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Environment-mediated vertical transmission fostered uncoupled phylogenetic relationships between longicorn beetles and their symbionts

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Running Head: Fungal symbionts of longicorn beetles

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ABSTRACT

The Coleoptera Cerambycidae (longicorn beetles) use wood under different states (living healthy, freshly snapped, completely rot, etc.) in a species-specific manner for their larval diet. Larvae of some Cerambycidae groups have mycetomes, accessory organs associated with the midgut that harbor fungal symbiont cells. The symbionts are thought to improve nutrient conditions; however, this has yet to be shown experimentally. To deduce the evolutionary history of this symbiosis, we investigated the characteristics of the mycetomes in the larvae of longicorn beetles collected in Japan. Lepturinae, Necydalinae, and Spondylidinae are the only groups that possess mycetomes, and these three groups' mycetomes and corresponding fungal cells exhibit different characteristics between the groups. However, the phylogenetic relationship of symbiont yeasts does not coincide with that of the corresponding longicorn beetle species, suggesting they have not co-speciated. The imperfect vertical transmission of symbiont yeasts from female to offspring is a mechanism that could accommodate the host-symbiont phylogenetic incongruence. Some Lepturinae species secondarily lost mycetomes. The loss is associated with their diet choice, suggesting that different conditions between feeding habits could have allowed species to discard this organ. We found that symbiont fungi encapsulated in the mycetomes are dispensable for larval growth if sufficient nutrients are given, suggesting that the role of symbiotic fungi could be compensated by the food larvae take. *Aegosoma sinicum* is a longicorn beetle classified to the subfamily Prioninae, which does not possess mycetomes. However, this species contains a restricted selection of yeast species in the larval gut, suggesting that the symbiosis between longicorn beetles and yeasts emerged before acquiring the mycetomes.

KEY WORDS

Longicorn beetle, fungi, yeast, symbiosis, mycetome, Lepturinae

53 **INTRODUCTION**

54

55 Heterotrophic animals use symbiotic microorganisms to improve their uptake
56 of nutrients. This kind of symbiosis is generally mutualism because host animals
57 provide a space for microorganisms to proliferate comfortably with essential nutrients,
58 while microorganisms support food digestion and supply nutrients (Lindsay et al., 2020;
59 Modolon et al., 2020). Through symbiosis, animals have found ways to adjust to
60 variable environments that are not always conducive to survival. Therefore, symbiotic
61 systems are crucial for understanding the flourishing of animals on Earth.

62 The wood-eating groups in the coleopteran insects, which comprise the most
63 prominent animal order, have significant relationships with fungi, particularly yeasts
64 (Vega and Blackwell 2005; Gibson and Hunter 2010; Hulcr et al., 2017; Biedermann
65 and Vega 2020). Many yeasts have been isolated from the frass, tunnel, and digestive
66 tubes of coleopteran larvae (Suh et al., 2005; Endoh et al., 2011; Davis et al., 2015). In
67 some groups, adults have a specific structure (the mycangium) that stores fungal cells
68 and passes them to the next generation during oviposition (Tanahashi et al., 2010;
69 Joseph et al., 2021). The fungi proliferate in the tunnel where larvae dwell and are
70 incorporated into the larval digestive tube. These fungi are consumed not only as part of
71 the larval diet (Toki et al., 2012) but are also thought to contribute to creating a suitable
72 condition for the larvae and assisting in the digestion of wood. Indeed, many yeasts
73 isolated from coleopteran larvae have cellulose-, cellobiose- and xylose-digesting
74 activities (Davis et al., 2015).

75 The Cerambycidae family (longicorn beetles) is a widespread coleopteran
76 group of wood-eating beetles. Larvae of longicorn beetles have a unique fungi-storing

77 organ, the mycetome, near the anterior end of the midgut (Schomann, 1937; Nardon and
78 Grenier, 1989; Grünwald et al., 2010). The mycetome consists of a mass of knob-shaped
79 sacs that attach to the outer membrane (serosa) of the gut. Mycetome sacs are filled with
80 nutritional particles, and fungal symbiont cells consisting primarily of yeasts dwell in
81 the lumen or mycetocytes (Jurzitza, 1960). The fungal cells are constantly transferred to
82 the gut lumen (Grünwald et al., 2010) and are thought to assist in the digestion of wood.
83 Although the mycetome seems essential for assisting the symbiosis with fungi,
84 its function is still obscure. Indeed, the mycetome is not conserved among all longicorn
85 beetle groups (Schomann, 1937). Therefore, this structure may have been acquired in
86 some groups that require an additional benefit from symbiont fungi to increase their
87 survival fitness, such as developing efficient digestion assisted by fungi. However, the
88 advantages that symbiont fungi confer to longicorn beetles still need to be resolved. To
89 deduce the evolutionary history of the symbiosis facilitated by the mycetome, we
90 investigated the distribution of this structure in various species from different
91 subfamilies of longicorn beetles in Japan. We inspected larvae from two families, six
92 subfamilies, 61 genera, 73 species, and 289 individuals of longicorn beetles. Species of
93 the subfamily Lepturinae are dominant among the species containing a mycetome;
94 however, some Lepturinae species do not have this structure. We found that the loss of a
95 mycetome is correlated with diet choice. The Japanese longicorn beetle groups/species
96 that had or that lacked a mycetome in our analysis were in good accordance with those
97 in a previous report of the presence/absence of symbiont yeasts in European species
98 (Schomann, 1937). *Aegosoma sinicum* belongs to the subfamily Prioninae, whose
99 members do not have a mycetome. We found that its larvae exhibit symbiosis with
100 limited yeast species classified into the genus *Scheffersomyces*, suggesting that

101 symbiosis with yeasts preceded the acquisition of mycetomes during the evolution of
102 Cerambicydae.
103
104 **MATERIALS AND METHODS**
105 ***Animals***
106 Most of the longicorn beetle larvae were collected from dead trees. We used
107 cultured larvae to analyze the species whose larvae are difficult to capture from the
108 wild. Their species names were identified by the cuticle structure on the thorax
109 (Cherepanov, 1988; Koiwaya, 2017). When the thoracic cuticle structure could not
110 identify species, genomic DNA was isolated from larval somatic tissues with a Wizard
111 Genomic DNA Purification kit (Promega), followed by PCR amplification of 28S rDNA
112 with ExTaq DNA polymerase hot start version (Takara-bio) and with the primers 5'-
113 GAACTTTGAAGAGAGAGTT-3' and 5'-CAGGCATAGTTCACCATCTT-3'. After
114 the PCR fragments were purified with the Qiaquick Gel Extraction kit (Qiagen), the
115 sequence was determined by the conventional Sanger method, which was used to
116 identify species with the aid of 28S rDNA sequence isolated from adults (Accession
117 number PP375410-PP375497). The larval midgut was surgically isolated with fine
118 tweezers in phosphate-buffered saline (PBS) to observe mycetomes and collect gut
119 materials. Frass was collected from the tunnel on wood with cotton swabs. Larvae in the
120 tunnel were isolated together with frass to identify species.
121
122 ***Identification of fungi***
123 Mycetome contents, gut materials, frass or fluid from adult genitalia were
124 spread on potato dextrose agar (PDA) plates (4-1126-05 Sani-Spec, Japan or 05709

125 Shimazu Diagnostics, Japan), including 15 μg/ml chloramphenicol and 50 μg/ml
126 ampicillin. 1L PDA agar broth contains 4g potato extract, 20g glucose, and 15g agar.
127 Eggs were immersed in PBS for a few minutes, and the supernatant was spread onto
128 PDA plates. The plates were incubated for a few days at 25ºC or longer at 4ºC. DNA
129 was extracted from yeast or yeast-like colonies by a microwave-based method (Izumitsu
130 et al., 2012) and was subjected to amplification of the internal transcribed spacer (ITS)
131 rDNA with ExTaq DNA polymerase hot start version (Takara-bio) and with the primers
132 5'-CTTGGTCATTTAGAGGAAGTAA-3' and 5'-TCCGTAGGTGAACCTGCGG-3'
133 according to the previous method (White et al., 1990; Gardes and Bruns 1993). In this
134 analysis, up to four colonies on a single plate were soaked together in 100 μl Tris-Cl
135 buffer if the colonies were available, before DNA extraction. Sequences were
136 determined from the amplified bands after purification with a Qiaquick Gel Extraction
137 kit (Qiagen). When sequences could not be determined due to the low quality of the
138 chromatographs, each colony was subjected to sequencing analysis separately. When the
139 mycetome contents did not yield colonies because the symbiont fungi were
140 unculturable, we isolated DNA from mycetomes with Isoplant II (Nippon-Gene)
141 according to the manufacturer's instructions, followed by PCR amplification and
142 sequencing of rDNA of fungal cells.
143 The identified rDNA sequences of fungal cells were compared with the
144 reported DNA sequences in the standard nucleotide collection (nr/nt) database using the
145 blastn program (Altschul et al., 1997). The species names of the source organisms of the
146 DNA sequence that exhibited the highest similarity in the blastn analysis are listed in
147 the "Blastn Best Hit" column in Table S1. We used the source organism name to refer to
148 the isolated fungal cells for further analyses. When an undescribed species (for example,

149 *Scheffersomyces* sp.) exhibited the best-hit, its strain identifier (such as strain Y13) is
150 referred. We have to note that it does not always guarantee the actual yeast species
151 because in many cases, our rDNA sequences did not exhibit a perfect match to a known
152 rDNA sequence (Table S1, "Blastn score" column).

153 The internal transcribed spacer (ITS) of *Scheffersomyces* sp. Y1052_SICYLG3
154 has one *Eco*RI restriction site. The yeasts isolated from adult genitalia of *Leptura*
155 *ochraceofasciata* females were identified by the presence of the *Eco*RI site. After PCR
156 amplification, the PCR fragments were digested by *Eco*RI, followed by agarose gel
157 electrophoresis.

158 Mycetome-possessing Lepturinae and mycetome-lacking longicorn beetle
159 groups are compared according to the number of larvae possessing symbiont yeasts, and
160 the number of yeast species characterized in these groups. We performed two-tailed
161 Chi-square test using R x64.4.1.2 and RStudio softwares (R Core Team 2021; RStudio
162 Team 2020). In the latter comparison, we counted the "Blastn Best Hit" names of yeasts
163 that formed colonies on PDA plates. *Encyclops olivaceus* and species in Necydalinae
164 and Spondylidinae have unculturable symbiont fungi in their mycetomes. On the other
165 hand, we collected only culturable yeasts from larvae in a mycetome-lacking group by
166 streaking gut contents or frass onto the PDA plates. For unbiased comparisons, the
167 identified unculturable fungi were excluded from these counts.

168

169 ***Phylogenetic analyses***

170 The sequences were aligned using the M-COFFEE program
171 (https://tcoffee.crg.eu/apps/tcoffee/do:mcoffee; Notredame et al., 2000; Wallace et al.,
172 2006). After removing the residues at the ends that seemed not to align properly due to

173 the differences in the lengths between the sequences, maximum likelihood trees were
174 constructed with the MEGA software version 11 (Tamura et al., 2021). Trees were
175 tested with 1,000 bootstrap pseudoreplicates. The aligned nucleotides used for
176 phylogenetic constructions are shown in Table S2.

