sencentalis Dominican Rep. MATT cMW2306 Pinus sencentalis Dominican Rep. MATT andfestum CMW2066 Picea glauca Canada MATT andfestum CMW2076 Pinus sencentalis Dominican Rep. MATT andfestum CMW2171 Pinus sencentalis Dominican Rep. MATT andfestum CMW2164 Pinus sylvestris Norway MATT andifestum CMW2164 Pinus sylvestris Norway MATT andifestum CMW2164 Pinus sylvestris Norway MATT andfestum CMW2164 Pinus sylvestris USA MATTI andfestum CMW2164 Pinus sylvestris USA MATTI andfestum CMW2164 Pinus sylvestris USA MATTI andfauma CMW2164 Pinus sylv	.T1-1	KT779227	HE
CMW 1006Hylurgus ligniperdaNew ZealandMAT1CMW 2015UnknownNew ZealandMAT1CMW 2842Pinus sp.USAMAT1koreanaCMW 14200Pinus densifloraKoreaMAT1piceiperda B*CMW 4201Pinus densifloraKoreaMAT1piceiperda B*CMW 4201Pieudotsuga merziesiiUSANApiceiperda C*CMW 446Pieca abiesNorwayNAobustaCMW 210UnknownUnknownMAT1cmW 34175Pinus ponderosaUSAMAT1erpensCMW 191Pinus pineaItalyMAT1CMW 290Pinus pineaItalyMAT1cMW 2019Pinus pineaItalyMAT1cMW 2020Pinus pineaItalyMAT1punnanensisCMW 2152Pinus yunnanensisChinaMAT1punnanensisCMW 2162Pinus yunnanensisChinaMAT1punnanensisCMW 2162Pinus yunnanensisChinaMAT1pibeitinumCMW 2866Abies balsameaUSAMAT1ibertinumCMW 2167Picea engelmanniiUSAMAT1ibertinumCMW 2167Pinus wullichianaBhutanMAT1ibertinumCMW 2167Pinus wullichianaBhutanMAT1ibertinumCMW 2167Pinus wullichianaBhutanMAT1ibertinumCMW 2167Pinus wullichianaBhutanMAT1ibertinumCMW 2167Pinus wullichianaBhutan	T1-2 KT779250		HE
CMW1015UnknownNew ZealandMAT1CMW2824Pinus sp.USAMAT1ckoreanaCMW14201Pinus densifloraKoreaMAT1piceiperda B*CMW452Pseudotsuga menziesiiUSANApiceiperda C*CMW462Picea abiesNorwayNApiceiperda C*CMW446Picea abiesNorwayNArobustaCMW710UnknownUNknownMAT1cMW3175Pinus pineaItalyMAT1cMW3192Pinus pineaItalyMAT1CMW291Pinus pineaItalyMAT1CMW292Pinus pineaItalyMAT1CMW294Pinus pineaItalyMAT1CMW295Pinus pineaItalyMAT1cMW296Pinus sylvestrisItalyMAT1unnanensisCMW2865Pinus yunanensisChinaMAT1ibieticolensCMW2865Pinus sylvestrisUSAMAT1ibietinumCMW2865Pinus walichianaBhutanMAT1uhtameseCMW3865Pinus walichianaBhutanMAT1ibietinumCMW2865Pinus walichianaBhutanMAT1ibitatoenseCMW3865Pinus walichianaBhutanMAT1ibitatoenseCMW18652Pinus walichianaBhutanMAT1ibitatoenseCMW2867Pinus walichianaBhutanMAT1ibitatiCMW2867Pinus walichianaBhutanMAT1ibitatiCMW2870Pinus senaonensis <td>T1-2 = KT779250</td> <td></td> <td>HE</td>	T1-2 = KT779250		HE
CMW2824Pinus sp.USAMAT1koreanaCMW14200Pinus densifloraKoreaMAT1CMW14201Pinus densifloraKoreaMAT1piceiperda B*CMW452Pseudotsuga menziesiiUSANAcMW2811Picea rubensUSANArobustaCMW446Picea abiesNorwayNArobustaCMW410UnknownUnknownMAT1cMW24175Pinus pineaItalyMAT1CMW24175Pinus pineaItalyMAT1CMW290Pinus pineaItalyMAT1CMW290Pinus pineaItalyMAT1CMW290Pinus pineaItalyMAT1CMW290Pinus pineaItalyMAT1CMW290Pinus pineaItalyMAT1unnanensisCMW2866Pinus sylvestrisItalyMAT1ibieticolensCMW2866Pinus sylvestrisUSAMAT1ibietinumCMW2767Pinus sulvestrisUSAMAT1uhtanenseCMW18650Pinus walichianaBhutanMAT1cMW18652Pinus walichianaBhutanMAT1cMW18652Pinus walichianaBhutanMAT1cMW18652Pinus seraonensisChinaMAT1cMW18652Pinus occidentalisDominican Rep.MAT1cMW18653Hylagus micklikiSpainMAT1cMW1865Pinus occidentalisDominican Rep.MAT1cMW2121Pinus seraonensisChinaMAT1 <t< td=""><td>T1-1</td><td>=KT779227</td><td>HE</td></t<>	T1-1	=KT779227	HE
koreana CMW14200 Pinus densiflora Korea MAT1 CMW14201 Pinus densiflora Korea MAT1 piceiperda B* CMW452 Pseudotsuga menziesii USA NA cMW2811 Picea rubens USA NA robusta CMW710 Unknown Unknown MAT1 CMW34175 Pinus ponderosa USA MAT1 CMW34175 Pinus ponderosa USA MAT1 CMW34175 Pinus pinea Italy MAT1 CMW34175 Pinus pinea Italy MAT1 CMW340 Pinus pinea Italy MAT1 CMW290 Pinus pinea USA MAT1 DMW290 Pinus pinea Italy MAT1 CMW290 Pinus pinea Italy MAT1 CMW290 Pinus pinea Italy MAT1 CMW280 Pinus sylvestris Italy MAT1 punnanensis CMW286 Abies balsamea USA MAT1 bieticiolens CMW2867 Pinus strobus USA MAT1 bietinum CMW2817 Picea engelmannii USA MAT1 bietinum CMW2817 Picea engelmannii USA MAT1 bietinum CMW2852 Pinus wallichiana Bhutan MAT1 astellanum CMW3865 Pinus wallichiana Bhutan MAT1 CMW3865 Pinus wallichiana Bhutan MAT1 CMW3865 Pinus wallichiana Bhutan MAT1 CMW1988 Hylurgus mickliki Spain MAT1 CMW1988 Hylurgus mickliki Spain MAT1 CMW2320 Pinus occidentalis Dominican Rep. MAT1 CMW2321 Pinus occidentalis Dominican Rep. MAT1 cMW2321 Pinus occidentalis Dominican Rep. MAT1 cMW2329 Pinus wallichiana Bhutan MAT1 actellanum CMW2873 Hylastes ater UK MAT1 racile CMW2873 Hylastes ater UK MAT1 racile CMW2316 Pinus armandii China MAT1 racile CMW2321 Pinus occidentalis Dominican Rep. MAT1 racile CMW2321 Pinus occidentalis Dominican Rep. MAT1 racile CMW2326 Pinus armandii China MAT1 racile CMW2377 Pinus armandii China MAT1 racile CMW2387 Hylastes ater UK MAT1 racile CMW2398 Pinus sp. Indonesia MAT1 racile CMW337 Pinus spluestris Sweden MAT1 racile CMW3387 Pinus spluestris Sweden MAT1 racifex CMW337 Pinus spluestris Sweden MAT1 racifex Pinus densiflora Japan MAT1 racifex Pinus densiflora Japan MAT1 racifex Pinus densiflora Japan MAT1 racifex Pinus spluestris USA MAT1 racifex Pinus spluestris Sweden MAT1 racifex Pinus spluestris Sweden MAT1 racifex Pinus densiflora Japan MAT1 racifex Pinus densiflora Japan MAT1 racifex Pi	T1-2 = KT779250		HE
CMW14201Pinus densifloraKoreaMAT1piceiperda B*CMW452Pseudotsuga menziesiiUSANApiceiperda C*CMW4452Picea rubensUSANArobustaCMW710UnknownUnknownMAT1cmW34175Pinus pinea onderosaUSAMAT1cmW34175Pinus pineaItalyMAT1cMW2802Pinus pineaItalyMAT1cMW2803Pinus pineaItalyMAT1cMW2804Pinus pineaItalyMAT1cMW2805Pinus pineaItalyMAT1cMW2806Abies balsameaUSAMAT1punnanensisCMW2866Abies balsameaUSAMAT1ibieticolensCMW2867Hjobius abietisUKMAT1ibietinumCMW2867Pinus strobusUSAMAT1ibietinumCMW2867Pinus wallichianaBhutanMAT1astellanumCMW18652Pinus wallichianaBhutanMAT1cMW18652Pinus wallichianaBhutanMAT1cMW18652Pinus socidentalisDominican Rep.MAT1cMW23200Pinus socidentalisDominican Rep.MAT1ibbsiiCMW2867Pisea daraUSAMAT1ibbsiiCMW28767Hylastes aterUKMAT1ibbsiiCMW2867Pinus socidentalisDominican Rep.MAT1ibbsiiCMW2867Pinus sylvestrisSaenaMAT1indersifloraeCMW2066Pieus armandiiChina	T1-2 KT779247		HE
piceiperda B* CMW452 Pseudotsuga menziesii USA NA CMW2811 Picea rubens USA NA piceiperda C* CMW466 Picea abies Norway NA robusta CMW710 Unknown Unknown MAT1 CMW34175 Pinus ponderosa USA MAT1 CMW34175 Pinus pinea Italy MAT1 CMW191 Pinus pinea Italy MAT1 CMW289 Pinus pinea Italy MAT1 CMW290 Pinus pinea Italy MAT1 CMW290 Pinus sylvestris USA MAT1 ibieticolens CMW2866 Abies balsamea USA MAT1 ibietinum CMW2817 Picea engelmannii USA MAT1 ibietinum CMW2867 Hylobius abietis UK MAT1 hutanense CMW18650 Pinus wallichiana Bhutan MAT1 CMW18652 Pinus wallichiana Bhutan MAT1 CMW18652 Pinus wallichiana Bhutan MAT1 CMW2320 Pinus vacidentalis Dominican Rep. MAT1 CMW2320 Pinus occidentalis Dominican Rep. MAT1 ibbisii CMW2076 Pseudotsuga menziesii USA MAT1 ibwisii CMW2076 Pseudotsuga menziesii SChina MAT1 matifestum CMW2040 Pinus armandii China MAT1 matifestum CMW2040 Pinus armandii China MAT1 matifestum CMW2040 Pinus densiftora Japan MAT1 mudbergii CMW217 Pinus sylvestris Sweden MAT1 mudbergii CMW216 Pinus densiftora Japan MAT1 mudv216 Pinus sylvestris SVeeden MAT1 mudv216 Pinus sylvestris USA MAT1 mudv216 Pinus sylvestris US	T1-1	KT779222	HE
CMW2811Picea rubensUSANApiceiperda C*CMW446Picea abiesNorwayNArobustaCMW710UnknownUnknownMAT1CMW34175Pinus ponderosaUSAMAT1cMW34175Pinus pineaItalyMAT1CMW192Pinus pineaItalyMAT1CMW289Pinus pineaItalyMAT1CMW290Pinus pineaItalyMAT1CMW2815Pinus sylvestrisItalyMAT1cMW2866Abies balsameaUSAMAT1ibieticolensCMW2866Picea engelmanniiUSAMAT1ibietinumCMW2865Pinus strobusUSAMAT1ibietinumCMW2865Pinus sunanensisUKMAT1ibietinumCMW2867Pinus walichianaBhutanMAT1cMW18650Pinus walichianaBhutanMAT1cMW18652Pinus walichikiaSpainMAT1cMW18652Pinus walichikiaSpainMAT1cMW2320Pinus occidentalisDominican Rep.MAT1cMW2321Pinus occidentalisDominican Rep.MAT1cMW2321Pinus secidentalisDominican Rep.MAT1louglasiiCMW2876Piseu armandiiChinaMAT1ibbsiiCMW2831Hylastes aterUKMAT1narcileCMW1855Pinus armandiiChinaMAT1cMW2066Pica alguacaCanadaMAT1nanjfestumCMW2046Pinus sylvestris <td>KT779246</td> <td>KT779224</td> <td>НО</td>	KT779246	KT779224	НО
