Species diversity of the entomopathogenic fungi Metarhizium anisopliae and M. flavoviride species complexes isolated from insects in Japan

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Abstract

Phylogenetic analyses of insect-derived isolates of the *Metarhizium anisopliae* and *M. flavoviride* species complexes in Japan were conducted to reveal their species diversity. Fifty-seven isolates were identified as nine species, including one species first reported for Japan. *Metarhizium pingshaense* was the most frequently isolated species from this genus, and the 29 isolates of *M. pingshaense* came from six orders and 14 families of insects. New host-pathogen associations were found for two species with relatively narrow host ranges: Hymenoptera-*M. pemphigi*, Orthoptera- and Phasmatodea-*M. majus*.

Keywords:

Clavicipitaceae

Green muscardine fungus

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New host

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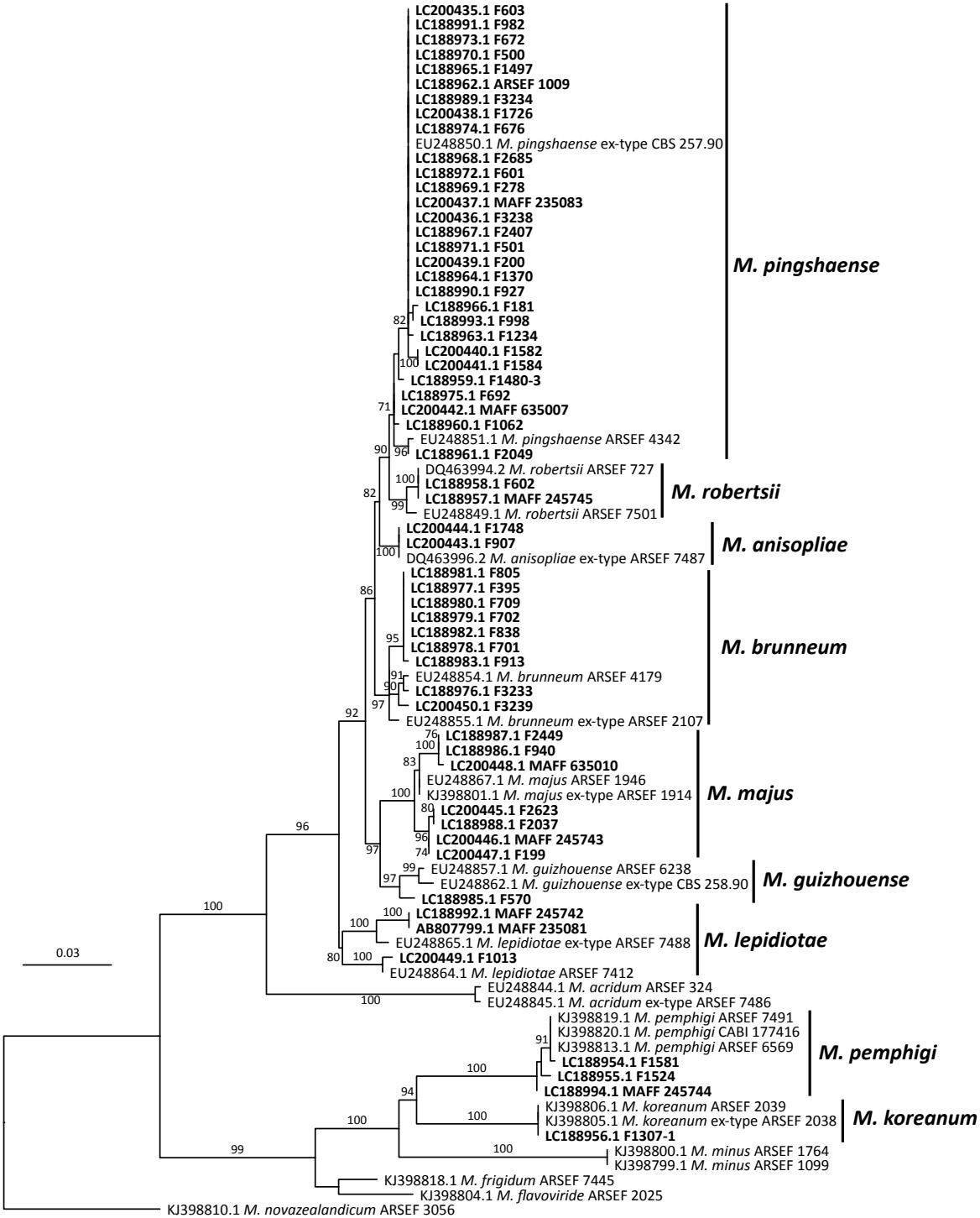