177 The phylogenetic tree for Lepturinae beetles was constructed using 28S rDNA
178 sequence. The phylogenetic trees of the yeasts isolated from longicorn beetle larvae
179 were constructed as follows. Referring to the results of the blastn search, the ITS
180 sequences of the yeast from the Lepturinae, Spondylidinae, and *Aegosoma sinicum*
181 larvae were used for the phylogenetic tree construction. When a yeast species was
182 detected from more than one larvae of the same longicorn beetle species, only one
183 sequence was used for the analysis. The ITS sequences from *Hyphopichia rhagii*
184 (isolated from *Rhagium femorale* and *R. japonicum*), *Candida wanchemiae* (isolated
185 from *Anastrangalia scotodes*), and uncultured fungus clone OUT_F389 (isolated from
186 *Megasemum quadricostulatum*) are considerably shorter than those from other yeasts
187 and were removed from the analysis in view of their potential influence on alignment.
188

189 ***Culture of Cerambycidae larvae***

190 Our artificial diet was developed based on the work of Gardiner (1970). The
191 artificial diet Insecta FII (Nosan Corporation, Japan) prevents the growth of fungi with
192 the aid of anti-fungal chemicals that are not open by the company. Nine grams of
193 Insecta FII, 6 g of dead *Symplocos coreana* powder, and 15 g of cellulose (ISC powder)
194 were kneaded in the following liquid to create ISC paste: 30 or 45 ml of water, 30 or 45
195 ml of PDA dissolved in water, or 30 or 45 ml of 1x PDA suspended with one of two
196 yeasts, *Candida vrieseae* or *Scheffersomyces* sp. Y1052_SICYLG3. These yeasts were

197 cultured on PDA plates until colonies grew to cover the surface of the broth. The
198 colonies were recovered with a cotton swab, and suspended in PDA liquid so that the
199 liquid becomes cloudy. We did not quantify the suspended yeast cells. The yeasts were
200 expected to improve larval growth; however, the effect was questionable and was not
201 analyzed in detail. The ISC paste was spread in a polypropylene box and was
202 autoclaved (20 min at 105°C) for sterilization. Eggs, hatched larvae or larvae collected
203 from dead woods were placed on the autoclaved diet and were kept at room temperature
204 until observation. For the introduction of selected yeast to larvae, eggs were sterilized
205 for more than one day by placing them on sterilized ISC paste. The powder of PDA
206 broth was suspended in distilled water and kept until agar powder sank. Then the
207 supernatant was collected to obtain 1 x PDA liquid. A 70 mm x 30 mm x 2 mm
208 rectangular wood plate of *Symplocos coreana* was sterilized by autoclaving, then
209 applied with 1 x PDA liquid in which yeast cells were suspended by the same method as
210 ISC paste. Sterilized eggs were cultured on the yeast-applied wood plate until hatching.
211 Hatched larvae were collected and cultured in sterilized ISC paste for about one month
212 until they became sufficiently large for the surgical isolation of mycetomes.
213
214
215 **RESULTS**
216 ***Distribution of mycetomes in Japanese Cerambycidae***
217 To deduce the evolutionary history of the mycetome in longicorn beetles, we
218 investigated the distribution of mycetomes in the Japanese species (Fig. 1). The majority
219 of the cerambycid species we inspected representing the subfamilies Lepturinae,
220 Necydalinae, and Spondylidinae, had mycetomes near the anterior end of the larval

221 midgut (Fig. 1 and Table 1). There are group-specific characteristics in the shapes of
222 mycetomes. The mycetomes of most Lepturinae species (Fig. 1B-C) and all
223 Spondylidinae (Fig. 1D) adhered tightly to the serosa of the midgut. By contrast, the
224 large mycetomes of Necydalinae species were well separated from the serosa with a
225 connection through several thin tubes (Fig. 1E). The mycetomes of Lepturinae form a
226 single circular ring surrounding the serosa (Fig. 1B). In contrast, those of Spondylidinae
227 larvae form two narrower rings (Fig. 1D). The characteristics of the appearance of
228 mycetomes in Lepturinae and Spondylidinae agree with those in the previous report
229 (Buchner 1965).

230 We found mycetomes in 22 out of 31 species of Lepturinae and all 3 of the
231 examined Necydalinae species (Table 1). *Paragaurotes doris*, *Japanocorus*
232 *caeruleipennis*, *Brachyta bifasciata,* and all *Pidonia* lacked mycetomes (Fig. 1F and
233 Fig. 2). In these cases, the mycetomes were probably lost secondarily during speciation.
234 Among the mycetome-lacking Lepturinae, larvae of *B. bifasciata* eat the roots of living
235 *Paeonia* (Table S1, "Feeding habit" column). All of the other mycetome-lacking
236 Lepturinae are inner bark-eaters at the larval stage. Inner bark-eating Lepturinae do not
237 necessarily lack mycetomes; two *Rhagium* species are inner bark-eaters but have
238 mycetomes. We found mycetomes in all examined Spondylidinae species (n=5; Table
239 1).

240

241 ***Characteristics of yeasts or yeast-like fungi in mycetomes***

242 We found that mycetomes and fungi dwelling in them had group-specific
243 characteristics. Each lobe of the Lepturinae mycetome contains tear-shaped fungal cells,
244 a membranous structure, and particles much smaller than yeast cells (Fig. 3A and B,

245 B'). The density of yeast cells in a mycetome is usually not high, and the cells do not
246 fully occupy the lumen. When cultured on potato dextrose agar (PDA) plates, almost all
247 of the fungi from the mycetomes of Lepturinae larvae formed colonies (Fig. 3C), and
248 their rDNA sequences indicated that they were yeasts.

249 We classified yeasts isolated from the Lepturinae mycetome or gut by
250 determining the sequence of the ITS site of mitochondria rDNA (Table S1). These
251 rDNA sequences were used as queries of blastn searches to estimate the species of
252 yeasts (Altschul et al., 1997). In the following section, we refer to the characterized
253 yeasts as the species exhibiting the highest similarity to the rDNA sequences in the
254 blastn analyses for simplicity.

255 In almost all cases, only a single yeast species was detected from the mycetome
256 of a Lepturinae larva (87%, n=109/125; Table 1). However, seven larvae had more than
257 one yeast species in the mycetome (rows 6, 8, 73-76, 107 in Table S1). Moreover, the
258 rDNA sequence derived from the mycetomes of single larvae exhibited nucleotide
259 variations in three cases (rows 10, 23, and 119 in Table S1). These results suggest that
260 the yeast cells in mycetomes are not always clones. There are species-specificities
261 between longicorn beetles and yeasts. For example, two Japanese *Rhagium* species had
262 a yeast whose rDNA exhibited the best nucleotide sequence similarity to that of
263 *Hyphopichia* (or *Candida*) *rhagii*, also found in a *Rhagium* species in Germany
264 (Grünwald et al., 2010).

265 The phylogenetic analysis (Fig. 4) classified the yeasts isolated from
266 Lepturinae mycetomes into eight species or unidentified species (*Scheffersomyces*
267 *ergatensis*, *Scheffersomyces paraergatensis*, *Scheffersomyces coipomoensis*,
268 *Scheffersomyces* sp. Y1052_SICYLG3, *Scheffersomyces* sp. Y6, *Scheffersomyces* sp.

269 Y13, *Candida tenuis*, *Candida vrieseae*). Two indentified species (*Scheffersomyces* sp.
270 Y6 and Y13) and *S. paraergatensis* are included into the branch of *S. ergatensis*. We did
271 not include two species (*Hyphopicha rhagii* and *Candida wanchemiae*) in this analysis
272 due to their short ITS sequences. In total, ten culturable yeast species were isolated from
273 22 mycetome-possessing Lepturinae species, suggesting that some yeast species were
274 shared among different Lepturinae species (Table 1, Fig. 2, and Fig. 4). However, the
275 Lepturinae species that share the same symbiont yeast are not always phylogenetically
276 close to each other (Fig. 2). For example, the larvae of *Leptura ochraceofasciata*
277 isolated from both hardwoods and conifers almost always had *Scheffersomyces* sp.
278 Y1052_SICYLG3 (Table S1). This yeast was also found in the larvae of *Caraphia*
279 *lepturoides*, although *L. ochracerofasciata* and *C. lepturoides* are phylogenetically
280 distant. Larvae of *Pyrrhona laeticolor*, *Ohbayashia nigromarginata*, *Ephies japonicas*,
281 *Pigostrangalia dulcis,* and *Mimostrangalia kurosonensis* had the yeast related to
282 *Candida vrieseae* in their mycetomes. All of these Lepturinae species use the hardwood
283 group Symplocaceae as their larval hosts. This was not due to the ubiquity of *C.*
284 *vrieseae* in the dead Symplocaceae tree since larvae of the stag beetle *Aesalus asiaticus*
285 isolated from a dead *Symplocos coreana* together with *Pyrrhona laeticolor* had a
286 different yeast (*Scheffersomyces ergatensis*) in their digestive tube (Table S1, stag
287 beetle). However, the relationships between yeast species and host plants of longicorn
288 beetles are not expected to be rigid since *Anastrangalia scotodes* and *Strangalia*
289 *koyaensis* use several conifers as hosts possessed *Candida vrieseae* (Table S1 and Fig.
290 4).