piceiperda C* CMW446 Picea abies Norway NA robusta CMW710 Unknown Unknown MAT1 CMW34175 Pinus ponderosa USA MAT1 CMW34175 Pinus pinea Italy MAT1 CMW192 Pinus pinea Italy MAT1 CMW289 Pinus pinea Italy MAT1 CMW290 Pinus pinea Italy MAT1 CMW290 Pinus pinea Italy MAT1 CMW290 Pinus pinea Italy MAT1 CMW290 Pinus sylvestris Italy MAT1 CMW290 Pinus sylvestris Italy MAT1 Unknown CMW2817 Picea engelmannii USA MAT1 bibeticolens CMW2866 Abies balsamea USA MAT1 bibetinum CMW2817 Picea engelmannii USA MAT1 thibetinum CMW2867 Pinus strobus USA MAT1 duban CMW2866 Pinus strobus USA MAT1 cMW18650 Pinus strobus USA MAT1 cMW18650 Pinus wallichiana Bhutan MAT1 CMW18650 Pinus wallichiana Bhutan MAT1 CMW18652 Pinus wallichiana Bhutan MAT1 CMW18652 Pinus wallichiana Bhutan MAT1 CMW18653 Pinus socidentalis Dominican Rep. MAT1 CMW18653 Hylurgus mickliki Spain MAT1 CMW2320 Pinus occidentalis Dominican Rep. MAT1 CMW2321 Pinus occidentalis Dominican Rep. MAT1 CMW2321 Pinus semaonensis China MAT1 CMW2320 Pinus orcidentalis Dominican Rep. MAT1 CMW2321 Pinus semaonensis China MAT1 CMW2320 Pinus orcidentalis Dominican Rep. MAT1 cMW2321 Pinus semaonensis China MAT1 cMW2320 Pinus semaonensis China MAT1 cMW2321 Pinus semandii China MAT1 cMW22607 Pinus semandii China MAT1 cMW20607 Pinus semandii China MAT1 cMW20607 Pinus densifiora Japan MAT1 cMW20607 Pinus densifiora Japan MAT1 cMW20616 Pinus densifiora Japan MAT1 cMW2162 Pinus selvestris Sweden MAT1 cMW2164 Pinus densifiora Japan MAT1 cMW2165 Pinus sensifora Japan MAT1 cMW2165 Pinus densifiora Japan MAT1 cMW2166 Pinus densifiora Japan MAT1 cMW2167 Pinus sylvestris US	KT779245	KT779223	НО
robusta CMW120 Unknown Unknown MAT1 CMW34175 Pinus pinea USA MAT1 CMW34175 Pinus pinea Italy MAT1 CMW192 Pinus pinea Italy MAT1 CMW290 Pinus pinea Italy MAT1 CMW290 Pinus pinea Italy MAT1 CMW290 Pinus pinea Italy MAT1 CMW304 Pinus sylvestris Italy MAT1 CMW304 Pinus sylvestris Italy MAT1 CMW304 Pinus sylvestris Italy MAT1 CMW304 Pinus sylvestris USA MAT1 bibiticolens CMW2866 Abies balsamea USA MAT1 bibitinum CMW2867 Pice angelmannii USA MAT1 ibipini CMW2065 Pinus sunanensis UKK MAT1 hihatanese CMW18650 Pinus wallichiana Bhutan MAT1 CMW18652 Pinus wallichiana Bhutan MAT1 CMW318652 Pinus wallichiana Bhutan MAT1 CMW318652 Pinus wallichiana Bhutan MAT1 CMW18852 Pinus wallichiana Bhutan MAT1 CMW18854 Pinus wallichiana Bhutan MAT1 CMW18854 Pinus scidentalis Dominican Rep. MAT1 CMW18854 Pinus scidentalis Dominican Rep. MAT1 CMW2320 Pinus occidentalis Dominican Rep. MAT1 CMW2320 Pinus occidentalis Dominican Rep. MAT1 CMW2321 Pinus armandii China MAT1 cMW2321 Pinus armandii China MAT1 cMW2331 Pinus armandii China MAT1 cMW2066 Picea glauca Canada MAT1 cMW2066 Picea glauca Canada MAT1 cMW2067 Pinus contorta Canada MAT1 cMW2060 Pinus contorta Canada MAT1 cMW2060 Pinus densiflora Japan MAT1 cMW2160 Pinus densiflora Japan MAT1 cMW2162 Pinus densiflora Japan MAT1 cMW2164 Pinus taeda South Africa MAT1 cMW21655 Nyssa sylvatica USA MAT1 cMW10555 Nyssa sylvatica USA MAT1 cMW10555 Nyssa sylvatica USA MAT1	KT779244	KT779221	НО
CMW14175 Pinus ponderosa USA MAT1 serpens CMW191 Pinus pinea Italy MAT1 CMW192 Pinus pinea Italy MAT1 CMW289 Pinus pinea Italy MAT1 CMW289 Pinus pinea Italy MAT1 CMW280 Pinus pinea Italy MAT1 CMW286 Pinus suluenta Italy MAT1 cMW286 Pinus strobus USA MAT1 ibieticolens CMW266 Pinus strobus USA MAT1 ibietinum CMW265 Pinus wallichiana Bhutan MAT1 CMW1865 Pinus wallichiana Bhutan MAT1 CMW1862 Pinus wallichiana Bhutan MAT1 CMW1888 Hylurgus mickliki Spain MAT1 CMW230 Pinus occidentalis Dominican Rep. MAT1 CMW230 Pinus occidentalis Dominican Rep. MAT1 CMW2321 Pinus occidentalis Dominican Rep. MAT1 italouglasii CMW2076 Pseudotsuga menziesii USA MAT1 italouglasii CMW2076 Pseudotsuga menziesii USA MAT1 racile CMW12316 Pinus armandii China MAT1 CMW2060 Pinus densiflora Japan MAT1 cMW2060 Pinus densiflora Japan MAT1 cMW2060 Pinus densiflora Japan MAT1 cMW216 Pinus densiflora Japan MAT1 cMW10555 Nyssa sylvatica USA MAT1 cMW10555 Nyssa sylvatica USA MAT1 cMW10555 Nyssa sylvatica USA MAT1 cMW2060 Pinus cerinosa USA MAT1 cMW2060 Pinus densiflora Pinus P	T1-1	KT779225	P-HF
serpens GMW311'3 Finas polaciosa USA MAT1 Serpens GMW191 Pinus pinea Italy MAT1 GMW289 Pinus pinea Italy MAT1 GMW289 Pinus pinea Italy MAT1 GMW200 Pinus sylvestris Italy MAT1 GMW304 Pinus sylvestris Italy MAT1 GMW304 Pinus sylvestris Italy MAT1 dW2066 Pinus sylvestris USA MAT1 ibietinum GMW2817 Picea engelmannii USA MAT1 ibiptini CMW2065 Pinus strobus USA MAT1 ibihatanense GMW18650 Pinus wallichiana Bhutan MAT1 GMW18652 Pinus wallichiana Bhutan MAT1 GMW18652 Pinus wallichiana Bhutan MAT1 GMW18652 Pinus wallichiana Bhutan MAT1 GMW18863 Hylurgus mickliki Spain MAT1 GMW2301 Pinus occidentalis Dominican Rep. MAT1 GMW2320 Pinus occidentalis Dominican Rep. MAT1 GMW2321 Pinus occidentalis Dominican Rep. MAT1 GMW2320 Pinus occidentalis Dominican Rep. MAT1 GMW2321 Pinus occidentalis Dominican Rep. MAT1 GMW2320 Pinus senzonensis China MAT1 GMW2321 Pinus senzonensis China MAT1 GMW2136 Hylastes ater UK MAT1 racile GMW12316 Pinus armandii China MAT1 GMW12319 Pinus contorta Canada MAT1 GMW20607 Pinus sylvestris Sweden MAT1 GMW20607 Pinus sylvestris Norway MAT1 maifestum GMW204 Pinus densiflora Japan MAT1 GMW2106 Pinus sylvestris Sweden MAT1 GMW2104 Pinus sylvestris Sweden MAT1 GMW216 Larix olgensis China MAT1 GMW216 Pinus sylvestris USA MAT1 GMW216 Pinus sylvestris USA MAT1 GMW1615 Pinus sylvestris USA MAT1 GMW1625 Carya sp. USA MAT1 GMW10555 Nyssa sylvatica USA MAT1 GMW10555 Nyssa sylvatica USA MAT1 GMW10555 Nyssa sylvatica USA MAT1 GMW10555 Nyssa sylvatica USA MAT1 GMW3089 Pinus rešinosa USA MAT1	T1-1	= KT779225	P-HF
CMW121Intus prindIdayMAT1CMW122Pinus pineaItalyMAT1CMW289Pinus pineaItalyMAT1CMW290Pinus pineaItalyMAT1CMW290Pinus pineaItalyMAT1CMW286Pinus sylvestrisItalyMAT1upunanensisCMW5152Pinus yunanensisChinaMAT1ubieticolensCMW2866Abies balsameaUSAMAT1ubietinumCMW2867Picea engelmanniiUSAMAT1ubietinumCMW2865Pinus strobusUSAMAT1ubtinumCMW3767Hylobius abietisUKMAT1ubtanenseCMW18650Pinus wallichianaBhutanMAT1cMW18650Pinus wallichianaBhutanMAT1astellanumCMW18650Pinus wallichianaBhutanMAT1cMW18652Pinus wallichianaBhutanMAT1cMW18652Pinus wallichianaBhutanMAT1cMW18652Pinus wallichianaBhutanMAT1cMW18652Pinus wallichianaBhutanMAT1cMW2321Pinus occidentalisDominican Rep.MAT1cMW2321Pinus occidentalisDominican Rep.MAT1ialglasiiCMW2206Pseudotsuga menziesiiUSAMAT1ialglasiiCMW2076Pseudotsuga menziesiiUSAMAT1iablasiiCMW2066Pinus armandiiChinaMAT1iablasiiCMW2037Pinus armandiiChinaMAT1		-KT770226	P_UF
CMW152 rhus pinea Italy MAT1 CMW289 Pinus pinea Italy MAT1 CMW290 Pinus pinea Italy MAT1 CMW304 Pinus sylvestris Italy MAT1 CMW304 Pinus sylvestris Italy MAT1 ubieticolens CMW5152 Pinus yunnanensis China MAT1 ubieticolens CMW2866 Abies balsamea USA MAT1 ubietinum CMW2817 Picea engelmannii USA MAT1 ulethinum CMW2816 Pinus strobus USA MAT1 ulethinum CMW3767 Hylobius abietis UK MAT1 hutanense CMW18650 Pinus wallichiana Bhutan MAT1 astellanum CMW18650 Pinus wallichiana Bhutan MAT1 cMW18650 Pinus wallichiana Bhutan MAT1 CMW1988 Hylurgus mickliki Spain MAT1 CMW2320 Pinus occidentalis Dominican Rep. MAT1 CMW2320 Pinus occidentalis Dominican Rep. MAT1 iuleglasii CMW2676 Pseudotsuga menziesii USA MAT1 pibbsii CMW853 Hylastes ater UK MAT1 cMW1376 Hylastes ater UK MAT1 cMW1219 Pinus armandii China MAT1 ongiclavatum CMW20606 Picea glauca Canada MAT1 ongiclovitum CMW2004 Pinus genisflora Japan MAT1 nanifestum CMW2004 Pinus densiflora Japan MAT1 rundbergii CMW217 Pinus sylvestris Sweden MAT1 rundbergii CMW216 Finus densiflora Japan MAT1 rundbergii CMW216 Pinus densiflora Japan MAT1 rundv216 Pinus densiflora Japan MAT1 rofanum CMW2162 Finus densiflora Japan MAT1 rofanum CMW2164 Pinus densiflora Japan MAT1 rundv216 Pinus densiflora Japan MAT1 rufanum CMW12436 Larix olgensis USA MAT1 rufanum CMW21655 Nyssa sylvestris USA MAT1 rufanum CMW21655 Nyssa sylvestris USA MAT1 rufanum CMW2168 Pinus teada South Africa MAT1 rufanum CMW2168 Pinus teifanai USA MAT1	T1 1	_KT770220	DIE