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LC188990.1 F927
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LC188988.1 F2037
LC200446.1 MAFF 245743
LC200447.1 F199
M. majus
EU248857.1 M. guizhouense ARSEF 6238
EU248862.1 M. guizhouense ex-type CBS 258.90
LC188985.1 F570
M. guizhouense
LC188992.1 MAFF 245742
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M. lepidiotae
0.03
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KJ398819.1 M. pemphigi ARSEF 7491
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LC188954.1 F1581
LC188955.1 F1524
LC188994.1 MAFF 245744
M. pemphigi
KJ398806.1 M. koreanum ARSEF 2039
KJ398805.1 M. koreanum ex-type ARSEF 2038
LC188956.1 F1307-1
M. koreanum
KJ398800.1 M. minus ARSEF 1764
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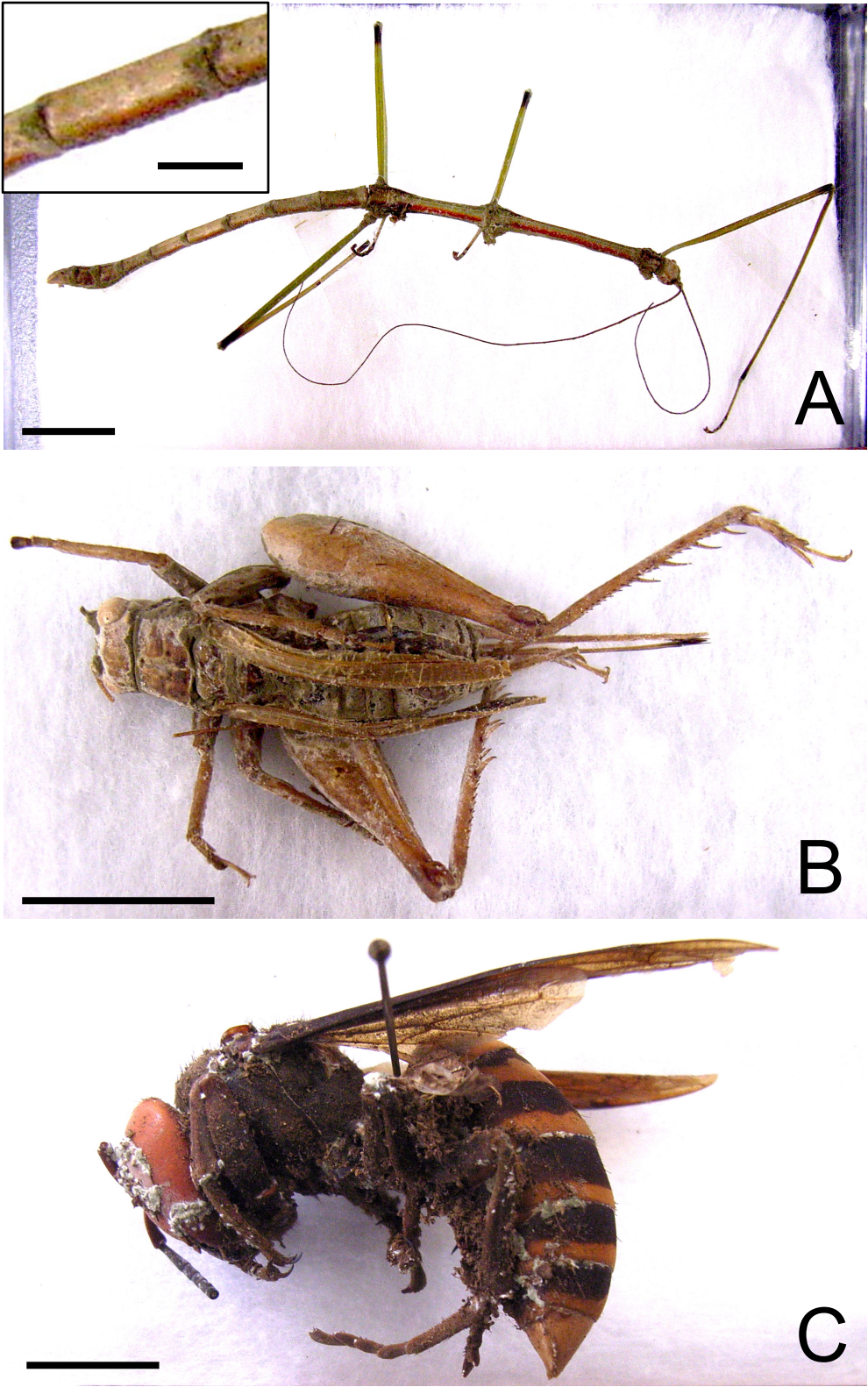
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Table 1 – The list of *Metarhizium* spp. isolated from insects in Japan.