291 The characteristics of the mycetome contents of the Lepturinae species
292 *Encyclops olivaceus* and the three examined Necydalinae species were quite different

293 from those of the other Lepturinae species. *Encyclops* and Necydalinae mycetomes were
294 fully occupied by fungal cells, and we could not find a membranous structure (Fig. 3).
295 The elongated fungal cells in these Necydalinae mycetomes were obviously larger than
296 the yeast cells of Lepturinae, and the Necydalinae symbionts did not form a colony on
297 PDA plates. The unculturable symbionts of *Encyclops* are similar in shape but smaller
298 than those of Necydalinae species (Fig. 3E). All analyzed larvae of Necydalinae species
299 are dependent on dead hardwood trees (Table S1). To further determine whether the
300 symbiont characteristics are shared among Necydalinae species, we examined the
301 symbiont of *N. sachalinensis*, which is dependent on the dead part of living conifers.
302 Because its larvae were unavailable, we isolated fungal cells from the oviducts of *N.*
303 *sachalinensis* females. The fungal cells had the same characteristics as those of the
304 fungi isolated from the other Necydalinae larvae (Fig. 3H' and Table S3).
305 We attempted to characterize the fungi from *Encyclops olivaceus* and
306 Necydalinae mycetomes by directly isolating DNA from their mycetomes. The DNA
307 fragments amplified from their mycetomes did not show sufficient similarity to those of
308 any known fungi (Table S1). The DNA fragments from *Encyclops olivaceus* and
309 *Necydalis sachalinensis* exhibited homology to the genome sequences of the other
310 Coleopteran species (Table S1 and S3), remaining the possibility that these DNAs were
311 not derived from the symbionts. Because Lepturinae symbiont yeasts yielded DNA
312 bands specific to them by the same methods, these results confirm that the symbionts of
313 the *Encyclops olivaceus* and Necydalinae species are distinct from yeasts.
314 The contents of Spondylidinae mycetomes were similar to those of typical
315 Lepturinae in that it contained membranous structures, and the density of fungal cells
316 was not high (Fig. 3J). The shape of Spondylidinae fungal cells was rod-like (Fig. 3K-

317 K'). None of these fungi made a colony on PDA plates (Fig. 3L). Unlike Necydalinae,
318 Spondylidinae fungal cells are classified into the yeast group
319 *Debaryomyces*/*Schwanniomyces* (Table S1 and Fig. 4). These yeasts derived from
320 Spondylidinae form a single clade in the phylogenetic tree of the yeasts isolated from
321 mycetome-positive Lepturinae/Spondylidinae (Fig. 4). Therefore, there is broad group-
322 specificity in the choice of symbiont fungi when Lepturinae, Necydalinae, and
323 Spondylidinae are compared. However, when viewed from the species level, Lepturinae
324 longicorn beetles' phylogenetic relationships do not reflect in those of the symbiont
325 yeasts (Fig. 2). This incongruence is our next target to address.
326
327 ***Imperfect delivery of yeasts across generations***
328 It has been suggested that the maternal parent provides the symbiont fungi in
329 the larval mycetome (Schomann, 1937; Douglas, 1989). We characterized yeasts
330 isolated from eight species of Lepturinae adult females. Although not perfectly matched,
331 the adult females had yeast species isolated from the same species of larvae (Fig. 5;
332 Table S3), confirming the vertical transfer of symbionts. We found that yeasts in the
333 female genitalia could be easily excreted by gently pushing the oviduct. This suggests
334 that yeasts are smeared around the eggs during oviposition, as previous studies
335 described (Schomann, 1937; Douglas, 1989). Hatched larvae may incorporate yeasts
336 with egg shells and nearby wood into their gut. Indeed, 1st instar larvae of *Ohbayashia*
337 *nigromarginata* and *Necydalis formosana* a few days after hatching already had
338 mycetome-like knobs at the anterior midgut and fungal cells in the structures (Fig. 6).
339 This process of yeast delivery does not guarantee the accurate transmission of
340 maternal yeasts to their descendants because larvae that accidentally move from the

341 oviposited area before yeast uptake may have a chance to incorporate a different yeast
342 species from the environment. For example, larvae of *L. ochraceofasciata* almost
343 always have *Scheffersomyces* sp. Y1052_SICYLG3; however, we found a single larva
344 with the yeast *Candida vrieseae* (Table S1). This individual was found in a dead tree of
345 Symplocaceae. As we stated above, Lepturinae species that depend on this tree group
346 usually have *Candida vrieseae*, and the larva of *L. ochraceofasciata* is suspected of
347 having accidentally incorporated *Candida vrieseae* supplied by another Lepturinae
348 species. We also found two larvae of *L. ochraceofasciata* and *P. laeticolor*, and five
349 larvae of *Ohbayashia nigromarginata* that had the yeast *Scheffersomyces coipomoensis*
350 (Table S1). These larvae were isolated from one dead tree of *Symplocos coreana* at Mt.
351 Amagi. We isolated *Scheffersomyces coipomoensis* from yellowish dead trees of
352 *Symplocos coreana* on the same mountain, suggesting that this yeast dwells in the
353 environment and could have been accidentally incorporated into the larvae.
354 If Lepturinae larvae could incorporate yeasts from environment, it would be
355 possible to generate yeast-free larvae or larvae having a yeast species different from that
356 dominantly possessed by larvae in the wild populations, by removing maternally
357 supplied yeasts. To show this, we developed a way to culture larvae under a condition
358 chemically suppressing fungal growth. After oviposition, we placed eggs on an artificial
359 diet containing anti-fungal chemicals for sterilization, so that the hatched larvae did not
360 come into contact with external yeasts during and after birth. Larvae of *Leptura*
361 *ochraceofasciata* and *Stictoleptura dichroa* grew well under this condition (Fig. 7A).
362 They had brownish mycetomes near the anterior end of the midgut (Fig. 7B and D). The
363 position of their mycetomes was identical to those of larvae from the wild. Microscopic
364 observations showed that these brown mycetomes did not include yeasts (Fig. 7C and

365 E), which was confirmed by culturing mycetome contents on PDA plates (Fig. 7C,
366 inset), suggesting that the yeasts needed to be supplied externally for larvae to acquire
367 yeasts in their mycetomes. This experiment also indicates that mycetomes can develop
368 without yeasts.

369 In the above culture experiments, some of the larvae had yeasts not found in
370 the mycetomes of larvae isolated from wild populations. In the experiment with *L.*
371 *ochraceofasciata*, we discovered that a few larvae (12.5%, n=2/16) had *Meyerozyma*
372 *caribbica*. *Meyerozyma* is an indigenous yeast group arising in our laboratory. In the
373 experiment using *S. dichroa*, a portion of eggs hatched on the wood onto which their
374 mothers had laid eggs before sterilization. We cultured the pre-hatched eggs and 1st
375 instar larvae in the same container. After one month, we observed that the larvae were
376 divided into three groups: larvae with yeast-free mycetomes (n=5; Fig. 7D and E),
377 larvae with mycetomes that included yeast cells with a low density (n=4; Fig. 7F and
378 G), and larvae with relatively yeast-rich mycetomes (n=9; Fig. 7H and I). The yeast
379 species isolated from the yeast-poor mycetomes differed from those isolated from yeast-
380 rich mycetomes: the former was *Meyerozyma caribbica,* while the latter was
381 *Scheffersomyces ergatensis*. *S. ergatensis* is the default species of this longicorn beetle
382 in the wild (Table S1). We suspected that the larvae with *S. ergatensis* hatched on the
383 egg-laid wood and had a chance to incorporate maternally gifted yeast.

384 To investigate the above hypothesis about environmental yeast acquisition, we
385 next tested whether we could deliver a specific yeast species into mycetomes. In this
386 experiment, sterilized eggs were placed on a wood plate to which a yeast species was
387 applied. After birth, we transferred the larvae to a plate with the artificial diet to avoid
388 further yeast incorporation during growth until the larvae reached a sufficient size to

389 isolate mycetomes. We examined 33 larvae, and 22 had the given yeast species (Table
390 S4). These yeasts were never detected in the control groups to which we did not provide
391 the yeasts (0%, n=50), suggesting that this experiment successfully replaced the
392 symbiont yeast with the arbitrary yeast. We concluded from these results that Lepturinae
393 longicorn beetles acquire yeasts externally after hatching. Although the yeasts are
394 usually maternally supplied, a different yeast could be obtained from the environment.
395

396 ***Lepturinae mycetomes protect symbionts from anti-fungal chemicals in food***

397 During the culture of longicorn beetle larvae with the artificial diet, we found
398 that the mycetomes of Lepturinae species protect the symbionts from the anti-fungal
399 chemicals in the artificial diet. A larva of *Etorofus vicaria* and *Leptura ochraceofasciata*
400 maintained their symbiont yeasts in the mycetomes even after culturing for about one
401 year (Fig. 8A and Table S5). By contrast, symbionts of *Necydalis formosana* larvae
402 were eliminated from their mycetomes (Fig. 8B) even though they increased their
403 weight during the culture (Table S5). These results suggest that the mycetome of this
404 species is weaker in protecting symbionts from chemicals taken with the food.
405

406 ***Yeast transmission between generations in the longicorn beetles without mycetomes***

407 There is an association between mycetome-possessing Lepturinae longicorn
408 beetles and yeast species. Our next question is how their symbiosis started, and notably,
409 how mycetomes contributed to the evolution of the symbiosis. To address these
410 questions, we investigated yeasts in the digestive tubes and frass of mycetome-lacking
411 longicorn beetles. We observed 135 larvae of mycetome-lacking longicorn beetles
412 (including Lepturinae) corresponding to 46 species from five families/subfamilies

413 (Table 1). Yeasts were detected in 46% (n=63/135) of larvae. This score is significantly
414 low compared to that of mycetome having Lepturinae (92%, n=116/125, $p<0.001$),
415 suggesting that mycetomes have a role in stabilizing symbiosis with yeasts. We isolated
416 in total 45 culturable yeast species from 46 mycetome-lacking longicorn beetle species
417 (Table 1). This score is two times more than mycetome-having Lepturinae species (10
418 out of 21 excluding *Encyclops olivaceus* whose symbiont is unculturable; $p<0.001$),
419 suggesting that yeasts habiting in the mycetome-lacking species are more variable than
420 mycetome-possessing species. These wobbles could be explained if mycetome-lacking
421 longicorn beetles do not usually possess a way to limit the species of symbiont yeasts,
422 such as a delivery system between generations.

423 We found an exceptional mycetome-lacking species that could transmit specific
424 yeasts between generations. *Aegosoma sinicum* is a kind of Prioninae that does not have
425 mycetomes (Fig. 1G). Two yeast groups belonging to the genus *Scheffersomyces* were
426 repeatedly isolated from larvae of *A. sinicum* collected in different locations and from
427 different host trees (Table S1 and Fig. 9), suggesting a strong correlation between *A.*
428 *sinicum* and the yeasts. Individual *A. sinicum* larvae exclusively possessed either of the
429 two *Scheffersomyces* groups.

430 Two stand out among the possible explanations for the relationship between *A.*
431 *sinicum* and *Scheffersomyces*. The first is that *A. sinicum* females might lay eggs on the
432 host trees in which the yeasts habit. The second possibility is that the yeasts were
433 transferred from adult females to their progeny. We isolated the colonies of the same
434 *Scheffersomyces* yeasts from the genitalia of females and the egg surfaces of *A. sinicum*
435 (Table S2). Therefore, it is likely that *A. sinicum* vertically transfers yeasts between
436 generations without mycetomes, although we cannot rule out the first possibility.

DISCUSSION

Many dead wood-eating beetles depend on yeasts or related fungi at their larval stage (Davis, 2015). Although the role of this symbiosis has yet to be fully understood, its suspected function is to increase efficiency in the nutrient absorption from dead wood that is less nutritious than living plants. Larvae of longicorn beetles, which form one of the most diversified groups in the Coleoptera, evolved a characteristic fungi-storing organ, the mycetome, at the midgut (Schomann, 1936; Buchner, 1965). Among six subfamilies of Cerambycidae in Japan, the species in Lepturinae, Necydalinae, and Spondylidinae have mycetomes. The Japanese species in Disteniidae, which is a sister group of Cerambycidae, does not have the mycetome, confirming the previous report (Svacha and Lawrence 2014). Svacha and Lawrence (2014) also reported that the larvae in the families Vesperidae and Oxypeltidae do not have mycetomes. Because mycetome-absent families are dominant in longicorn beetles, mycetome was likely to have evolved in the subfamilies of Cerambycidae. If this is true, the mycetome emerged at least twice in Spondylidinae and Lepturinae/Necydalinae according to the phylogenetic tree of the Cerambycidae subfamilies (Fig. 1; Haddad et al., 2018; Nie et al., 2021). The emergence of the mycetome in Lepturinae and Necydalinae is likely to have been the single event at the root of these groups. This is supported by the recent phylogenetic study suggesting that Necydalinae is regarded to be included in Lepturinae (Nie et al., 2021). However, we cannot rule out the possibility that these mycetomes were evolved independently because Necydalinae mycetomes have characteristics that are not seen in Lepturinae mycetomes.