CMW289Pinus pineaItalyMAT1CMW290Pinus pineaItalyMAT1CMW2904Pinus sylvestrisItalyMAT1ubieticolensCMW5152Pinus yunnanensisChinaMAT1ubietinumCMW2866Abies balsameaUSAMAT1ubietinumCMW2817Picea engelmanniiUSAMAT1ubietinumCMW2867Hylobius abietisUKMAT1ubietinumCMW2867Hylobius abietisUKMAT1ubitatinumCMW2867Hylobius abietisUKMAT1ubitatinumCMW18650Pinus wallichianaBhutanMAT1ubitatinumCMW18652Pinus wallichianaBhutanMAT1cMW1898Hylurgus micklikiSpainMAT1cMW2300Pinus occidentalisDominican Rep.MAT1cMW2320Pinus occidentalisDominican Rep.MAT1louglasiiCMW2376Pseudotsuga menziesiiUSAMAT1louglasiiCMW2376Pisus armandiiChinaMAT1racileCMW12421Pinus armandiiChinaMAT1cMW12319Pinus armandiiChinaMAT1ongiconidiophorumCMW2004Pinus armandiiChinaMAT1cMW20607Pinus sylvestrisSwedenMAT1inietiCMW2383Pinus densifloraJapanMAT1inietiCMW2387Pinus densifloraJapanMAT1rofarumCMW1245Larix olgensisChinaMAT1 <td>T1 1</td> <td>=K1//9226</td> <td>P-HE</td>	T1 1	=K1//9226	P-HE
CMW290Prinus pineaItalyMAT1CMW304Pinus sylvestrisItalyMAT1CMW3052Pinus sylvestrisItalyMAT1ibieticolensCMW2866Abies balsameaUSAMAT1ibietinumCMW2817Picea engelmanniiUSAMAT1ibietinumCMW2867Pinus strobusUSAMAT1ibiopiniCMW2867Pinus strobusUSAMAT1ibitatinumCMW3767Hylobius abietisUKMAT1ibitatinumCMW38650Pinus wallichianaBhutanMAT1cMW18652Pinus wallichianaBhutanMAT1cMW18654Pinus wallichianaBhutanMAT1cMW18854Hylurgus micklikiSpainMAT1cMW18859Hylurgus micklikiSpainMAT1cMW2320Pinus occidentalisDominican Rep.MAT1cMW2321Pinus occidentalisDominican Rep.MAT1louglasiiCMW2076Pseudotsuga menziesiiUSAMAT1ibibiiCMW2076Pseudotsuga menziesiiUSAMAT1collastatumCMW2076Pieca glaucaCanadaMAT1collastatumCMW2060Picea glaucaCanadaMAT1collapticitCMW2167Pinus densifloraJapanMAT1collapticitCMW2167Pinus densifloraJapanMAT1collapticitCMW2167Pinus densifloraJapanMAT1collapticitCMW2167Pinus densifloraJapanMAT1<	.11-1 	=K17/9226	P-HE
CMW304Prinus sylvestrisItalyMAT1yunnanensisCMW5152Pinus yunnanensisChinaMAT1ubieticolensCMW2866Abies balsameaUSAMAT1ubietinumCMW2817Picca engelmanniiUSAMAT1uloopiniCMW2065Pinus strobusUSAMAT1ulotinumCMW3767Hylobius abietisUKMAT1uhutanenseCMW18650Pinus wallichianaBhutanMAT1cMW18652Pinus wallichianaBhutanMAT1astellanumCMW1988Hylurgus micklikiSpainMAT1cMW1989Hylurgus micklikiSpainMAT1cMW2320Pinus occidentalisDominican Rep.MAT1cMW2321Pinus occidentalisDominican Rep.MAT1louglasiiCMW2076Pseudotsuga menziesiiUKMAT1ibbsiiCMW2176Hylastes aterUKMAT1racileCMW1376Hylastes aterUKMAT1ongiclavatumCMW20600Picea glaucaCanadaMAT1cMW20607Pinus armandiiChinaMAT1ongiclavatumCMW217Pinus sylvestrisSwedenMAT1nanifestumCMW217Pinus densifloraJapanMAT1nanifestumCMW2161Pinus densifloraJapanMAT1rocerumCMW217Pinus densifloraJapanMAT1rocerumCMW216Pinus densifloraJapanMAT1rofanumCMW216Pinus densif	.11-1	=K1779226	P-HE
yunnanensis CMW5152 Pinus yunnanensis China MAT1 hbieticolens CMW2866 Abies balsamea USA MAT1 hbietinum CMW2817 Picea engelmannii USA MAT1 ubietinum CMW2867 Pinus strobus USA MAT1 alethinum CMW3767 Hylobius abietis UK MAT1 ohutanense CMW18650 Pinus wallichiana Bhutan MAT1 CMW18652 Pinus wallichiana Bhutan MAT1 CMW18652 Pinus wallichiana Bhutan MAT1 CMW18652 Pinus wallichiana Bhutan MAT1 CMW18684 Hylurgus mickliki Spain MAT1 CMW1988 Hylurgus mickliki Spain MAT1 CMW2320 Pinus occidentalis Dominican Rep. MAT1 CMW2321 Pinus occidentalis Dominican Rep. MAT1 CMW2321 Pinus occidentalis Dominican Rep. MAT1 CMW2321 Pinus occidentalis Dominican Rep. MAT1 for CMW2321 Pinus semaonensis China MAT1 for CMW2321 Pinus armandii China MAT1 for CMW12316 Pinus armandii China MAT1 for CMW12316 Pinus armandii China MAT1 for CMW12319 Pinus armandii China MAT1 for Pinus contorta Canada MAT1 for Pinus sylvestris Sweden MAT1 for Pinus sylvestris Norway MAT1 for Pinus sylvestris Norway MAT1 for CMW2100 Pinus sylvestris Norway MAT1 for CMW2135 Pinus arp. Indonesia MAT1 for CMW2136 Pinus densiflora Japan MAT1 for CMW2136 Pinus densiflora Japan MAT1 for CMW2136 Pinus sylvestris Norway MAT1 for CMW2136 Pinus sylvestris Norway MAT1 for CMW2136 Pinus sylvestris Norway MAT1 for CMW2136 Pinus densiflora Japan MAT1 for CMW2136 Pinus densiflora Japan MAT1 for CMW2136 Pinus sylvestris USA MAT1 for CMW3162 Pinus densiflora Japan MAT1 for CMW3162 Pinus densiflora Japan MAT1 for CMW3162 Pinus densiflora Japan MAT1 for CMW3164 Pinus densiflora Japan MAT1 for CMW3165 Pinus densiflora Japan MAT1 for CMW3165 Pinus densiflora Japan MAT1 for CMW3165 Pinus densiflora Japan MAT1 for W3166 Pinus teeda South Africa MAT1 for W3167 Pinus sylvestris USA MAT1 for MW3188 Pinus resinosa USA MAT1 for W3188 Pinus resinosa USA MAT1	T1-1	KT779226	P-HE
bieticolens CMW2866 Abies balsamea USA MAT1 bietinum CMW2817 Picea engelmannii USA MAT1 ulbojni CMW2065 Pinus strobus USA MAT1 ulbojni CMW3767 Hylobius abietis UK MAT1 phutanense CMW18650 Pinus wallichiana Bhutan MAT1 CMW18652 Pinus wallichiana Bhutan MAT1 CMW18852 Pinus wallichiana Bhutan MAT1 CMW1888 Hylurgus mickliki Spain MAT1 CMW1989 Hylurgus mickliki Spain MAT1 CMW2300 Pinus occidentalis Dominican Rep. MAT1 CMW2320 Pinus occidentalis Dominican Rep. MAT1 CMW2321 Pinus semaonensis China MAT1 louglasii CMW2076 Pseudotsuga menziesii USA MAT1 racile CMW12316 Pinus armandii China MAT1 CMW1376 Hylastes ater UK MAT1 CMW1376 Hylastes ater UK MAT1 CMW12319 Pinus armandii China MAT1 CMW20607 Pinus octorta Canada MAT1 cMW20606 Picea glauca Canada MAT1 cMW20607 Pinus contorta Canada MAT1 cMW20607 Pinus schiflora Japan MAT1 cMW20607 Pinus sylvestris Sweden MAT1 cMW20607 Pinus densiflora Japan MAT1 cMW20190 Pinus sylvestris Sweden MAT1 cMW20190 Pinus sylvestris Sweden MAT1 cMW2190 Pinus armandii China MAT1 cMW2190 Pinus sylvestris Sweden MAT1 cMW20607 Pinus sylvestris USA MAT1 rini-densiflorae CMW5157 Pinus densiflora Japan MAT1 rini-densiflorae CMW5157 Pinus densiflora Japan MAT1 cMW5162 Pinus densiflora Japan MAT1 cMW5162 Pinus densiflora Japan MAT1 rofmum CMW5157 Pinus sylvestris USA MAT1 cMW5165 Pinus sylvestris USA MAT1 rofanum CMW10555 Nyssa sylvatica USA MAT1 rofanum CMW10555 Nyssa sylvatica USA MAT1 rofMW1689 Pinus resinosa USA MAT1	T1-2 KT779249		P-HE
IbietinumCMW2817Picea engelmanniiUSAMAT1IlbopiniCMW2065Pinus strobusUSAMAT1IlbopiniCMW3767Hylobius abietisUKMAT1IchtinumCMW3767Hylobius abietisUKMAT1ohutanenseCMW18650Pinus wallichianaBhutanMAT1astellanumCMW1988Hylurgus micklikiSpainMAT1CMW1988Hylurgus micklikiSpainMAT1CMW2320Pinus occidentalisDominican Rep.MAT1CMW2321Pinus occidentalisDominican Rep.MAT1louglasiiCMW2076Pseudotsuga menziesiiUSAMAT1ibbsiiCMW2076Pseudotsuga menziesiiUSAMAT1racileCMW12421Pinus armandiiChinaMAT1ongiclavatumCMW20606Picea glaucaCanadaMAT1ongiconidiophorumCMW2004Pinus contortaCanadaMAT1ongiconidiophorumCMW204Pinus sylvestrisSwedenMAT1nanifestumCMW217Pinus sylvestrisSwedenMAT1nini-densifloraeCMW3157Pinus densifloraJapanMAT1rocerumCMW3157Pinus densifloraJapanMAT1rocerumCMW3157Pinus densifloraJapanMAT1rofanumCMW3157Pinus densifloraJapanMAT1rofanumCMW3157Pinus densifloraJapanMAT1rofanumCMW3162Pinus sylvestrisUSA	T1-1	KT779228	P-HE
albopiniCMW2065Pinus strobusUSAMAT1alethinumCMW3767Hylobius abietisUKMAT1bhutanenseCMW18650Pinus wallichianaBhutanMAT1CMW18652Pinus wallichianaBhutanMAT1cMw18852Pinus wallichianaBhutanMAT1cMW18862Pinus wallichianaBhutanMAT1cMW1988Hylurgus micklikiSpainMAT1CMW1989Hylurgus micklikiSpainMAT1CMW2320Pinus occidentalisDominican Rep.MAT1cMW2321Pinus occidentalisDominican Rep.MAT1louglasiiCMW2076Pseudotsuga menziesiiUSAMAT1louglasiiCMW2076Pseudotsuga menziesiiUSAMAT1ibbsiiCMW2131Pinus armandiiChinaMAT1cMW12316Pinus armandiiChinaMAT1ongiclavatumCMW20606Picea glaucaCanadaMAT1ongiconidiophorumCMW2044Pinus densifloraJapanMAT1undbergiiCMW217Pinus sylvestrisSwedenMAT1nanifestumCMW1246Larix olgensisChinaMAT1rorecrumCMW5157Pinus densifloraJapanMAT1rofanumCMW216Pinus sensifloraJapanMAT1rofanumCMW5157Pinus sensifloraJapanMAT1rofanumCMW5157Pinus sensifloraJapanMAT1rofanumCMW5162Pinus sensiflora <td< td=""><td>T1-2 KT779264</td><td></td><td>P-HE</td></td<>	T1-2 KT779264		P-HE