Species	**Strains**	**Isolation sources (order: family)**	**Locations**	**Genbank Accession Nos.**
M. anisopliae	F907 [a]	Coleoptera	Okinawa	LC200443
	F1748 (NBRC 112627)	Coleoptera: Scarabaeidae	Miyako-jima island, Okinawa	LC200444
M. brunneum	F395 (NBRC 112628)	Coleoptera: Scarabaeidae	Nagano	LC188977
	F701 (NBRC 112629)	Coleoptera: Scarabaeidae	Unknown (Japan)	LC188978
	F702 (NBRC 112630)	Hymenoptara: Pamphiliidae	Unknown (Japan)	LC188979
	F709 (NBRC 112631)	Coleoptera: Scarabaeidae	Hokkaido	LC188980
	F805 (NBRC 112632)	Coleoptera: Scarabaeidae	Unknown (Japan)	LC188981
	F838 (NBRC 112633)	Coleoptera: Scarabaeidae	Hokkaido	LC188982
	F913 [a]	Coleoptera: Scarabaeidae	Unknown (Japan)	LC188983
	F3233 (NBRC 112634)	Homoptera: Cydnidae	Saga	LC188976
	F3239 (NBRC 112635)	Homoptera: Cydnidae	Fukuoka	LC200450
M. guizhouense	F570 [a]	Coleoptera: Scarabaeidae	Ibaraki	LC188985
M. koreanum	F1307-1 (NBRC 112636)	Homoptera: Tropiduchidae	Bonin islands, Tokyo	LC188956
M. lepidiotae	MAFF 235081	Coleoptera: Scarabaeidae	Fukuoka	AB807799
	MAFF 245742	Coleoptera: Curculionoidea	Fukuoka	LC188992
	F1013 [a]	Coleoptera: Scarabaeidae	Hiroshima	LC200449
M. majus	F940 (NBRC 112637)	Phasmatodea	Ibaraki	LC188986
	F2037 (NBRC 112638)	Orthoptera: Glyllidae	Okinawa	LC188988

	F2449 (NBRC 112639)	Phasmatodea: Phasmatidae	Hiroshima	LC188987
	F2623 (NBRC 112640)	Orthoptera: Glyllidae	Okinawa	LC200445
	F199 [a]	Coleoptera: Scarabaeidae	Shizuoka	LC200447
	MAFF 245743	Coleoptera: Scarabaeidae	Ibaraki	LC200446
	MAFF 635010	Phasmatodea: Phasmatidae	Ibaraki	LC200448
M. pemphigi	F1524 (NBRC 112641)	Hymenoptera: Tenthredinidae	Iwate	LC188955
	F1581 (NBRC 112642)	Hymenoptera: Formicidae	Unknown (Japan)	LC188954
	MAFF 245744	Hymenoptera: Vespidae	Ibaraki	LC188994
M. pingshaense	ARSEF 1009	Orthoptera: Gryllidae	Unknown (Japan)	LC188962
	F181 [a]	Lepidoptera: Noctuidae	Saitama	LC188966
	F200 (NBRC 112643)	Coleoptera: Scarabaeidae	Shizuoka	LC200439
	F278 (NBRC 112644)	Orthoptera: Gryllidae	Saitama	LC188969
	F500 (NBRC 112645)	Orthoptera: Gryllidae	Ibaraki	LC188970
	F501 [a]	Coleoptera: Cerambycidae	Ibaraki	LC188971
	F601 (NBRC 112646)	Coleoptera: Scarabaeidae	Ibaraki	LC188972
	F603 [a]	Coleoptera: Scarabaeidae	Ibaraki	LC200435
	F672 [a]	Orthoptera: Glyllidae	Ibaraki	LC188973
	F676 [a]	Orthoptera: Glyllidae	Ibaraki	LC188974
	F692 [a]	Coleoptera: Scarabaeidae	Okinawa	LC188975
	F927 [a]	Coleoptera: Scarabaeidae	Unknown (Japan)	LC188990
	F982 (NBRC 112647)	Diptera: Tabanidae	Unknown (Japan)	LC188991