461 The symbionts of Necydalinae species and that of *Encyclops olivaceus* are
462 unculturable and fully occupy the lumens of the mycetomes. The shared characteristics
463 of the mycetomes and symbionts among *Encyclops* and Necydalinae species suggest
464 that they are phylogenetically related. The previous study showed that the Lepturinae
465 species *Oxymirus cursor* has gigantic mycetomes fully occupied with slender tear-
466 shaped and unculturable symbiont fungal cells (Buchner 1965). These described
467 characteristics are also similar to those in Necydalinae. The phylogenetic relationships
468 between Necydalis and these Lepturinae species should be considered in future studies.
469 The emergence of mycetomes in Spondylidinae and Lepturinae/Necydalinae
470 suggests that the acquisition of this organ is beneficial for these groups. The benefits of
471 this symbiosis need to be considered from the respective perspectives of the symbionts
472 and longicorn beetles. Establishing symbiotic relationships between Cerambycidae
473 through mycetomes would have apparent advantages for fungi. Our study and previous
474 studies have shown that the mycetomes of some species include fungi that cannot
475 proliferate on a PDA plate, although such a plate is sufficient for many yeasts to grow
476 (Müller, 1934). From this result, one of the functions of the mycetome is to provide
477 fungi with nutrients necessary for their proliferation.
478 Moreover, we here showed that the mycetomes of larval Lepturinae possessed
479 yeast cells even when the larvae ate an artificial diet containing anti-fungal chemicals,
480 which can inactivate yeast cells by overnight incubation, suggesting that another role of
481 the mycetome is to protect fungi from the chemicals that could be included in plants or
482 secreted from competitive fungi. Unlike larvae of the species in Lepturinae, *Necydalis*
483 *formosana* larvae lost symbionts from their mycetomes when cultured with a diet
484 containing anti-fungal chemicals, suggesting that the mycetomes of Lepturinae and

485 Necydalinae are functionally and structurally different. In most cases, a single yeast
486 species occupies the mycetome of a larva, even though the larvae have a chance to
487 incorporate external yeasts or yeast-like fungi that are spontaneously present in dead
488 trees. Therefore, the mycetome can protect symbiotic fungi from competition with other
489 fungi.

490 In contrast to the advantages for fungi, the benefits conferred to the longicorn
491 beetles remain obscure. Because the mycetome is a longicorn beetle-derived organ and
492 fungi are not necessary for its formation, symbiosis with fungi is advantageous for
493 longicorn beetles. It has been proposed that symbiont fungi are nutritionally beneficial
494 for host beetles, as in the case of many wood-eating insects and their symbiotic gut
495 microorganisms (Gibson and Hunter, 2010). Our study provided evidence suggesting
496 that symbiosis is related to nutrition and/or feeding habits. All species in Spondylidinae
497 examined in this study and most of the mycetome-possessing species in Lepturinae eat
498 brown rot supwood. This shared food choice would have been made possible by the
499 acquisition of mycetomes. Some Lepturinae species do not have mycetomes, and the
500 loss of this organ occurred over several rounds of speciation (Fig. 2). The loss of the
501 mycetome is also strongly associated with the choice of diet. All mycetome-lacking
502 Lepturinae species eat inner bark or living roots. Inner bark and living roots have
503 different nutritional or organismal conditions from those in rot supwood. For example,
504 inner bark and living plant tissue contain more nutrients than supwood (Schowalter
505 1992). The change in diet preference may have enabled the Lepturinae species to be free
506 from mycetome-dwelling yeasts due to the improved nutrient uptake. When fed an
507 artificial diet, we also showed that mycetome-possessing Lepturinae larvae can grow
508 without symbiont yeasts. For this experiment, we used an artificial diet based on the

509 silkworm diet, which is much richer in nutrients than dead wood. Therefore, the species
510 having mycetomes do not require symbiont yeasts under such improved nutritional
511 conditions, which paradoxically supports the idea that the benefits from symbiont fungi
512 are associated with nutrients. To confirm more conclusively, we need to compare the
513 growth rate between larvae having mycetomes with or those having mycetomes without
514 yeast when fed a diet having low nutritional value. Our method of obtaining larvae with
515 yeast-free mycetomes will assist in future experiments.

516 Among the Cerambycidae group, many Lamiinae and Cerambycinae species
517 are known to eat inner bark of fresh dead trees or live trees (Svacha and Lawrence
518 2014). The species in Lamiinae and Cerambycinae do not have mycetomes, probably
519 because their diets are more nutritive than those of mycetome-possessing groups.
520 Lamiinae is the sister group of Spondylidinae, which is dominant in mycetome-
521 possessing species. Unlike Lamiinae, Spondylidinae longicorn beetles in Japan depend
522 on brown rot supwoods (Table S1). The symbiosis strategy with fungi in these two
523 groups may have differed in their diet choices after the split. We have to note that the
524 tendency of diet choice is not always supported since several mycetome-lacking
525 Prioninae (such as *Psephactus remiger*), Cerambycinae (such as *Thranius variegatus*,
526 *Paraclytus excultus*, *Xylotrechus cuneipennis*) and Lamiinae (such as *Nanohammus*
527 *rufescens*) species eat strongly rot wood or outer bark. These species may have another
528 way to strengthen their nutritional condition, including establishing symbiosis with
529 another microorganism group, such as bacteria, as reported previously (Grünwald et al.,
530 2010; Reid et al., 2011).

531 In our experiments, mycetome-lacking larvae of longicorn beetles tended not to
532 have yeast in their gut compared to those in mycetome-possessing larvae (Table 1),

533 suggesting that many of mycetome-lacking species do not transfer yeasts between
534 generations. When yeasts were found in these larvae, the assortment of yeast species
535 usually varied among individuals. A possible explanation is that mycetome-lacking
536 larvae uptake yeasts from their environment, and yeast species that can withstand larval
537 digestive systems proliferate in the gut. Such environment-dependent acquisition of
538 yeast is likely to be the ancestral form of the symbiosis between longicorn beetles and
539 yeasts, as was suggested previously (Douglas, 1989), and the external delivery of yeasts
540 observed in mycetome-possessing species is probably the remnant of this ancestral
541 symbiosis. Because the mycetome-lacking longicorn beetle *A. sinicum* can share a
542 limited number of yeast species between generations, the emergence of mycetomes is
543 not requisite for longicorn beetles to initiate symbiosis with specific yeasts.
544 Because mycetome-possessing longicorn beetles tend to have a single preferred
545 yeast or fungal species/group as their symbiosis partner, mycetomes could limit the
546 symbiont to a specific species. This role of mycetomes should be in concert with the
547 development of the sac in the female genitalia that stores symbiont yeasts (Schomann,
548 1937; Buchner, 1965). With the aid of the mechanism of vertical transmission, the
549 mycetomes and symbionts have group-specific features. Larvae of the species in
550 Lepturinae tend to have yeasts culturable on the PDA plate and the yeast cells do not
551 fully occupy the lumen of the lobes of mycetomes. Larvae of the species in
552 Spondylidinae have elongated yeasts unculturable on the PDA plate. Larvae of the
553 species in Necydalinae have unculturable fungal cells that are larger than the yeast cells
554 in Lepturinae. The Necydalinae mycetomes are well-developed whose lumen is fully
555 occupied by the fungal cells. The presence of these group-specific characteristics
556 suggests that some limitations worked to prevent the loss of these characteristics even as

speciation occurred within these groups. It is possible that the constraint is associated with group-specific diet choices. These group-specific diet choices include the hardness/softness and degree of moisture of rotten wood, and dependency on specific fungal infestation (Svacha and Lawrence, 2014).

However, our data showed that the vertical transmission of symbionts between generations is imperfect, and larvae sometimes have a different yeast from the symbiont species. These exceptions could be explained by somewhat imperfect manner of yeast transmission between generations. Moreover, this accidental "mismatch" could disturb the phylogenetic relationship between symbiont yeasts and longicorn beetle species. During the speciation of longicorn beetles, the acquisition of symbiont fungi from the environment allowed the diversification of symbiont yeast species that is not always associated with longicorn beetle speciation. We found that the Lepturinae species using Symplocaceae as their host predominantly have the symbiont yeast related to *Candida vrieseae*, even though these Lepturinae species are not monophyletic. Probably the use of the same dead tree promoted the sharing of the yeast species between the beetle species. The mismatch between the phylogenies of host beetle species and symbiont yeasts has also been reported in stag beetles (Kubota et al., 2020), suggesting that this incongruent evolution is shared among Coleopteran groups. Such incongruent evolution was probably permitted because symbiont yeasts have similar physiological characteristics and because the function of yeasts may be to provide inessential dietary supplementation, which may have been dispensable when longicorn beetles switched their food from dead wood to inner bark.

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COMPETING INTERESTS

The authors have no competing interest to declare.

AUTHOR CONTRIBUTIONS

YS, NY, KM, and MN conceived and designed the study. YS and MN performed most experiments. NY, and KM performed sampling. JY provided the phylogenetic analyses. TH performed molecular experiments. YS wrote the manuscript with the aid of the other authors.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online.

Table S1.

Symbiont fungi isolated from larvae of longicorn beetles in Japan. Each row corresponds to the results of a single larva. "Culture" indicates the cultured larvae used for the analyses. The digits at the columns of "*Aegosoma sinicum*" correspond to those in Figure 9. The numbers of colonies on the plates are indicated when a few colonies appeared.

605

606 **Table S2.**

607 Alignment of nucleotide sequences used for phylogenetic tree construction.

608

609 **Table S3.**

610 Symbiont fungi isolated from female adults of longicorn beetles in Japan. Each row

611 corresponds to the results of a single female.

612

613 **Table S4.**

614 Artificial transfer of yeasts to larvae. The same Exp number indicates the experiment

615 using the eggs from the same female individual.

616

617 **Table S5.**

618 Possession of symbiont cells in the mycetome after long culture in the artificial diet

619 with anti-fungal chemicals.

620

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FIGURE LEGENDS AND TABLE CAPTIONS

Fig. 1.