alethinumCMW3767Hylobius abietisUKMAT1chutanenseCMW18650Pinus wallichianaBhutanMAT1cMW18652Pinus wallichianaBhutanMAT1cMW18652Pinus wallichianaBhutanMAT1cMW1988Hylurgus micklikiSpainMAT1cMW1989Hylurgus micklikiSpainMAT1cMW2320Pinus occidentalisDominican Rep.MAT1cMW2321Pinus occidentalisDominican Rep.MAT1louglasiiCMW2076Pseudotsuga menziesiiUSAMAT1ibbsiiCMW853Hylastes aterUKMAT1gracileCMW1376Hylastes aterUKMAT1cMW1376Hylastes aterUKMAT1gracileCMW12310Pinus armandiiChinaMAT1cMW20607Pinus armandiiChinaMAT1cMW20607Pinus contortaCanadaMAT1cMW20607Pinus contortaCanadaMAT1cMW20607Pinus sylvestrisNorwayMAT1ongiconidiophorumCMW217Pinus sylvestrisNorwayMAT1inietiCMW2180Pinus sylvestrisNorwayMAT1inietiCMW3837Pinus sylvestrisUSAMAT1iniedensifloraeCMW5157Pinus densifloraJapanMAT1iniedensifloraeCMW5157Pinus sylvestrisUSAMAT1iniedensifloraeCMW10552Carya sp.USAMAT1irofanumCMW216 <td>T1-2 KT779251</td> <td></td> <td>P-HE</td>	T1-2 KT779251		P-HE
chutanenseCMW18650Pinus wallichianaBhutanMAT1CMW18652Pinus wallichianaBhutanMAT1cMW1985Hylurgus micklikiSpainMAT1CMW1989Hylurgus micklikiSpainMAT1CMW2320Pinus occidentalisDominican Rep.MAT1CMW2321Pinus occidentalisDominican Rep.MAT1cMW2321Pinus occidentalisDominican Rep.MAT1louglasiiCMW2421Pinus semaonensisChinaMAT1louglasiiCMW2767Pseudotsuga menziesiiUSAMAT1ibbsiiCMW1076Pseudotsuga menziesiiUSAMAT1racileCMW1376Hylastes aterUKMAT1cMW12319Pinus armandiiChinaMAT1cMW20607Pinus armandiiChinaMAT1cMW20607Pinus contortaCanadaMAT1ongiclavatumCMW2004Pinus densifloraJapanMAT1cMW2190Pinus sylvestrisNorwayMAT1iniefetiCMW337Pinus sylvestrisNorwayMAT1iniedensifloraeCMW5157Pinus densifloraJapanMAT1ini-densifloraeCMW5157Pinus sylvestrisUSAMAT1rofanumCMW216Pinus sylvestrisUSAMAT1rofanumCMW216Pinus sylvestrisUSAMAT1rofanumCMW10552Carya sp.USAMAT1rofanumCMW10555Nysas sylvaticaUSAMAT1r	T1-1	KT779229	P-HE
CMW18652Pinus wallichianaBhutanMAT1cmw1988Hylurgus micklikiSpainMAT1CMW1989Hylurgus micklikiSpainMAT1CMW2320Pinus occidentalisDominican Rep.MAT1CMW2321Pinus occidentalisDominican Rep.MAT1CMW2321Pinus occidentalisDominican Rep.MAT1louglasiiCMW2076Pseudotsuga menziesiiUSAMAT1louglasiiCMW2076Pseudotsuga menziesiiUSAMAT1ibbsiiCMW1376Hylastes aterUKMAT1gracileCMW12316Pinus armandiiChinaMAT1cMW12319Pinus armandiiChinaMAT1ongiclavatumCMW20606Picea glaucaCanadaMAT1ongiconidiophorumCMW2004Pinus contortaCanadaMAT1undbergiiCMW217Pinus sylvestrisSwedenMAT1nanifestumCMW2136Larix olgensisChinaMAT1nini-densifloraeCMW5157Pinus densifloraJapanMAT1rocerumCMW216Pinus sylvestrisSouth AfricaMAT1rofanumCMW216Pinus sylvestrisUSAMAT1rofanumCMW216Pinus sylvestrisUSAMAT1rofanumCMW216Pinus sylvestrisUSAMAT1rofanumCMW216Pinus sylvestrisUSAMAT1rofanumCMW216Pinus sylvestrisUSAMAT1rofanumCMW10552Nysa sylv	T1-1	KM491450	HE
astellanumCMW1988Hylurgus micklikiSpainMAT1CMW1989Hylurgus micklikiSpainMAT1CMW2320Pinus occidentalisDominican Rep.MAT1CMW2321Pinus occidentalisDominican Rep.MAT1louglasiiCMW2076Pseudotsuga menziesiiUSAMAT1louglasiiCMW2076Pseudotsuga menziesiiUSAMAT1ibbsiiCMW2076Pseudotsuga menziesiiUSAMAT1racileCMW1376Hylastes aterUKMAT1cMW12316Pinus armandiiChinaMAT1ongiclavatumCMW20606Picea glaucaCanadaMAT1ongiconidiophorumCMW20607Pinus densifloraJapanMAT1undbergiiCMW217Pinus densifloraJapanMAT1nanifestumCMW2136Larix olgensisChinaMAT1rocerumCMW12436Larix olgensisChinaMAT1rororanCMW5157Pinus sylvestrisNorwayMAT1rororanCMW458Pinus sylvestrisUSAMAT1rororanCMW5162Pinus sylvestrisUSAMAT1rorfanumCMW10552Carya sp.USAMAT1rofanumCMW10555Nysas sylvaticaUSAMAT1rofanumCMW169Pinus resinosaUSAMAT1Pinus iefferniUSAPinus iefferniUSAMAT1	T1-2 KM491428		HE
CMW 1989Hylurgus mickliktOpunMAT1CMW 1989Hylurgus micklikiSpainMAT1CMW 2320Pinus occidentalisDominican Rep.MAT1CMW 2321Pinus occidentalisDominican Rep.MAT1louglasiiCMW 2076Pseudotsuga menziesiiUSAMAT1louglasiiCMW 2076Pseudotsuga menziesiiUSAMAT1ibbsiiCMW 2076Pseudotsuga menziesiiUSAMAT1ibbsiiCMW 2076Pseudotsuga menziesiiUSAMAT1racileCMW 12316Hylastes aterUKMAT1comgiclavatumCMW 203606Picea glaucaCanadaMAT1ongiconidiophorumCMW 20606Picea glaucaCanadaMAT1ongiconidiophorumCMW 2004Pinus densifloraJapanMAT1undbergiiCMW 217Pinus sylvestrisSwedenMAT1inietiCMW 216Larix olgensisChinaMAT1inietiCMW 2157Pinus densifloraJapanMAT1inietiCMW 2162Pinus densifloraJapanMAT1rofanumCMW 5157Pinus densifloraJapanMAT1rofanumCMW 45Pinus sylvestrisUSAMAT1rofanumCMW 2052Carya sp.USAMAT1rofanumCMW 10555Nyssa sylvaticaUSAMAT1rofanumCMW 169Pinus resinosaUSAMAT1CMW 169Pinus resinosaUSAMAT1PinusPin	T1-1	=KT779231	P-HE
CMW 2320Pinus occidentalisDominican Rep.MAT1CMW 2321Pinus occidentalisDominican Rep.MAT1CMW 2321Pinus occidentalisDominican Rep.MAT1louglasiiCMW 2076Pseudotsuga menziesiiUSAMAT1louglasiiCMW 2076Pseudotsuga menziesiiUSAMAT1ibbsiiCMW 1236Hylastes aterUKMAT1cmw 1236Pinus armandiiChinaMAT1racileCMW 12316Pinus armandiiChinaMAT1cmw 12319Pinus armandiiChinaMAT1ongiclavatumCMW 20606Picea glaucaCanadaMAT1ongiconidiophorumCMW 2004Pinus densifloraJapanMAT1undbergiiCMW 217Pinus sylvestrisSwedenMAT1inetiCMW 217Pinus sylvestrisNorwayMAT1inetiCMW 216Larix olgensisChinaMAT1inetiCMW 5157Pinus densifloraJapanMAT1ini-densifloraeCMW 5157Pinus densifloraJapanMAT1rofanumCMW 5152Pinus sylvestrisUSAMAT1rofanumCMW 5152Pinus sylvestrisUSAMAT1rofanumCMW 216Pinus sylvestrisUSAMAT1rofanumCMW 10555Nyssa sylvaticaUSAMAT1rofanumCMW 10555Nyssa sylvaticaUSAMAT1rofanumCMW 169Pinus resinosaUSAMAT1CMW 169<	T1-1	= KT770231	P-HF
CMW 2520Finus occidentalisDominican Rep.MAT1CMW2321Pinus occidentalisDominican Rep.MAT1louglasiiCMW2076Pseudotsuga menziesiiUSAMAT1louglasiiCMW2076Pseudotsuga menziesiiUSAMAT1ibbsiiCMW853Hylastes aterUKMAT1gracileCMW12316Pinus armandiiChinaMAT1ongiclavatumCMW20606Picea glaucaCanadaMAT1ongiconidiophorumCMW20607Pinus contortaCanadaMAT1undbergiiCMW217Pinus vylvestrisSwedenMAT1nanifestumCMW2160Pienus sylvestrisSwedenMAT1nanifestumCMW217Pinus sylvestrisSwedenMAT1nini-densifloraeCMW5157Pinus densifloraJapanMAT1rocerumCMW5157Pinus densifloraJapanMAT1rocerumCMW5157Pinus densifloraJapanMAT1rofanumCMW5157Pinus densifloraJapanMAT1rofanumCMW5157Pinus densifloraJapanMAT1rofanumCMW216Pinus sylvestrisUSAMAT1rofanumCMW10552Carya sp.USAMAT1ryrinumCMW10555Nyssa sylvaticaUSAMAT1rofanumCMW1099Pinus resinosaUSAMAT1rofanumCMW1099Pinus resinosaUSAMAT1rofanumCMW1699Pinus resinosaUSAMAT1 <td></td> <td>-KT770221</td> <td>P_UF</td>		-KT770221	P_UF
CMW 2521Pinus occidentatisDominican Rep.MAT1retereCMW12421Pinus semaonensisChinaMAT1douglasiiCMW2076Pseudotsuga menziesiiUSAMAT1gibbsiiCMW853Hylastes aterUKMAT1gibbsiiCMW1376Hylastes aterUKMAT1gracileCMW12316Pinus armandiiChinaMAT1cMW12319Pinus armandiiChinaMAT1ongiclavatumCMW20606Picea glaucaCanadaMAT1ongiconidiophorumCMW20404Pinus densifloraJapanMAT1undbergiiCMW217Pinus sylvestrisSwedenMAT1nanifestumCMW12436Larix olgensisChinaMAT1nanifestumCMW5157Pinus densifloraJapanMAT1rocerumCMW5162Pinus densifloraJapanMAT1rocerumCMW5162Pinus sylvestrisUSAMAT1rofanumCMW5157Pinus sylvestrisUSAMAT1rofanumCMW10552Carya sp.USAMAT1ryrinumCMW10555Nyssa sylvaticaUSAMAT1CMW1089Pinus resinosaUSAMAT1CMW1089Pinus resinosaUSAMAT1	T1 1	-K1779231	
tetereCMW 12421Pinus semaonensisChinaMAT1louglasiiCMW2076Pseudotsuga menziesiiUSAMAT1gibbsiiCMW853Hylastes aterUKMAT1gibbsiiCMW1376Hylastes aterUKMAT1racileCMW12316Pinus armandiiChinaMAT1ongiclavatumCMW20606Picea glaucaCanadaMAT1ongiconidiophorumCMW20607Pinus contortaCanadaMAT1ongiconidiophorumCMW20404Pinus densifloraJapanMAT1undbergiiCMW217Pinus sylvestrisSwedenMAT1nanifestumCMW21837Pinus sylvestrisNorwayMAT1nini-densifloraeCMW5157Pinus densifloraJapanMAT1rocerumCMW5157Pinus densifloraJapanMAT1rofanumCMW5162Pinus sylvestrisUSAMAT1rofanumCMW5157Pinus densifloraJapanMAT1rofanumCMW5157Pinus densifloraJapanMAT1rofanumCMW458Pinus sylvestrisUSAMAT1rofanumCMW10552Carya sp.USAMAT1ryrinumCMW1089Pinus resinosaUSAMAT1Pinus istefforaiUSAPinus refirariUSAMAT1	T1 0 VT770064	K1//9231	P-HE