	F998 [a]	Coleoptera: Scarabaeidae	Unknown (Japan)	LC188993
	F1062 (NBRC 112648)	Coleoptera: Scarabaeidae	Kagoshima	LC188960
	F1234 (NBRC 112649)	Coleoptera: Scarabaeidae	Ibaraki	LC188963
	F1370 [a]	Lepidoptera: Geometridae (pupa)	Hachijo-jima island, Tokyo	LC188964
	F1480 (NBRC 112650)	Coleoptera: Curculionoidea	Aomori	LC188959
	F1497 (NBRC 112651)	Coleoptera: Lucanidae	Nagano	LC188965
	F1582 (NBRC 112652)	Hymenoptera: Formicidae	Unknown (Japan)	LC200440
	F1584 (NBRC 112653)	Hymenoptera: Formicidae	Unknown (Japan)	LC200441
	F1726 (NBRC 112654)	Coleoptera: Scarabaeidae (adult)	Kanagawa	LC200438
	F2049 (NBRC 112655)	Homoptera: Cydnidae	Unknown (Japan)	LC188961
	F2407 (NBRC 112656)	Homoptera: Pentatomidae	Ibaraki	LC188967
	F2685 (NBRC 112657)	Hymenoptera: Vespidae	Ibaraki	LC188968
	F3234 (NBRC 112658)	Homoptera: Dinidridae	Ibaraki	LC188989
	F3238 (NBRC 112659)	Homoptera: Cydnidae	Fukuoka	LC200436
	MAFF 235083	Homoptera: Largidae	Fukuoka	LC200437
	MAFF 635007	Coleoptera: Curculionoidea	Ibaraki	LC200442
M. robertsii	F602 (NBRC 1126560)	Coleoptera: Elateridae	Ibaraki	LC188958
	MAFF 245745	Coleoptera: Scarabaeidae (adult)	Ibaraki	LC188957

[a] Living cultures were not available for 14 of these strains. The strains were not recovered either from glycerol stocks (–80 °C) or water stocks (7 °C). The DNA samples of these strains were prepared from the agar blocks of the glycerol stocks.

41 **1. Introduction**

42 The genus *Metarhizium* (Ascomycota: Hypocreales: Clavicipitaceae) is largely
43 composed of entomopathogenic fungi (Kepler et al. 2014). Most species produce green
44 conidia on the corpses of arthropod hosts and are known as “green muscardine fungus”
45 (Roberts and St. Leger 2004). This fungus has a global distribution and has been
46 isolated from more than 200 species in 17 families of insects and acari (Roberts and St.
47 Leger 2004; Zimmermann 2007). Many species have been isolated from soil where they
48 often show a close association with plant roots (Hu and St. Leger 2002; Nishi et al.
49 2011; Wyrebek et al. 2011). Species in this genus are used as biological control agents
50 for various pests in agriculture and forestry and insect vectors of human disease (e.g.,
51 Zimmermann 1993; Milner and Pereire 2000; Lomer et al. 2001; Scholte et al. 2005).
52 *Metarhizium* is one of the most important groups of entomopathogenic fungi for
53 commercially developed microbial pesticides: *M. anisopliae* sensu lato comprises
54 33.9% of microbial pesticides made of entomopathogenic fungi (Faria and Wraight
55 2007).

56 The current taxonomy of *Metarhizium* is based on multi-locus phylogenetic
57 DNA sequence analyses. *Metarhizium anisopliae* and *M. flavoviride*, which are
58 relatively common species in the genus, are currently recognized as species complexes,
59 comprised of 10 and six species, respectively, according to Bischoff et al. (2006, 2009),
60 Kepler et al. (2014), and Montalva et al. (2016). *Metarhizium pingshaense*, *M.*
61 *anisopliae*, *M. robertsii*, and *M. brunneum* comprise the *M. anisopliae* species complex,
62 which are called the PARB clade, and have particularly wide host ranges and global
63 distributions (e.g., Bischoff et al. 2009). These species comprised majority groups
64 among *Metarhizium* spp. isolated from soil in Brazil, Canada, Denmark, and Japan

65 (Nishi et al. 2011; Wyrebek et al. 2011; Rocha et al. 2013; Steinwender et al. 2014). On
66 the other hand, species placed outside the PARB clade are specialists or species that
67 have not been sufficiently characterized in terms of host-associations and geographical
68 distributions due to their scarcity (e.g., Bischoff et al. 2006, 2009; Kepler et al. 2014;
69 Keyser et al. 2015).