Detection of mycetomes in Japanese cerambycid larvae. **(A)** The phylogenetic relationships of Japanese longicorn beetle families/subfamilies. Red circles indicate groups including mycetome-possessing species. The number of species examined is shown in parentheses. This phylogenetic tree follows that of Hadded et al. (2018), although the branch length does not reflect phylogenetic distances. **(B)** Mycetomes (arrow) on the anterior midgut serosa of the Lepturinae *Caraphia lepturoides*. The anterior is toward the top. Scale bar, 500 μm. **(C)** Mycetomes of *Encyclops olivaceus*. Scale bar, 500 μm. **(D)** Mycetomes of the Spondylidinae *Asemum striatum*. Scale bar, 500 μm. The mycetomes of this group form two rings surrounding the serosa, which are shown by two arrows. **(E)** Giant mycetomes (My) of *Necydalis formosana*. Scale bar, 500 μm. MG, midgut; Th, thorax. The arrowhead indicates a thin tube that connects mycetomes with the serosa. **(F)** Mycetome-lacking midgut of the Lepturinae *Paragaurotes doris*. Scale bar, 500 μm. **(G)** Anterior midgut of the Prioninae *Aegosoma sinicum*. Scale bar, 500 μm. The mycetome is absent.

Fig. 2.

Distribution of mycetomes in the subfamilies Lepturinae and Necydalinae. **(A)** This

730 phylogenetic tree was generated by the maximum likelihood method based on the
731 alignment of 28S rDNA sequences. Crossed circles indicate mycetome-lacking species.
732 Open circles indicate Lepturinae species that preferentially include the yeast
733 *Scheffersomyces* sp. Y1052_SICYLG3 in their mycetomes. Black circles indicate
734 Lepturinae species that preferentially include the yeast *Scheffersomyces ergatensis*.
735 Squares indicate Lepturinae species that preferentially include the yeast *Candida*
736 *vrieseae* with the information of their host trees. These three yeasts were frequently
737 isolated from various species. Note that this tree does not include all species examined
738 in this study for simplicity. The numbers beside branches indicate the percentage of
739 times that a node was supported in 1,000 bootstrap pseudoreplication. The scores that
740 are equal to or exceed 50% are shown. Species names are based on Danilevsky (2020).
741 The accession numbers of the DNA sequences used for the phylogenetic tree
742 construction are shown at the right side of the species names. **(B)** A simplified image of
743 (A), highlighting the distribution of the symbionts and the loss of mycetomes.
744
745 **Fig. 3.**
746 Fungal cells dwelling in the mycetomes. **(A-C)** Results for typical Lepturinae species.
747 The names of the examined longicorn beetles are shown at the bottom of each panel.
748 **(A)** Mycetome contents and yeast cells. Membranous structures are shown by arrows.
749 An arrowhead indicates an example of symbiont yeast cells. Yeast cells are shown in
750 higher-magnified images in **(B)** and **(B')**. Scale bar, 50 μm in **(A)** and 10 μm in **(B)**. **(C)**
751 Yeast colonies on a PDA plate. **(D-F)** The results for Lepturinae *Encyclops olivaceus*. In
752 **(F)**, no colony was detected. **(G-I)** The results for Necydalinae species. The fungal cell
753 in **(H')** was isolated from a female oviduct. **(J-L)** The results for Spondylidinae species.

754 Arrows indicate the membranous structures.

755

756 **Fig. 4.**

757 Phylogenetic relationships of yeasts isolated from the mycetome-possessing longicorn
758 beetle larvae in this study. This phylogenetic tree was generated by the maximum
759 likelihood method based on the alignment of ITS rDNA sequences. *Hyphopichia rhagii*
760 (isolated from *Rhagium femorale* and *R. japonicum*), *Candida wanchemiae* (isolated
761 from *Anastrangalia scotodes*), and uncultured fungus clone OUT_F389 (isolated from
762 *Megasemum quadricostulatum*) were excluded from this tree because the yeast has
763 much shorter ITS rDNA sequence than those of the other yeasts. The species names of
764 yeasts are abbreviated. For example, "*Judolidia bangi* Se OP537542" indicates
765 "*Scheffersomyces ergatensis* isolated from *Judolidia bangi*, and the accession number of
766 the sequence used for the phylogenetic tree construction". The number noted at a given
767 branch indicates the percentage of times that a node was supported in 1,000 bootstrap
768 pseudoreplications. The scores that are equal to or exceed 50% are shown.

769

770 **Fig. 5.**

771 Phylogenetic relationships of yeasts isolated from larvae and adults of the Lepturinae
772 beetles. This phylogenetic tree was generated by the maximum likelihood method based
773 on the alignment of ITS rDNA sequences. The sequences of the yeasts isolated from the
774 larvae are the same as the ones used in Fig. 4. Ad, yeast isolated from adult; Lv, yeast
775 isolated from larva. The yeast species names are shown on the right side of the tree. For
776 example, "*Pachytodes cometes* Lv OP537597" indicates "*Scheffersomyces ergatensis*
777 isolated from a larva of *Pachytodes cometes*, and the accession number of the sequence

778 used for the phylogenetic tree construction". The clades that include the yeasts from
779 both adults and larvae of the same Lepturinae species are marked with black dots. The
780 number noted at a given branch indicates the percentage of times that a node was
781 supported in 1,000 bootstrap pseudoreplications. The scores that are equal to or exceed
782 50% are shown.
783
784 **Fig. 6.**
785 Fungal cells in the 1st instar larvae of Lepturinae/Necydalinae beetles. The observation
786 was carried out over the first few days after hatching. **(A-A')** The anterior midgut of
787 *Ohbayashia nigromarginata* and yeast cells isolated from the gut. The bracket indicates
788 mycetomes. Bar, 50 μm for A and 25 μm for A'. **(B-B')** The anterior midgut of
789 *Necydalis formosana* and a fungal cell isolated from the gut. Mycetome-like knobbed
790 structures are shown by the bracket. Bar, 50 μm for B and 25 μm for B'.
791
792 **Fig. 7.**
793 Yeast-free mycetomes in Lepturinae larvae. **(A)** *Leptura ochraceofasciata* larvae
794 cultured with an artificial diet. The images were obtained approximately one month
795 after hatching. **(B)** Yeast-free mycetomes (knobs shown by the bracket). **(C)** A
796 magnified image of the contents of yeast-free mycetomes. The inset shows the results of
797 culturing of the contents on a PDA plate. No colony appeared. **(D-I)** The results for
798 *Stictoleptura dichroa*. **(D-E)** Yeast-free mycetomes. **(F-G)** Yeast-poor mycetomes. Note
799 that the color of the mycetomes is somewhat whitish. Four examples of yeast cells are
800 shown by arrowheads. The yeast *Meyerozyma caribbica* was identified from the
801 mycetomes. **(H-I)** Yeast-rich mycetomes. The yeast *Scheffersomyces ergatensis* was

802 identified from these mycetomes.

803

804 **Fig. 8.**

805 The presence of symbionts in the mycetomes after long-time culture on the artificial diet
806 with anti-fungal chemicals. **(A)** *Etorofus vicarius* mycetome. Arrowheads are examples
807 of symbiont yeasts. Bar, 100 μm. **(B)** *Necydalis formosana* mycetome. Bar, 100 μm.

808

809 **Fig. 9.**

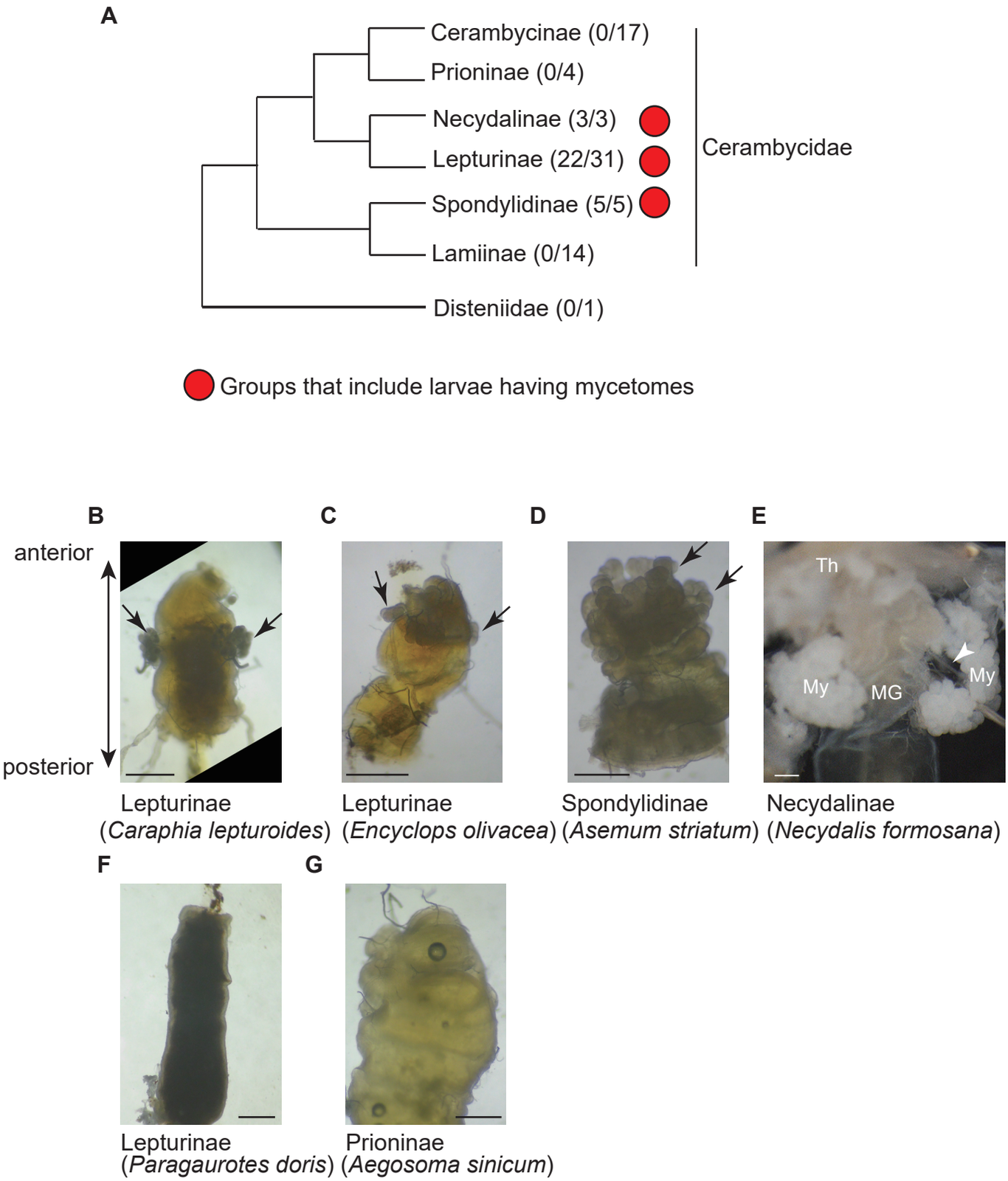
810 *Aegosoma sinicum* larvae have a symbiotic relationship with the two yeast groups. This
811 phylogenetic tree was generated by the maximum likelihood method based on the
812 alignment of ITS rDNA sequences. The yeasts from mycetome-possessing longicorn
813 beetles used in Fig. 4 are also shown in this phylogenetic tree to investigate the
814 phylogenetic relationships between mycetome-dependent and *Aegosoma* yeasts. The
815 yeasts isolated from larvae of *Aegosoma sinicum* are indicated by the sampling location
816 and identification numbers corresponding to those in Table S1. The number noted at a
817 given branch indicates the percentage of times that a node was supported in 1,000
818 bootstrap pseudoreplications. The scores that are equal to or exceed 50% are shown.