tougiasiiCMW20/6Pseudotsuga menziesiiUSAMAT1jibbsiiCMW853Hylastes aterUKMAT1cMW1376Hylastes aterUKMAT1cMW1376Hylastes aterUKMAT1cMW12316Pinus armandiiChinaMAT1cMW12319Pinus armandiiChinaMAT1cMW20606Picea glaucaCanadaMAT1cMW20607Pinus contortaCanadaMAT1ongiconidiophorumCMW2004Pinus densifloraJapanMAT1undbergiiCMW217Pinus sylvestrisSwedenMAT1nanifestumCMW2180Pinus sylvestrisNorwayMAT1nanifestumCMW12436Larix olgensisChinaMAT1ininetiCMW5157Pinus densifloraJapanMAT1rocerumCMW5157Pinus densifloraJapanMAT1rofanumCMW45Pinus sylvestrisUSAMAT1rofanumCMW10552Carya sp.USAMAT1yrinumCMW10955Nyssa sylvaticaUSAMAT1CMW1089Pinus resinosaUSAMAT1CMW13880Pinus referariUSAMAT1OMW1089Pinus referariUSAMAT1Pinus referariUSAMAT1CMW1888Pinus referariUSAMAT1	KT7/9261		P-HE
DibbsitCMW853Hylastes aterUKMAT1CMW1376Hylastes aterUKMAT1CMW1376Hylastes aterUKMAT1pracileCMW12316Pinus armandiiChinaMAT1CMW12319Pinus armandiiChinaMAT1compiclavatumCMW20606Picea glaucaCanadaMAT1comgiconidiophorumCMW20007Pinus contortaCanadaMAT1ongiconidiophorumCMW2004Pinus densifloraJapanMAT1undbergiiCMW217Pinus sylvestrisSwedenMAT1cMW2190Pinus sylvestrisNorwayMAT1nanifestumCMW12436Larix olgensisChinaMAT1ninetiCMW3837Pinus spl.IndonesiaMAT1rocerumCMW5157Pinus densifloraJapanMAT1rocerumCMW45Pinus sylvestrisUSAMAT1rofanumCMW10552Carya sp.USAMAT1ryinumCMW1099Pinus resinosaUSAMAT1CMW3889Pinus refinosaUSAMAT1CMW3889Pinus refinosaUSAMAT1		KT779230	P-HE
CMW1376Hylastes aterUKMAT1gracileCMW12316Pinus armandiiChinaMAT1CMW12319Pinus armandiiChinaMAT1ongiclavatumCMW20606Picea glaucaCanadaMAT1cMW20607Pinus contortaCanadaMAT1ongiconidiophorumCMW2004Pinus densifloraJapanMAT1undbergiiCMW217Pinus sylvestrisSwedenMAT1cMW2190Pinus sylvestrisNorwayMAT1nanifestumCMW12436Larix olgensisChinaMAT1ninetiCMW3837Pinus spl.IndonesiaMAT1rocerumCMW5157Pinus densifloraJapanMAT1rocerumCMW5162Pinus densifloraJapanMAT1rofanumCMW45Pinus sylvestrisUSAMAT1rofanumCMW10552Carya sp.USAMAT1yrinumCMW1099Pinus resinosaUSAMAT1CMW3889Pinus resinosaUSAMAT1CMW3889Pinus resinosaUSAMAT1	T1-2 = KT779258		P-HE
gracileCMW12316Pinus armandiiChinaMAT1CMW12319Pinus armandiiChinaMAT1ongiclavatumCMW20606Picea glaucaCanadaMAT1cMW20607Pinus contortaCanadaMAT1ongiconidiophorumCMW2004Pinus densifloraJapanMAT1undbergiiCMW217Pinus sylvestrisSwedenMAT1cMW2190Pinus sylvestrisSwedenMAT1nanifestumCMW12436Larix olgensisChinaMAT1nini-densifloraeCMW3837Pinus spl.IndonesiaMAT1cMW5157Pinus densifloraJapanMAT1rocerumCMW5157Pinus densifloraJapanMAT1rofanumCMW45Pinus sylvestrisUSAMAT1rofanumCMW1655Nyssa sylvaticaUSAMAT1yrinumCMW169Pinus resinosaUSAMAT1	T1-2 KT779258		P-HE
CMW12319Pinus armandiiChinaMAT1ongiclavatumCMW20606Picea glaucaCanadaMAT1CMW20607Pinus contortaCanadaMAT1ongiconidiophorumCMW2004Pinus densifloraJapanMAT1undbergiiCMW217Pinus sylvestrisSwedenMAT1CMW2190Pinus sylvestrisNorwayMAT1nanifestumCMW12436Larix olgensisChinaMAT1nini-densifloraeCMW12436Larix olgensisChinaMAT1nini-densifloraeCMW5157Pinus densifloraJapanMAT1rocerumCMW5162Pinus densifloraJapanMAT1rofanumCMW252Carya sp.USAMAT1rofanumCMW10555Nyssa sylvaticaUSAMAT1ryinumCMW169Pinus resinosaUSAMAT1CMW3889Pinus refireruiUSAMAT1	T1-2 KM491429		P-HE
ongiclavatumCMW20606Picea glaucaCanadaMAT1CMW20607Pinus contortaCanadaMAT1ongiconidiophorumCMW2004Pinus densifloraJapanMAT1undbergiiCMW217Pinus sylvestrisSwedenMAT1CMW2190Pinus sylvestrisNorwayMAT1nanifestumCMW12436Larix olgensisChinaMAT1ininetiCMW3837Pinus sp.IndonesiaMAT1inin-densifloraeCMW5157Pinus densifloraJapanMAT1rocerumCMW5162Pinus sylvestrisUSAMAT1rofanumCMW216Pinus sylvestrisUSAMAT1yrinumCMW10955Nyssa sylvaticaUSAMAT1CMW1099Pinus resinosaUSAMAT1CMW3889Pinus iefferviUSAMAT1	T1-2 KM491436		P-HE
CMW20607Pinus contortaCanadaMAT1ongiconidiophorumCMW2004Pinus densifloraJapanMAT1undbergiiCMW217Pinus sylvestrisSwedenMAT1CMW2190Pinus sylvestrisNorwayMAT1nanifestumCMW12436Larix olgensisChinaMAT1ninetiCMW3837Pinus sp.IndonesiaMAT1nini-densifloraeCMW5157Pinus densifloraJapanMAT1rrocerumCMW5162Pinus densifloraJapanMAT1rrofanumCMW45Pinus sylvestrisUSAMAT1rofanumCMW10552Carya sp.USAMAT1yrinumCMW169Pinus resinosaUSAMAT1	T1-2 KT779255		HE
ongiconidiophorumCMW2004Pinus densifloraJapanMAT1undbergiiCMW217Pinus sylvestrisSwedenMAT1CMW2190Pinus sylvestrisNorwayMAT1nanifestumCMW12436Larix olgensisChinaMAT1inietiCMW3837Pinus sp.IndonesiaMAT1ini-densifloraeCMW5157Pinus densifloraJapanMAT1rrocerumCMW5162Pinus densifloraJapanMAT1rrofanumCMW45Pinus sylvestrisUSAMAT1rofanumCMW10552Carya sp.USAMAT1yrinumCMW169Pinus resinosaUSAMAT1	T1-1	KT779232	HE
undbergii CMW217 Pinus sylvestris Sweden MAT1 CMW2190 Pinus sylvestris Norway MAT1 nanifestum CMW12436 Larix olgensis China MAT1 ineti CMW3837 Pinus sp. Indonesia MAT1 ini-densiflorae CMW5157 Pinus densiflora Japan MAT1 CMW5162 Pinus densiflora Japan MAT1 rocerum CMW45 Pinus sylvestris USA MAT1 CMW216 Pinus taeda South Africa MAT1 rofanum CMW10552 Carya sp. USA MAT1 CMW10555 Nyssa sylvatica USA MAT1 CMW109 Pinus resinosa USA MAT1	T1-1	KM491452	P-HE
CMW2190 Pinus sylvestris Norway MAT1 nanifestum CMW12436 Larix olgensis China MAT1 ineti CMW3837 Pinus sp. Indonesia MAT1 ini-densiflorae CMW5157 Pinus densiflora Japan MAT1 CMW5162 Pinus densiflora Japan MAT1 CMW5162 Pinus sylvestris USA MAT1 CMW216 Pinus taeda South Africa MAT1 rofanum CMW10552 Carya sp. USA MAT1 CMW10555 Nyssa sylvatica USA MAT1 CMW109 Pinus resinosa USA MAT1	T1-1	KT779233	HE
Internet Inter	T1-2 KT779252		HE
Initia Initia INITIA INITIA INITIA Initia CMW3837 Pinus sp. Indonesia MAT1 Initia CMW3837 Pinus sp. Indonesia MAT1 Initia CMW5157 Pinus sp. Indonesia MAT1 CMW5162 Pinus densiflora Japan MAT1 Introcerum CMW45 Pinus sylvestris USA MAT1 Introcerum CMW216 Pinus taeda South Africa MAT1 Introcerum CMW10552 Carya sp. USA MAT1 Introcerum CMW10555 Nyssa sylvatica USA MAT1 Introcerum CMW1089 Pinus resinosa USA MAT1	T1-1	KT779234	P-HF
viniti GMW 3657 Pinus sp. Indonesia MATT pini-densiflorae CMW 5157 Pinus densiflora Japan MATT CMW 5162 Pinus densiflora Japan MATT CMW 5162 Pinus sylvestris USA MATT CMW 216 Pinus taeda South Africa MATT CMW 10552 Carya sp. USA MATT CMW 10555 Nyssa sylvatica USA MATT yrinum CMW 169 Pinus resinosa USA MATT	T1-1	KT770225	P_UF
Intracensitionae CMW5157 Pinus densitiona Japan MAT1 CMW5162 Pinus densiflora Japan MAT1 procerum CMW45 Pinus sylvestris USA MAT1 CMW216 Pinus taeda South Africa MAT1 profanum CMW10552 Carya sp. USA MAT1 cMW10555 Nyssa sylvatica USA MAT1 yrinum CMW169 Pinus resinosa USA MAT1	T1 1	K1//9233	P-DL UF
CMW5162 Pinus densifiora Japan MAT1 procerum CMW45 Pinus sylvestris USA MAT1 CMW216 Pinus taeda South Africa MAT1 rofanum CMW10552 Carya sp. USA MAT1 vyrinum CMW10555 Nyssa sylvatica USA MAT1 vyrinum CMW169 Pinus resinosa USA MAT1	T1 0 77 401 400	KIVI491453	HE
vrocerum CMW45 Pinus sylvestris USA MAT1 CMW216 Pinus taeda South Africa MAT1 vrofanum CMW10552 Carya sp. USA MAT1 CMW10555 Nyssa sylvatica USA MAT1 vyrinum CMW169 Pinus resinosa USA MAT1	T1-2 KM491438		HE
CMW216 Pinus taeda South Africa MAT1 profanum CMW10552 Carya sp. USA MAT1 CMW10555 Nyssa sylvatica USA MAT1 pyrinum CMW169 Pinus resinosa USA MAT1 CMW3889 Pinus ieffravi USA MAT1	TT1-2 KC883455		HE
brofanum CMW10552 Carya sp. USA MAT1 CMW10555 Nyssa sylvatica USA MAT1 Pyrinum CMW169 Pinus resinosa USA MAT1 CMW13889 Pinus ieffravi USA MAT1	T1-1	KC883456	HE
CMW10555 Nyssa sylvatica USA MAT1 yrinum CMW169 Pinus resinosa USA MAT1 CMW3889 Pinus ieffravi USA MAT1	T1-2 KC883457		HE
yrinum CMW169 Pinus resinosa USA MAT1 CMW3889 Pinus ieffravi USA MAT4	T1-1	KC883458	HE
CMW3889 Dinus jeffroni LICA MATI	T1-2 KT779262		HE
GIVI VV JOOD FILIUS JEJJIEVI UJA MATI	T1-1	KT779236	HE
		,	