70 Improving our understanding of *Metarhizium* species diversity and the
71 association between each species and host insects is beneficial for pest management and
72 taxonomic studies. A phylogenetic analysis of DNA sequences is necessary to identify
73 *Metarhizium* spp. Bischoff et al. (2009) recommended the DNA sequence of the 5'
74 partial region of translation elongation factor 1 alpha (5TEF) for the molecular
75 phylogenetic species identification of *M. anisopliae* species complex. *Metarhizium* in
76 east Asia seem to be particularly genetically diverse because all known teleomorphs
77 collected to date are restricted to this area (Kepler et al. 2012), and the diversity of
78 *Metarhizium* soil isolates in Japan is larger than those in Brazil, Canada, Denmark, and
79 USA (Nishi et al. 2011; Wyrebek et al. 2011; Rocha et al. 2013; Steinwender et al. 2014;
80 Kepler et al. 2015; Keyser et al. 2015). Thus, investigating new isolates in eastern Asia
81 may help reveal additional diversity in this genus. Eight species have been isolated from
82 soil in Japan (Nishi et al. 2011). However, few insect-derived isolates in Japan have
83 been identified using molecular phylogenetic methods, despite that isolates from various
84 insects have been deposited at some research institutes. The *M. anisopliae* species
85 complex (MASC) and *M. flavoviride* species complex (MFSC) are highly diversified in
86 host insect species, distributions, habitat types, and emergence seasons, which make it
87 difficult to collect insect cadavers infected by the fungi efficiently by narrowing
88 investigation areas or periods. Thus phylogenetic analysis of *Metarhizium* isolates

deposited in culture collections in addition to ones collected in our investigation is a practical approach for a better estimation of their species diversity. In this study, molecular phylogenetic analysis of 5TEF was conducted for 57 fungal isolates of *Metarhizium* spp. that we have collected during the course of the investigation of entomopathogenic fungi in Japan and that we obtained from culture collections for improving our understanding of species diversity of MASC and MFSC and the association between each species and host insects.

2. Materials and methods

2.1. Fungal isolates

The fungal strains used in this study are listed in Table 1. Isolates used in the study are deposited in the fungal culture collections of the Agricultural Research Service Collection of Entomopathogenic Fungal Cultures (Ithaca, NY, USA) (ARSEF strains), the Forestry and Forest Product Research Institute (FFPRI, Tsukuba, Ibaraki, Japan) (F strains), and Genebank of the National Agriculture and Food Research Organization (NARO, Tsukuba, Ibaraki, Japan) (MAFF strains), the Biological Resource Center of the National Institute of Technology and Evaluation (NBRC, Kisarazu, Chiba, Japan) (NBRC strains). Living cultures were confirmed to have cylindrical or ellipsoidal conidia produced in chain from cylindrical or clavate phialides, which are typical morphological characteristics of MASC and MFSC. Living cultures were not available for 14 of the F strains; vital signs were not observed in cultures either from glycerol stocks (–80 °C) or water stocks (7 °C).

2.2. DNA extraction, amplification and sequencing

The DNA sequences of the 5′ partial region of translation elongation factor 1 alpha (5TEF) of the isolates were analyzed to identify the species, as recommended by Bischoff et al. (2006, 2009). Crude DNA samples were prepared as follows. Mycelia from a pure culture on potato dextrose agar medium (2.1% dextrose, 1.4% agar, 0.4% potato extract, and 0.03% chloramphenicol) (PDA) for 2 d or over were picked with a sterile micropipette tip and suspended in 50–100 µL of TE buffer containing RNase A (10 µM pH 8.0 Tris-HCl, 1 µM pH 8.0 EDTA, and 10 mg/mL RNase A). The crude DNA solutions were kept at −20 °C. The suspensions were frozen at least once before use in the PCR reaction. DNA samples from the 14 strains whose living cultures were not available were prepared from agar blocks of glycerol stocks preserved at −80 °C using the same method as that used for the living cultures.

PCR was performed in 10–20 µL reaction volumes comprising 0.1–10% (v/v) of crude DNA solution, 1× PCR buffer for KOD FX Neo (Toyobo, Osaka, Japan), 0.2 mM dNTPs, 20 µM of each primer, and 0.02 U/µL KOD FX Neo (DNA polymerase) (Toyobo). The EF1T (5′-ATGGGTAAGGARGACAAGAC) and EF2T (5′-GGAAGTACCAGTGATCATGTT) primer pair from Bischoff et al. (2006) was used for this reaction. The conditions of amplification were initial denaturation for 2 min at 94 °C, followed by 35–38 cycles of 10 s at 98 °C, annealing for 30 s at 50–56 °C, elongation for 50 s at 68 °C, and a final holding step for 2 min at 68 °C.