819

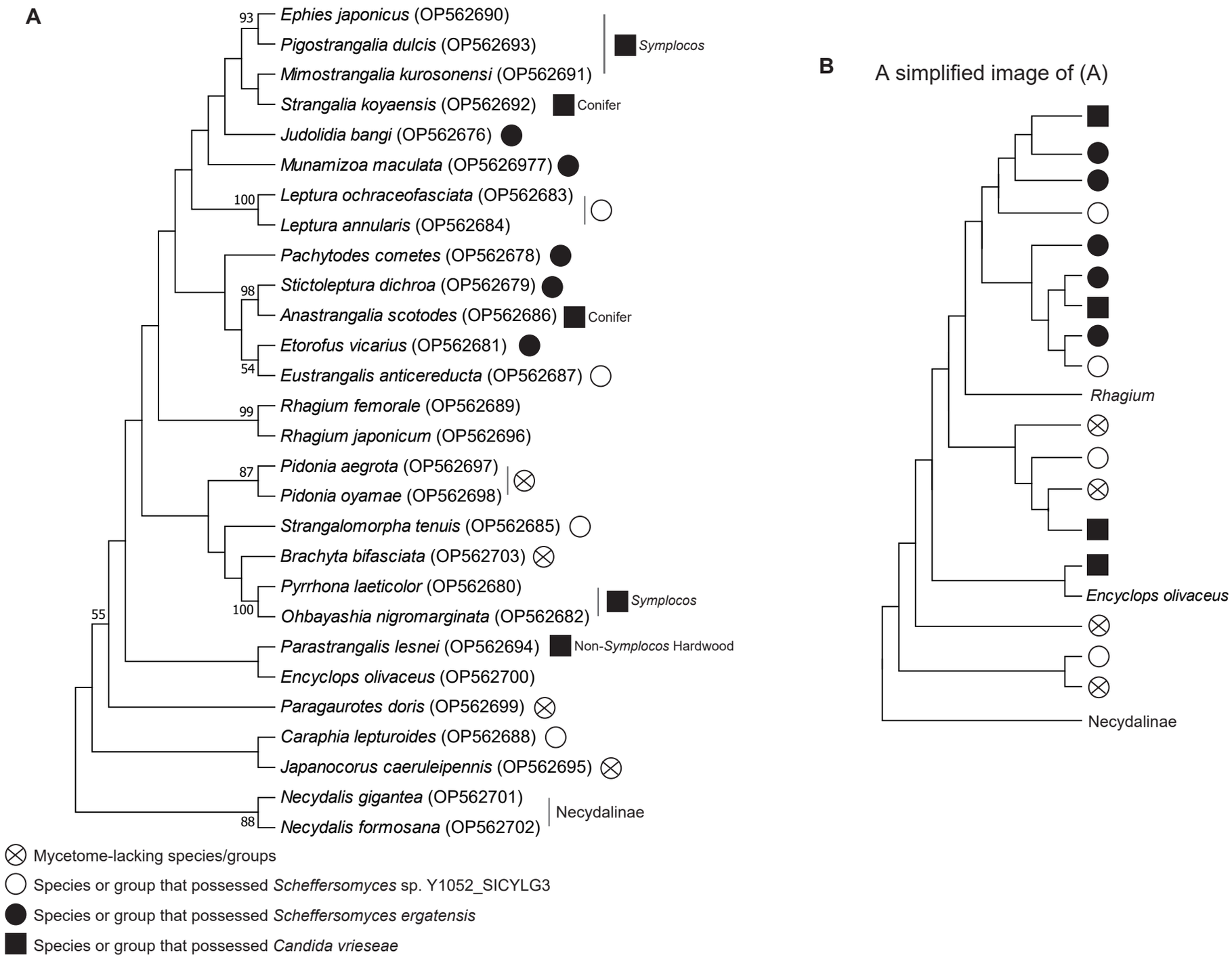
820 **Table 1.**

821 Summary of symbiont characterization among longicorn beetle groups collected in
822 Japan. The sum of yeast species isolated from Lepturinae having mycetome,
823 Necydalinae and Spondylidinae, excluding duplicates between groups. The sum of yeast
824 species isolated from Lepturinae without mycetome, Prioninae, Cerambycinae,
825 Lamiinae, and Disteniidae, excluding duplicates between groups.

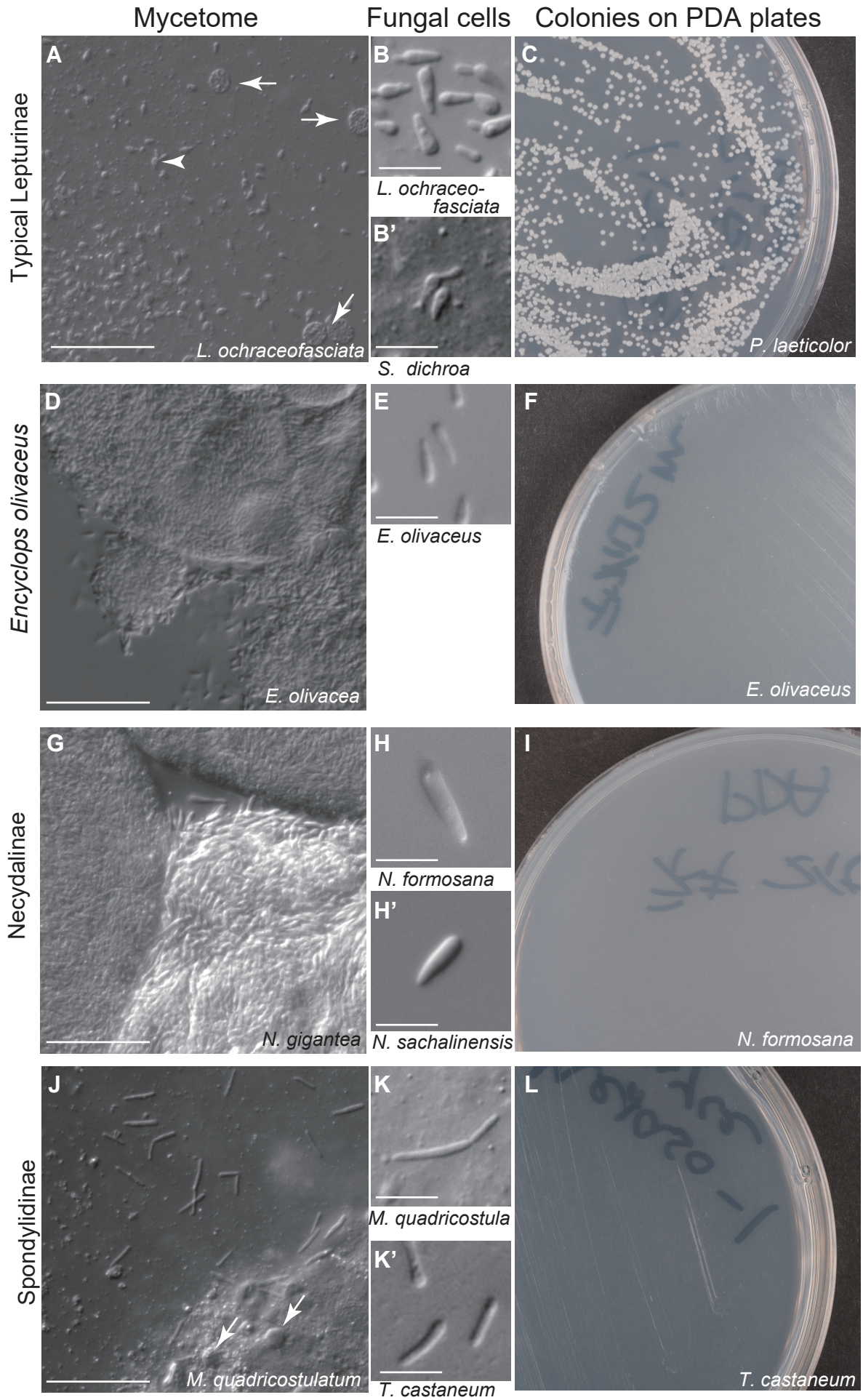
Sasakura et al., Figure 1.