Please cite this article in press as: Duong TA, et al., Mating type markers reveal high levels of heterothallism in Leptographium sensu lato, Fungal Biology (2016), http://dx.doi.org/10.1016/j.funbio.2016.01.001

T. A. Duong et al.

Table 1 – (continued)							
Species	Isolate number	Insect/Host	Origin	Mating type	MAT1-2-1	MAT1-1-3	Thallism
L. sibiricum	CMW4481	Abies sibirica	Russia	MAT1-2	KM491443		P-HE
L. sinense	CMW38172	Pinus elliottii	China	MAT1-2	KM491433		P-HE
L. sinoprocerum	CMW26230	Pinus tabuliformis	China	MAT1-1		KM491460	HE
	CMW29990	Pinus tabuliformis	China	MAT1-2	KM491447		HE
L. terebrantis	SS394	Pinus contorta banksiana hybrid	Canada	MAT1-2	JX402935		HE
	SS403	Pinus contorta	Canada	MAT1-1		JX402956	HE
L. truncatum	CMW644	Hylastes sp.	UK	MAT1-2	KT779248		HE
	CMW2402	Pinus resinosa	Canada	MAT1-1		KT779237	HE
L. wageneri v. ponderosae	CMW279	Pinus ponderosae	USA	MAT1-2	KT779259		HE
	CMW307	Pinus contorta	USA	MAT1-1		=KT779239	HE
L. wageneri v. pseudotsugae	CMW1533	Pseudotsuga menziesii	USA	MAT1-1		=KT779239	HE
	CMW154	Pseudotsuga menziesii	USA	MAT1-1		KT779239	HE
	CMW1541	Pseudotsuga menziesii	USA	MAT1-1		=KT779239	HE
	CMW2087	Pseudotsuga menziesii	USA	MAT1-2	KT779260		HE
L. wageneri v. wageneri	CMW53	Pinus ponderosa	USA	MAT1-2	=KT779259		HE
	CMW493	Pinyon Pine	USA	MAT1-1		KT779240	HE
	CMW1828	Pinus edulis	USA	MAT1-1		=KT779240	HE
L. wingfieldii	CMW2096	Pinus sylvestris	France	MAT1-1		JX402949	HE
	CMW10221	Pinus strobus	USA	MAT1-2	KT779256		HE
L. yamaokae	CMW1935	Pinus sp.	Japan	MAT1-1		=KT779241	P-HE
	CMW1944	Pinus sp.	Japan	MAT1-1		=KT779241	P-HE
	CMW4726	Pinus densiflora	Japan	MAT1-1		KT779241	P-HE
	CMW4727	Pinus densiflora	Japan	MAT1-1		=KT779241	P-HE
	CMW4728	Pinus densiflora	Japan	MAT1-1		=KT779241	P-HE
	CMW4729	Pinus densiflora	Japan	MAT1-1		=KT779241	P-HE
Leptographium sp. X	CMW15470	Pinus contorta	Canada	MAT1-2	KT779257		HE
	CMW15493	Pinus contorta	Canada	MAT1-1		KT779242	HE
O. novo-ulmi sub. novo-ulmi	H327	Ulmus sp.	Slovakia	MAT1-1		FJ858801	HE
	V19	Ulmus sp.	Russia	MAT1-2	AY887029		HE
O. quercus	CMW27845	Quercus sp.	Canada	MAT1-1		JQ319596	HE
	CMW27847	Quercus sp.	UK	MAT1-2	FJ865429		HE

CMW = Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. NA = Not applicable; HE = Heterothallic; HO = Homothallic; P-HE = Putative heterothallic. * = Isolates referred to as 'G. piceiperda B' and 'G. piceiperda C' have been shown to represent distinct species in the G. piceiperda species complex (Linnakoski et al. 2012).

extension for 60 s, with a final extension at 72 °C for 8 min. In most cases, the annealing temperature was at 55 °C for both MAT1-1 and MAT1-2 primers. However, when the PCR failed or when non-specific amplification was observed, the annealing temperature was adjusted in the range of 52 °C–60 °C. Resulting PCR products were separated using 2 % agarose gel electrophoresis, and gels were stained with GelRed (Biotium, Inc., California, USA) and examined under UV light.