The PCR products were purified by polyethylene glycol precipitation. The nucleotide sequences of the PCR products were determined by a DNA sequencing

137 service (Eurofins Genomics, Tokyo, Japan) or by using a BigDye Terminator Cycle
138 sequencing kit (Applied Biosystems, Foster City, CA, USA) and an ABI 3100
139 automated sequencer (Applied Biosystems). The sequence data were deposited in
140 GenBank. The accession numbers of the DNA sequence used for the following
141 phylogenetic analysis are listed in Table 1 and Supplementary Table S1.
142
143 ***2.3. Phylogenetic analysis***
144
145 Multiple sequence alignment of the dataset was conducted using the default settings in
146 MUSCLE (Edgar 2005) attached to MEGA7.0 (Kumar et al. 2016). The 5TEF dataset
147 was composed of the 57 DNA sequences and 25 reference DNA sequences listed in
148 Supplementary Table S1. *Metarhizium novazealandicum* ARSEF 3056 were included in
149 the reference DNA sequences as outgroups for both MASC and MFSC. The alignment
150 length was 696 bp.
151 The most appropriate partitioning scheme for the DNA sequence data was
152 determined with the PartitionFinder program using the greedy search option (Lanfear et
153 al. 2012). The Bayesian Information Criterion was used to evaluate the partition scheme.
154 As a result, three partitions (intron, codon 1 + codon 2, and codon 3) with
155 GTRGAMMA was selected for the dataset.
156 Maximum likelihood analyses were conducted with the RAxML 7.4.4
157 program (Stamatakis 2006) using the selected partition scheme and models and support
158 for each branch was evaluated by 1,000 bootstrap replicates (Felesenstein 1985). The
159 resulting tree was edited with Figtree (ver. 1.4.2, Rambaut A., Institute of Evolutionary
160 Biology, University of Edinburgh, http://tree.bio.ed.ac.uk/software/figtree/). The results

161 of the multiple sequence alignment and the phylogenetic tree were deposited in
162 TreeBASE (http://treebase.org/treebase-web/home.html) as no. S19983.
163
164 **3. Results and Discussion**
165
166 The 5TEF phylogenetic analysis revealed that the 57 Japanese isolates belonged to
167 clades of nine known species (Fig. 1). We identified the 57 isolates as nine species
168 based on this result (Table 1). Among the nine species, seven and two species belonged
169 to MACS and MFSC, respectively. The nine species included eight species isolated
170 previously from soils in Japan by Nishi et al. (2011) and *M. koreanum*, which was
171 confirmed in Japan for the first time.
172 Among the nine species identified in this study, *M. pingshaense* was the most
173 frequently isolated, to which 29 isolates from six orders (14 families) of insects were
174 identified. *Metarhizium pingshaense* is also the most frequently detected species from
175 soils in Japan (Nishi et al. 2011). Thus, this high frequency of *M. pingshaense* in Japan
176 may be due in part to its ability to infect a wide range of insects. The second most
177 abundant species was *M. brunneum*, to which nine isolates from three orders (three
178 families) of insects were identified. Both *M. pingshaense* and *M. brunneum* were
179 members of the PARB clade, which is a group of generalist insect pathogens that are
180 morphologically indistinguishable from *M. anisopliae* sensu stricto (Bischoff et al.
181 2009). The high frequency of occurrence of these two species suggests that most of the
182 Japanese isolates that have been morphologically identified as *M. anisopliae* sensu lato
183 may actually be either *M. pingshaense* or *M. brunneum*. *Metarhizium anisopliae* sensu
184 stricto appears to have a small population or a very restricted distribution in Japan