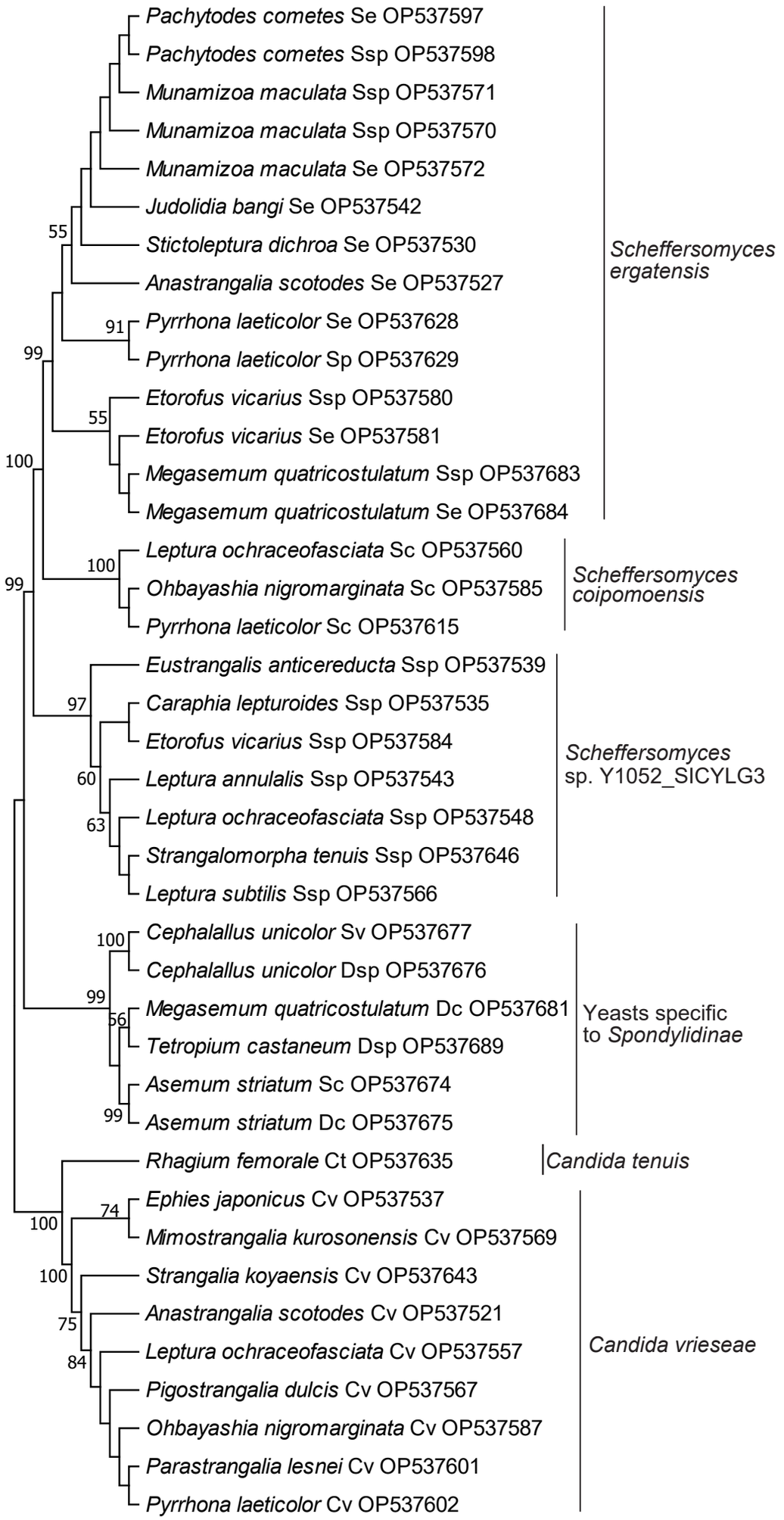
Sasakura et al., Figure 2



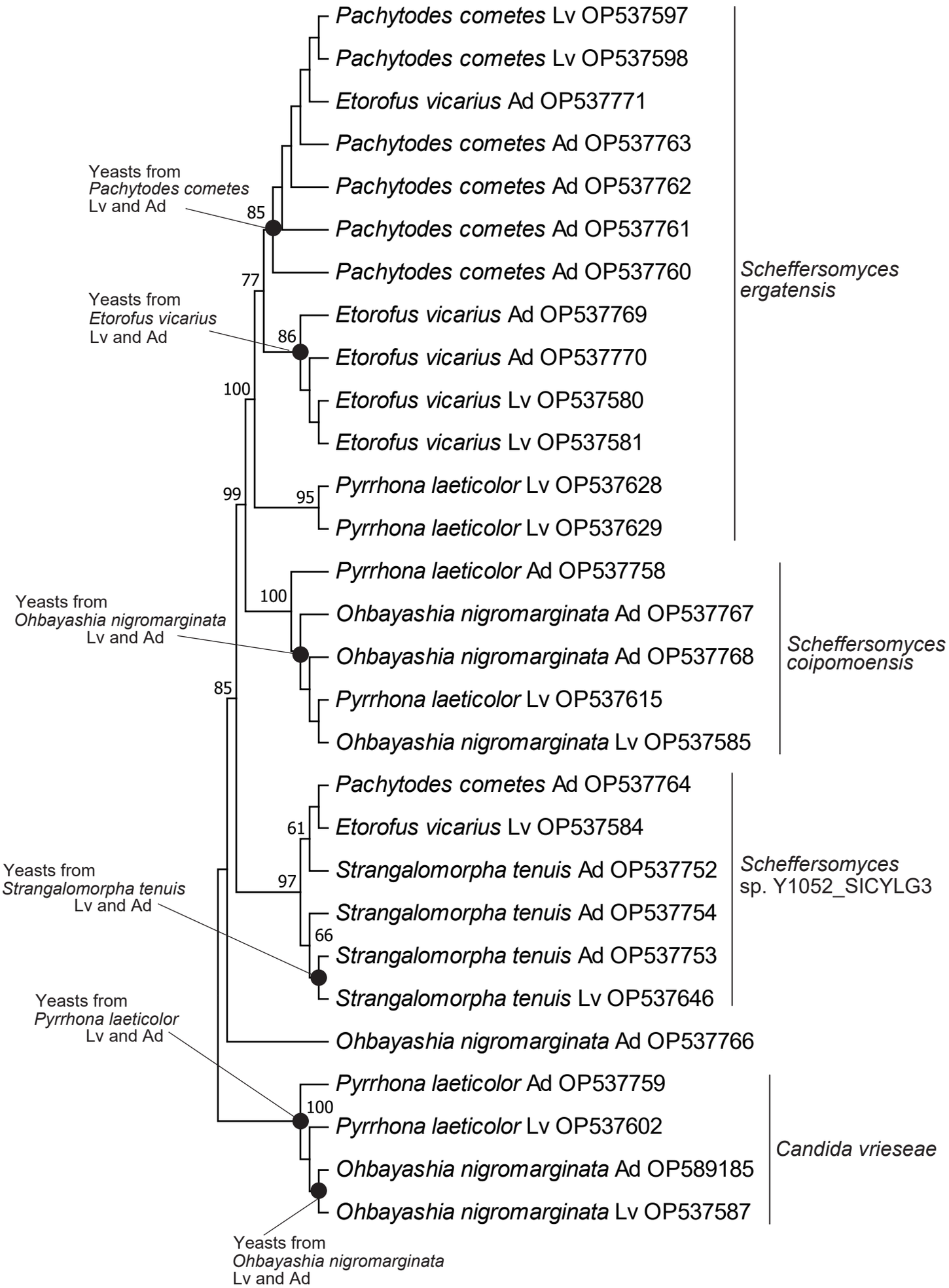
Sasakura et al., Figure 3



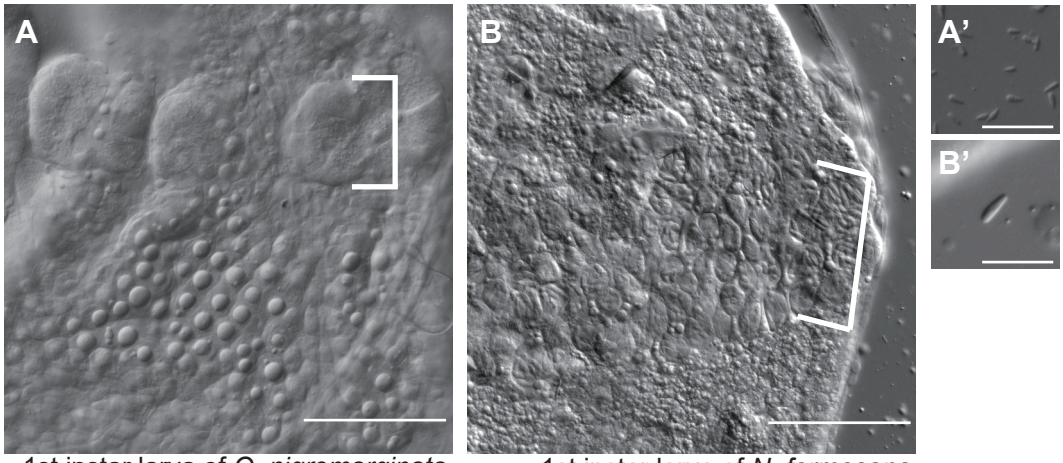
Sasakura et al., Figure 4



Sasakura et al., Figure 5



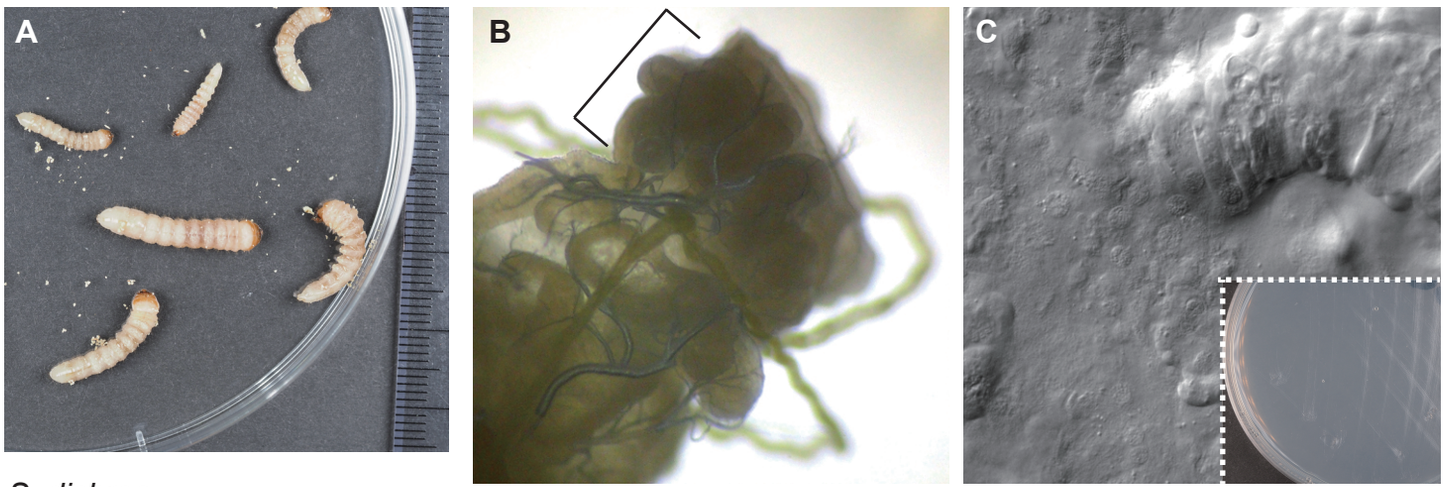
Sasakura et al., Figure 6



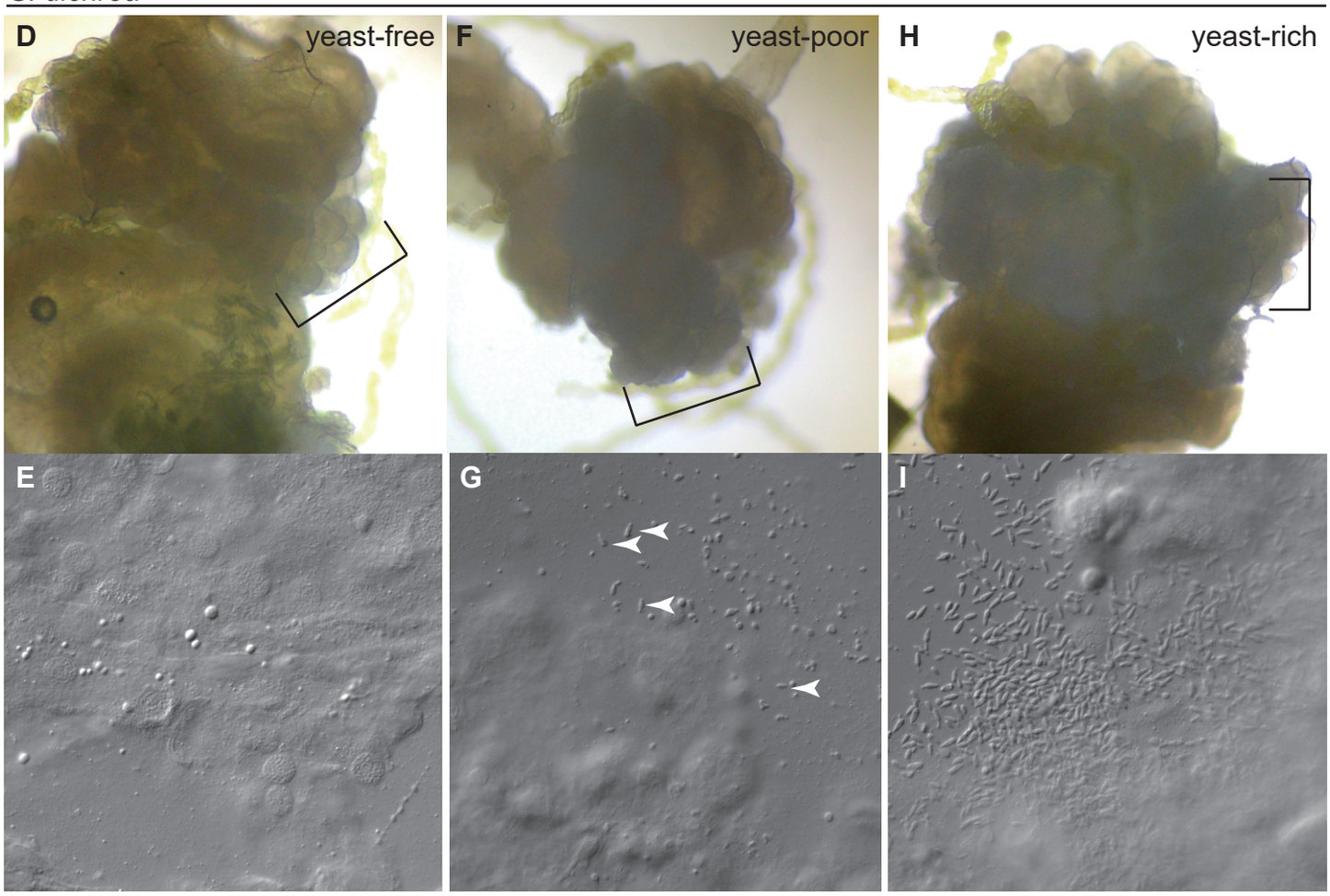
1st instar larva of *O. nigromarginata* 1st instar larva of *N. formosana*