The success of MAT marker amplification was confirmed by sequencing the obtained PCR amplicons. In cases where a single product was obtained, the PCR products were treated with exonuclease I and shrimp alkaline phosphatase (Exo-SAP) (Fermentas Inc., Hanover, MD, USA) following the manufacturer's instructions, to remove excess primers and dNTPs. Where multiple bands were present, fragments of expected size were excised from the gel and sequenced using the same protocol. The treated or excised PCR products were directly sequenced using the same primers that were used for PCR amplification and the Big Dye[®] Terminator v. 3.1 cycle sequencing premix kit (Applied Biosystems, Foster City, California, USA).

Species that showed amplification products for both MAT1-1 and MAT1-2 primer combinations in a single isolate were designated as being homothallic. Species displaying amplification for both MAT1-1 and MAT1-2 primer combinations, but where only MAT1-1 or MAT1-2 could be detected in an individual isolate, were likewise designated as being heterothallic. Species where only the MAT1-2 or MAT1-1 idiomorph was detected were considered to be putatively heterothallic.

Phylogenetic analyses

In order to investigate the phylogenetic relationship between homothallic and heterothallic species investigated, phylogenetic analyses were conducted on a combined dataset of ITS2-LSU, partial MAT1-2-1 and MAT1-1-3 genes. The ITS2-LSU sequences for all species were obtained from GenBank, representing type isolates of each species, sequence accession numbers for these are presented elsewhere (De Beer & Wingfield 2013; Yin *et al.* 2015). Sequences for regions of MAT1-2-1 and MAT1-1-3 genes were generated as described above (Table 1). All there gene regions were combined and aligned using an online version of MAFFT v. 7 (Katoh & Standley 2013).

ARTICLE IN PRESS

FUNBIO672_proof = 4 February 2016 = 5/9



Maximum likelihood (ML) and Bayesian inference (BI) analyses were carried out on the aligned dataset. Maximum likelihood analysis was conducted using RaxML v8.1.15 (Stamatakis et al. 2005) applying GTR + G model. A ML search for best-scoring ML tree followed by one thousand rapid bootstrap analysis, was conducted. Bayesian inference analyses were performed using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003) applying the same models as used in the ML analysis. Four MCMC chains were run simultaneously for 5 million generations and tree sampling was conducted after every 100th generation. Twenty five percent of the trees sampled at the burn-in phase were discarded and posterior probabilities were calculated from the remaining trees.

Results

Mating type markers

MAT primer design, PCR amplification, and mating type assignment

MAT1-2 and MAT1-1 primers were successfully used in PCRs to amplify portions of MAT1-2-1 and MAT1-1-3 genes respectively. Primers Oph-HMG1 and Oph-HMG2 amplified part of HMG box of MAT1-2-1 gene, resulting in PCR products of about 230 bp. Primers Oph-MAT1F1 and Oph-MAT1R2 amplified part of the MAT1-1-3 gene, resulting in PCR products of about 450 bp. In cases where this MAT1-1 primer combination failed to amplify, for example in Grosmannia aenigmatica, 'Grosmannia piceiperda B', and 'G. piceiperda C', primers Oph-MAT1R1 were successfully used in place of Oph-MAT1R2, resulting in slightly shorter PCR products of the MAT1-1-3 gene. The relative primer binding positions on each of the MAT loci are presented in Fig 1.

In most cases, a single PCR product was obtained from each positive reaction. The identities of PCR products were confirmed as part of MAT1-2-1 or MAT1-1-3 genes by sequencing all positive amplifications. All obtained sequences were deposited in GenBank and accession numbers are presented in Table 1. Portions of the MAT genes for a total of 42 species residing in *Leptographium sensu lato* species were successfully amplified (Table 1). Based on the amplification profile, 20 species were identified as heterothallic, three species were homothallic and 19 species were tentatively assigned as heterothallic. Examples of MAT PCR amplification for some of the tested isolates are presented in Fig 2.

Phylogenetic analyses

The alignment of the ITS2-LSU region contained 604 characters, of which 450 characters were constant and 114 characters were parsimony informative. The alignment of MAT1-1-3 gene region contained 493 characters, of which 191 characters were constant and 251 characters were parsimony



Fig 2 – Agarose gel electrophoresis (2 % w/v) of MAT1-1-3 (larger size bands-around 500 bp) and MAT1-2-1 (smaller size bands, about 230 bp) PCR fragments from representative Leptographium sensu lato species. 'G. piceiperda C' CMW446 (1); G. aenigmatica CMW2199 (2); 'G. piceiperda B' CMW2811 (3); G. alacris: CMW623 (4), CMW621 (5); G. koreana: CMW14200 (6), CMW14201 (7); G. huntii: CMW1006 (8), CMW1015 (9); L. bhutanense: CMW18650 (10), CMW18652 (11); L. longiclavatum: CMW20606 (12), CMW20607 (13); L. wageneri var. wageneri CMW53 (14), CMW493 (15); L. wageneri var. ponderosae CMW279 (16), CMW307 (17); L. wageneri var. pseudotsugae CMW154 (18), CMW2087 (19). The molecular weight marker (M) used was GeneRuler[™] 100 bp Plus DNA Ladder (Fermentas), the 1000 and 500 bp size fragments are indicated on the figure.

L. wingfieldii AY553398





Fig 3 — Phylogram derived from RaxML analysis of combined dataset of ITS2-LSU, MAT1-1-3 and MAT1-2-1 gene regions. Phylogenetic support is presented at nodes as Bayesian posterior probabilities (≥0.95)/ML bootstrap (≥70). Homothallic species are marked with green circles, heterothallic species are marked with blue squares and putatively heterothallic species are marked with yellow squares. ITS-LSU GenBank accession numbers are presented next to the species name. GenBank accession numbers for MAT1-1-3 and MAT1-2-1 used in phylogenetic analyses are presented in italic in Table 1.

67

68

69

70

71

72

73

74

75

76 77

78 79

80

81

82

83

84

85

86

87

88

89

90

91

92

93

94

95

96

97

98

99

100

101

102

103

104

105

106

107

108

109

110

111

112

113

114

115

116

117

118

119

120

121

122

123

124

125

126

127

128

129

130

1

informative. The alignment of MAT1-2-1 gene region contained 215 characters, of which 90 were constant and 121 were parsimony informative.

ML and BI phylogenetic analyses of the combined dataset of ITS2-LSU, partial MAT1-2-1 and MAT1-1-3 genes resulted in trees with similar topology. In most cases, the species investigated grouped together to form species complexes as defined by De Beer & Wingfield (2013). Collectively, species from seven species complexes in Leptographium sensu lato were considered. Notably, three homothallic species as identified using MAT markers, Grosmannia aenigmatica, 'Grosmannia piceiperda B', and 'G. piceiperda C', resided in a single clade with high BI posterior probability and ML bootstrap support (Fig 3). All the remaining species that were heterothallic or putatively heterothallic resided in six different species complexes (Fig 3). These were the Grosmannia clavigera complex, Leptographium lundbergii complex, Grosmannia wageneri complex, Grosmannia serpens complex, Leptographium procerum complex, and Grosmannia penicillata complex. Of these, only the G. wageneri and G. penicillata complexes were well supported by BI and ML analyses.

Discussion

Molecular markers developed in this study made it possible to amplify and identify the mating strategy of 42 species residing in *Leptographium sensu lato*. Many species previously considered to be asexual were shown to be either heterothallic or putatively heterothallic, with individual isolates having only a single idiomorph. It will now be possible to attempt to induce sexual structures for these fungi in culture, by pairing isolates known to represent opposite mating types. Where this can be achieved, various genetic studies could also then be undertaken on these species that would otherwise not have been possible.

The lack of opposite mating type isolates in our possession negated the possibility for us to recover both MAT idiomorphs in a number of species included in this study. Thus, 19 species for which only MAT1-1 or MAT1-2 idiomorph could be recovered were designated as putatively heterothallic. With the MAT makers now available, it will be possible to confirm the heterothallic nature of these species when additional isolates become available for them. It is important to also recognize that primers described in this study could fail to amplify both MAT idiomorphs in some of these species and thus they could be homothallic. Although this is unlikely, the thallism of these species will need to be treated as putative until the opposite MAT idiomorphs to those detected in this study can be found.

Prior to this study, the mating types were known for only a small number of species residing in *Leptographium sensu lato*. Thus a particularly interesting outcome of this study was that the majority of species tested were either heterothallic or putatively heterothallic and this was in contrast to a relatively small number (three) of homothallic species detected. This finding is consistent with the fact that the greater number of species in *Leptographium sensu lato* have long been considered as asexual species (De Beer & Wingfield 2013). Based on the results of this study, we believe that many species found only in the asexual form in nature are probably capable of reproducing sexually. It is plausible that their sexual states have not been seen due to their heterothallic nature and the fact that they have been collected in the absence of an opposite mating strain. This is similar to the situation for various other fungi, thought to be asexual but later shown to be heterothallic and where sexual states have recently been discovered for some of the species (O'Gorman et al. 2008; Horn et al. 2009; Seidl et al. 2009).

Duong et al. (2012) were able to show that Grosmannia alacris is heterothallic by randomly crossing different isolates in all possible combinations. Thus, of the five species in the Grosmannia serpens complex (Duong et al. 2012), sexual sates have been found only in the case of G. alacris. Efforts to induce sexual states in the other four species did not result in ascomata. The present study has provided molecular evidence confirming that G. alacris is heterothallic, as are the other species in the G. serpens complex (Duong et al. 2012). In the present study, only a single mating type was found for isolates of G. serpens (MAT1-1), Leptographium castellanum (MAT1-1), Leptographium yamaokae (MAT1-1), and Leptographium gibbsii (MAT1-2) and it will not be possible to attempt to produce sexual structures until strains of opposite mating type have been found. The results of this study explain why these fungi failed to produce sexual states in the study by Duong et al. (2012).