185 because only two isolates from coleopteran insects and a soil isolate from Nishi et al.
186 (2011) were identified as this species and all were from the southwestern islands.
187 This study has revealed that two isolates from crickets and three isolates from
188 stick insects belong to the *M. majus* clade (Fig. 2). The *M. majus* clade is mainly
189 comprised of isolates from scarabaeid insects, some of which are clearly specialized to
190 their original hosts, such as isolates from coconut rhinoceros beetles and fruit beetles
191 (Ferron 1972; Nishi et al. 2015; Supplementary Table S2). *Metarhizium majus* has been
192 estimated to have diverged before the emergence of generalist species and has
193 intermediate host range between specialists and generalists (Hu et al. 2014). Thus, it
194 was unexpected that the cricket and stick insect isolates belonged to the *M. majus* clade.
195 These isolates may also be specialized to their hosts just like the isolates from
196 scarabaeid hosts. A similar example was also reported for a pathogen of a cockroach; *M.*
197 *blattodeae* was the only species isolated from cockroaches in MFSC and an isolate of
198 this species had pathogenicity against cockroaches (Montalva et al. 2016). The mean
199 dimensions of F2037 and F940 conidia produced on PDA were 5.7 × 2.6 (n = 20) and
200 5.7 × 2.7 (n = 20), respectively, which are clearly smaller than those of *M. majus*
201 isolated from scarabaeid insects, according to Nishi et al. (2015). These differences also
202 support differentiating these two isolates from other *M. majus* isolates. This discovery
203 indicates the necessity for reconsidering the phylogenetic relationships and host
204 preferences of the *M. majus* clade.
205 This study has revealed for the first time that hymenopteran insects are the
206 host of *M. pemphigi* (Fig. 2). *Metarhizium pemphigi* have been isolated from root
207 aphids in Britain and bark beetles in Australia (Driver et al. 2000; Brownbridge et al.
208 2010; Supplementary Table S2). This species is distantly related to the PARB clade,

209 suggesting that it has a relatively restricted host range. The isolation of three *M.*
210 *pemphigi* from insects of three different hymenopteran families suggests that *M.*
211 *pemphigi* in Japan prefers hymenopteran insects, which distinguishes them from the
212 foreign isolates. However, the 5TEF DNA sequences of the three isolates were not
213 clearly differentiated from those of *M. pemphigi* from root aphids. The analysis of four
214 additional loci of F1524 and F1581 also did not support local differentiation of the
215 Japanese isolates (data not shown). A comparative virulence analysis is necessary for
216 further discussion of the possible differentiation of *M. pemphigi* in Japan.

217 An isolate from a plant hopper (Homoptera: Tropiduchidae) on the Bonin
218 Islands (F1307-1) was identified as *M. koreanum*. This is the first discovery of this
219 species in any country outside of Korea, where two isolates from brown plant hoppers
220 (*Nilaparvata lugens*) (Homoptera: Delphacidae) were identified. The similarity of the
221 host species in the two countries suggests that *M. koreanum* is specific to plant hoppers.
222 The Bonin Islands have been geographically isolated for a long time and many endemic
223 species have been discovered there including species of Tropiduchidae (Karube et al.
224 2011). Thus, *M. koreanum* on the islands may also be differentiated from *M. koreanum*
225 in Korea to be specific to its original host, although it is unknown whether the *M.*
226 *koreanum* host is an endemic species of the islands. Further research on *M. koreanum*
227 pathogenicity is important for the taxonomy of this species and for biological control of
228 the brown plant hopper because it is a serious rice pest in East Asia.

229 This study has reported new associations of *Metarhizium* spp. and host groups
230 in the two *Metarhizium* species complexes as well as a species first discovered in Japan.
231 Although it is unresolved how such host-pathogen associations discovered only in Japan
232 should be incorporated into the latest taxonomy, further investigation of the local

differences will be helpful when the species diversity of this genus is re-evaluated.

Disclosures

The authors declare no conflict of interest. All the experiments undertaken in this study comply with the current laws of Japan.

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Figure legends

Fig. 1 – A maximum likelihood phylogeny inferred from the analysis of the 5TEF of the

353 *Metarhizium anisopliae* species complex and *M. flavoviride* species complex. The
354 support values were obtained from 1,000 bootstrap replicates, and values > 70% are
355 indicated above or below branches. *Metarhizium novazealandicum* ARSEF 3056 was
356 used as an outgroup taxon.
357
358 Fig. 2 – Cadavers recognized as new host insects of *Metarhizium majus* and *M.*
359 *pemphigi*. A: *Phraortes illepidus* (Phasmatodea: Phasmatidae) (the isolation source of
360 *M. majus* F2449). The inset shows the higher magnification of the fourth abdominal
361 segment of the same cadaver. Masses of conidia of *Metarhizium* are observed between
362 the segments. B: *Cardiodactylus guttulus* (Orthoptera: Glyllidae) (the isolation source
363 of *M. majus* F2623). C: A species of Vespidae (Hymenoptera) (the isolation source of *M.*
364 *pemphigi* MAFF 245744). *Bars*: A–C 1 cm; A (inset) 2 mm.

Supplementary Table S1 Reference isolates for phylogenetic analyzes.

Species	Isolates	Isolation source (order)	Location	Genbank ID of TEF
M. acridum	ARSEF 324	Orthoptera	Australia	EU248844
	ARSEF 7486	Orthoptera	Niger	EU248845
M. anisopliae	ARSEF 7487	Orthoptera	Ethiopia	DQ463996
M. brunneum	ARSEF 2107	Coleoptera	USA	EU248855
	ARSEF 4179	soil	Australia	EU248854
M. flavoviride	ARSEF 2025	soil	Germany	KJ398804
M. frigidum	ARSEF 7445	Isoptera	Australia	KJ398818
M. guizhouense	ARSEF 6238	Lepidoptera	China	EU248857
	CBS 258.90	Lepidoptera	China	EU248862
M. koreanum	ARSEF 2038	Hemiptera	Korea	KJ398805
	ARSEF 2039	Hemiptera	Korea	KJ398806
M. lepidiotae	ARSEF 7412	Coleoptera	Australia	EU248864
	ARSEF 7488	Coleoptera	Australia	EU248865
M. majus	ARSEF 1914	Coleoptera	Philippines	KJ398801
	ARSEF 1946	Coleoptera	Philippines	EU248867
M. minus	ARSEF 1099	Hemiptera	Philippines	KJ398799
	ARSEF 1764	Hemiptera	Solomon Island	KJ398800
M. novazealandicum	ARSEF 3056	Coleoptera	New Zealand	KJ398810

M. pemphigi	ARSEF 6569	Hemiptera	United Kingdom	KJ398813
	ARSEF 7491	Hemiptera	United Kingdom	KJ398819
	CABI 177416	Hemiptera	United Kingdom	KJ398820
M. pingshaense	ARSEF 4342	Coleoptera	Solomon Island	EU248851
	CBS257.90	Coleoptera	China	EU248850
M. robertsii	ARSEF 727	Orthoptera	Brazil	DQ463994
	ARSEF 7501	Coleoptera	Australia	EU248849

Supplementary Table S2 List of insect-derived *M. pemphigi* and *M. majus* strains

Species	Strain	Location	Host	Reference
M. majus	ARSEF 297	Western Samoa	Coleoptera: Scarabaeidae: Dynastinae (*Xyloryctes jamaicensis*)	Nishi et al. (2015)
	ARSEF 978	France	Coleoptera: Scarabaeidae: Dynastinae (*Oryctes* sp.)	Bischoff et al. (2009)
	ARSEF 1015	Japan	Lepidoptera: Bombycidae (*Bombyc mori*)	Bischoff et al. (2009)
	ARSEF 1858	Poland	Coleoptera: Scarabaeidae (Scarabaeid species)	Bischoff et al. (2009)
	ARSEF 1914	Philippines	Coleoptera: Scarabaeidae: Dynastinae (*Oryctes* sp.)	Bischoff et al. (2009)
	ARSEF 1946	Philippines	Coleoptera: Scarabaeidae: Dynastinae (*Orycetas rhinoceros*)	Bischoff et al. (2009)
	ARSEF 2151	Indonesia	Coleoptera: Scarabaeidae: Dynastinae (*Orycetas rhinoceros*)	Nishi et al. (2015)
	ARSEF 3145	France	Coleoptera: Scarabaeidae: Dynastinae (*Orycetas rhinoceros*)	Nishi et al. (2015)
	ARSEF 4566	Australia	Coleoptera: Scarabaeidae: Rutelinae (*Anoplognathus* sp.)	Bischoff et al. (2009)
	ARSEF 7505	Australia	Coleoptera: Scarabaeidae: Rutelinae (*Anoplognathus* sp.)	Bischoff et al. (2009)
	Hn1	Japan	Coleoptera: Scarabaeidae: Cetoninae (*Protaetia orientalis*)	Nishi et al. (2015)
	PRC27	Ethiopia	Coleoptera: Scarabaeidae: Cetoninae (*Pachnoda interrupta*)	Ment et al. (2012)
M. pemphigi	ARSEF 6569	Britain	Homoptera: Apididae (*Pemphigus trehernei*)	Driver et al. (2000)
	ARSEF 7491	Britain	Homoptera: Apididae (*Pemphigus trehernei*)	Driver et al. (2000)
	CABI 177416	Britain	Homoptera: Apididae (*Pemphigus trehernei*)	Kepler et al. (2014)
	AgR F652	Australia	Coleoptera: Curculionidae (*Hylastes ater*)	Brownbridge et al. (2010)
	AgR F658	Australia	Coleoptera: Curculionidae (*Hylastes ater*)	Brownbridge et al. (2010)

Brownbridge M, Reay SD, Cummings NJ, 2010. Association of entomopathogenic fungi with exotic bark beetles in New Zealand pine plantations. *Mycopathologia* 169: 75–80.

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