Goheen & Cobb (1978) reported the discovery of a sexual state in the important conifer root pathogen *Grosmannia wageneri*, which was found in the galleries of *Hylastes macer*. This form of the fungus has never again been seen and there has been doubt as to whether these authors had possibly collected a sexual state of some other ophiostomatoid fungus (Harrington & Cobb 1988). The results of our study show clearly that *G. wageneri* is a heterothallic fungus and thus has the capacity to undergo sexual outcrossing. This provides strong evidence to suggest that Goheen & Cobb (1978) correctly identified the ascomata of this fungus in nature. Thus, it serves as an interesting example of a *Leptographium* sp. for which a sexual state has been found in nature only once and could never be produced in the laboratory (Wingfield, unpubl.).

Species in Leptographium sensu lato have been assigned to different complexes based on their relatedness in phylogeny, morphological characters, as well as their ecology (Linnakoski et al. 2012; De Beer & Wingfield 2013). Results of the present study showed that those species belonging to the same complex consistently share the same mode of sexual reproduction. Likewise, the only three homothallic species ('Grosmannia piceiperda B', 'G. piceiperda C', and Grosmannia aenigmatica) considered in this study grouped in a single, well supported clade, consistent with the G. piceiperda complex previously defined (Linnakoski et al. 2012; De Beer & Wingfield 2013). This suggests that these species might share a common homothallic ancestor. The remaining 39 heterothallic (or putatively heterothallic species) reside in six different species complexes. A number of other species residing in these six species complexes could not be included in this study but based on the patterns observed, it is likely that they will also have a heterothallic mating system.

Patterns of distribution of sexual compatibility have previously been used to better understand the evolution of fungal mating systems in other fungi (Yun *et al.* 1999; Inderbitzin

ARTICLE IN PRESS

T. A. Duong et al.

et al. 2005; Nygren *et al.* 2011). Likewise, the distribution of homothallic and heterothallic species provides an opportunity to gain insights into the origin and evolution of homothallism and heterothallism in *Leptographium sensu lato*. From the results of this study, it is reasonable to hypothesize that homothallism in *G. piceiperda* complex has evolved once from a heterothallic ancestor. A common heterothallic ancestor would thus best explain the current patterns of sexual compatibility in *Leptographium sensu lato*. However, the detailed structure of the MAT loci of species in the *G. piceiperda* complex, together with that in closely related heterothallic species such as those in the *Grosmannia clavigera* and *Leptographium lundbergii* complexes, will be required to confirm this hypothesis.

Acknowledgements

This work was funded by members of the Tree Protection Cooperative Programme (TPCP) and the DST-NRF Centre of Excellence in Tree Health Biotechnology (CTHB) at the Forestry and Agricultural Biotechnology Institute (FABI), the National Research Foundation (NRF), and the University of Pretoria, 95 Pretoria, South Africa.

REFERENCES

- Comeau AM, Dufour J, Bouvet GF, Jacobi V, Nigg M, Henrissat B, Laroche J, Levesque RC, Bernier L, 2015. Functional annotation of the Ophiostoma novo-ulmi genome: insights into the phytopathogenicity of the fungal agent of Dutch elm disease. *Genome* Biology and Evolution 7: 410–430.
- De Beer ZW, Wingfield MJ, 2013. Emerging lineages in the Ophiostomatales. In: Seifert KA, De Beer ZW, Wingfield MJ (eds), The Ophiostomatoid Fungi: expanding frontiers. CBS Biodiversity Series 12, Utrecht, The Netherlands, pp. 21–46.
- Duong TA, de Beer ZW, Wingfield BD, Eckhardt LG, Wingfield MJ, 2015. Microsatellite and mating type markers reveal unexpected patterns of genetic diversity in the pine root-infecting fungus Grosmannia alacris. Plant Pathology **64**: 235–242.
- Duong TA, de Beer ZW, Wingfield BD, Wingfield MJ, 2012. Phylogeny and taxonomy of species in the Grosmannia serpens complex. Mycologia **104**: 715–732.
- Duong TA, de Beer ZW, Wingfield BD, Wingfield MJ, 2013. Characterization of the mating-type genes in Leptographium procerum and Leptographium profanum. Fungal Biology **117**: 411–421.
- Goheen DJ, Cobb FW, 1978. Occurrence of Verticicladiella wagenerii and its perfect state, Ceratocystis wageneri sp. nov., in insect galleries. Phytopathology 68: 1192–1195.
- Groenewald M, Groenewald JZ, Harrington TC, Abeln ECA, Crous PW, 2006. Mating type gene analysis in apparently asexual Cercospora species is suggestive of cryptic sex. Fungal Genetics and Biology **43**: 813–825.
- Harrington TC, Cobb FW, 1988. Leptographium Root Diseases on Conifers. APS Press, St. Paul, Minnesota.
- Horn BW, Ramirez-Prado JH, Carbone I, 2009. The sexual state of Aspergillus parasiticus. Mycologia **101**: 275–280.
- Inderbitzin P, Harkness J, Turgeon BG, Berbee ML, 2005. Lateral transfer of mating system in Stemphylium. Proceedings of the National Academy of Sciences of the United States of America **102**: 11390–11395.

- Jacobs K, Wingfield M, Wingfield B, Yamaoka Y, 1998. Comparison of Ophiostoma huntii and O. europhioides and description of O. aenigmaticum sp. nov. Mycological Research **102**: 289–294.
- Katoh K, Standley DM, 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780.
- Kück U, Pöggeler S, 2009. Cryptic sex in fungi. Fungal Biology Reviews 23: 86–90.
- Linde CC, Zala M, Ceccarelli S, McDonald BA, 2003. Further evidence for sexual reproduction in Rhynchosporium secalis based on distribution and frequency of mating-type alleles. Fungal Genetics and Biology 40: 115–125.
- Linnakoski R, Dee Beer ZW, Duong TA, Niemelä P, Pappinen A, Wingfield MJ, 2012. Grosmannia and Leptographium spp. associated with conifer-infesting bark beetles in Finland and Russia, including Leptographium taigense sp. nov. Antonie Van Leeuwenhoek **102**: 375–399.
- Masuya H, Kim JJ, Wingfield MJ, Yamaoka Y, Kaneko S, Breuil C, Kim GH, 2005. Discovery and description of a teleomorph for Leptographium koreanum. Mycotaxon 94: 159–173.
- Metzenberg RL, Glass NL, 1990. Mating type and mating strategies in Neurospora. Bioessays **12**: 53–59.
- Nygren K, Strandberg R, Wallberg A, Nabholz B, Gustafsson T, García D, Cano J, Guarro J, Johannesson H, 2011. A comprehensive phylogeny of *Neurospora* reveals a link between reproductive mode and molecular evolution in fungi. *Molecular Phylogenetics and Evolution* **59**: 649–663.
- O'Gorman CM, Fuller HT, Dyer PS, 2008. Discovery of a sexual cycle in the opportunistic fungal pathogen Aspergillus fumigatus. Nature **457**: 471–474.
- Paoletti M, Buck KW, Brasier CM, 2005a. Cloning and sequence analysis of the MAT-B (MAT-2) genes from the three Dutch elm disease pathogens, Ophiostoma ulmi, O. novo-ulmi and O. himalulmi. Mycological Research **109**: 983–991.
- Paoletti M, Rydholm C, Schwier EU, Anderson MJ, Szakacs G, Lutzoni F, Debeaupuis J-P, Latgé J-P, Denning DW, Dyer PS, 2005b. Evidence for sexuality in the opportunistic fungal pathogen Aspergillus fumigatus. Current Biology 15: 1242–1248.
- Ronquist F, Huelsenbeck JP, 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Santos JM, Correia VG, Phillips AJL, 2010. Primers for mating-type diagnosis in Diaporthe and Phomopsis: their use in teleomorph induction in vitro and biological species definition. Fungal Biology 114: 255–270.
- Seidl V, Seibel C, Kubicek CP, Schmoll M, 2009. Sexual development in the industrial workhorse Trichoderma reesei. Proceedings of the National Academy of Sciences of the United States of America 106: 13909–13914.
- Stamatakis A, Ludwig T, Meier H, 2005. RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. Bioinformatics **21**: 456–463.
- Tsui CK-M, DiGuistini S, Wang Y, Feau N, Dhillon B, Bohlmann J, Hamelin RC, 2013. Unequal recombination and evolution of the mating-type (MAT) loci in the pathogenic fungus Grosmannia clavigera and relatives. G3: Genes|Genomes|Genetics **3**: 465–480.
- Turgeon BG, Yoder OC, 2000. Proposed nomenclature for mating type genes of filamentous ascomycetes. *Fungal Genetics and* Biology **31**: 1–5.
- Wada R, Maruyama J-i, Yamaguchi H, Yamamoto N, Wagu Y, Paoletti M, Archer DB, Dyer PS, Kitamoto K, 2012. Presence and functionality of mating type genes in the supposedly asexual filamentous fungus Aspergillus oryzae. Applied and Environmental Microbiology **78**: 2819–2829.
- Wilken PM, Steenkamp ET, Hall TA, de Beer ZW, Wingfield MJ, Wingfield BD, 2012. Both mating types in the heterothallic fungus Ophiostoma quercus contain MAT1-1 and MAT1-2 genes. Fungal Biology 116: 427–437.

1

2

3

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

10

11

12

13

14

15

16

- Yamaoka Y, Masuya H, Chung W-H, Goto H, To-Anun C, Tokumasu S, Zhou X, Wingfield M, 2008. The teleomorph of *Leptographium yunnanense*, discovered in crosses among isolates from Thailand, China, and Japan. Mycoscience **49**: 233–240.
- Yin M, Duong TA, Wingfield MJ, Zhou X, de Beer ZW, 2015. Taxonomy and phylogeny of the Leptographium procerum complex, including Leptographium sinense sp. nov. and Leptographium longiconidiophorum sp. nov. Antonie Van Leeuwenhoek 107: 547–563.
- Yun S-H, Berbee ML, Yoder OC, Turgeon BG, 1999. Evolution of the fungal self-fertile reproductive life style from self-sterile ancestors. Proceedings of the National Academy of Sciences of the United States of America **96**: 5592–5597.
- Zipfel RD, De Beer ZW, Jacobs K, Wingfield BD, Wingfield MJ, 2006. Multi-gene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*. Studies in Mycology **55**: 75–97.