Sasakura et al., Figure 7

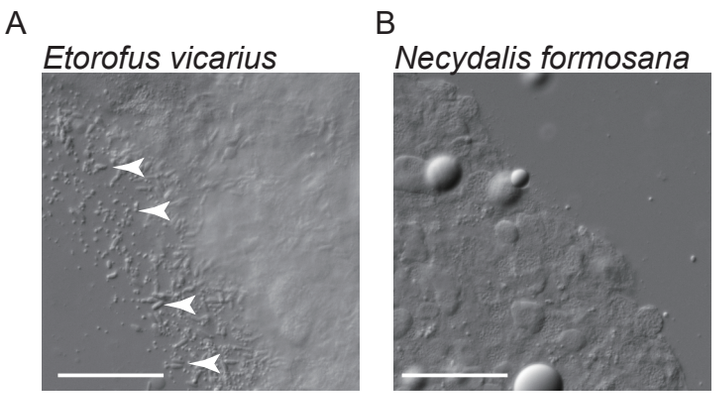
L. ochraceofasciata



S. dichroa



Sasakura et al., Figure 8



Sasakura et al., Figure 9

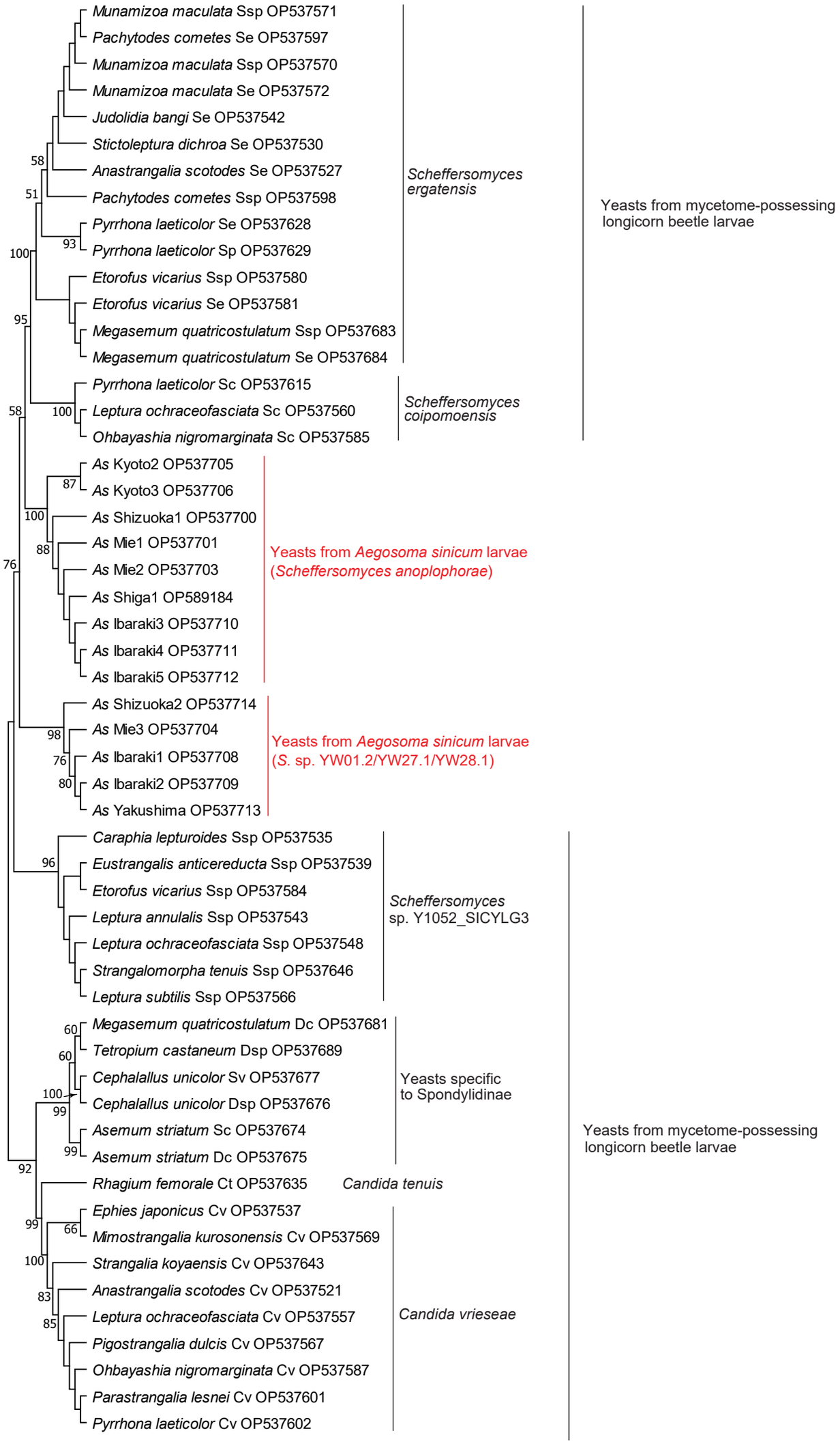


Table 1. Summary of symbiont characterization among Longicorn beetle groups in Japan

Groups	Number of observed species	Number of observed larvae	N of yeast colony yielded larvae	N of larvae which had more than one yeast species	N of yeast species
Lepturinae having mycetome	22	125	116	7	10
Necydalinae	3	12	0	0	0
Spondylidinae	5	19	3	1	8
Lepturinae without mycetome	9	38	13	5	15
Prioninae	4	25	20	2	11
Cerambycinae	17	38	15	3	13
Lamiinae	14	29	11	3	12
Disteniidae	1	5	4	0	4
Mycetome present groups	30	156	119	8	18
Mycetome absent groups	46	135	63	13	46

Table S1. Symbiont fungi isolated from larvae of Longicorn beetles in Japan

Group	Species	Collected location*	host	mycetome	Analyzed region	Colonies on plate**	Blastn Best Hit	Blastn Score	Accession Number
Lepturinae with mycetome	*Anastrangalia scotodes*	Shizuoka	*Pinus thunbergii*	y	mycetome	y 10	[*Candida*] *vrieseae* CBS 10829	592/606	OP537521
Lepturinae with mycetome	*Anastrangalia scotodes*	Shizuoka	*Pinus thunbergii*	y	mycetome	no yeast colony	[*Candida*] *vrieseae* CBS 10829	578/593	OP537522
Lepturinae with mycetome	*Anastrangalia scotodes*	Shizuoka	*Pinus thunbergii*	y	mycetome	no yeast colony	not analyzed		
Lepturinae with mycetome	*Anastrangalia scotodes*	Nara	*Pinus densiflora*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829 *Candida wanchemiae* CBS 12090	612/630 371/420	OP537523 OP537524
Lepturinae with mycetome	*Anastrangalia scotodes*	Gifu	*Betula platyphylla*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537525
Lepturinae with mycetome	*Anastrangalia scotodes*	Gifu	*Abies veitchii*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829 *Schefferomyces ergatensis* strain ATCC MYA-4655	614/629 614/615	OP537526 OP537527
Lepturinae with mycetome	*Anastrangalia scotodes*	Gifu	*Abies veitchii*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537528
Lepturinae with mycetome	*Stictoleptura dichroa*	Yamanashi	*Larix kaempferi*	y	mycetome	y	*Schefferomyces ergatensis* strain ATCC MYA-4655	615/615	OP537529
Lepturinae with mycetome	*Stictoleptura dichroa*	Yamanashi	*Larix kaempferi*	y	mycetome	y	*Schefferomyces ergatensis* strain ATCC MYA-4655	615/615	OP537530
Lepturinae with mycetome	*Stictoleptura dichroa*	Yamanashi	*Larix kaempferi*	y	mycetome	y	*Schefferomyces ergatensis* strain ATCC MYA-4655	615/615	OP537531
Lepturinae with mycetome	*Stictoleptura dichroa*	Yamanashi	*Larix kaempferi*	y	mycetome	y	*Schefferomyces ergatensis* strain ATCC MYA-4655	615/615	OP537532
Lepturinae with mycetome	*Stictoleptura dichroa*	Yamanashi	*Larix kaempferi*	y	mycetome	y	*Schefferomyces ergatensis* strain ATCC MYA-4655	614/616	OP537533
Lepturinae with mycetome	*Stictoleptura dichroa*	Yamanashi	*Larix kaempferi*	y	mycetome	y	*Schefferomyces ergatensis* strain ATCC MYA-4655	614/615	OP537534
Lepturinae with mycetome	*Caraphia lepturoides*	Aichi	*Clerodendrum trichotomum*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	618/619	OP537535
Lepturinae with mycetome	*Caraphia lepturoides*	Aichi	*Clerodendrum trichotomum*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	618/619	OP537536
Lepturinae with mycetome	*Ephies japonicus*	Yamaguchi	*Symplocos myrtacea*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	584/597	OP537537
Lepturinae with mycetome	*Ephies japonicus*	Yamaguchi	*Symplocos myrtacea*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	584/597	OP537538
Lepturinae with mycetome	*Eustrangalis anticereducta*	Gifu	*Robinia pseudoacacia*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	617/619	OP537539
Lepturinae with mycetome	*Eustrangalis anticereducta*	Gifu	*Robinia pseudoacacia*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	587/587	OP537540
Lepturinae with mycetome	*Eustrangalis anticereducta*	Gifu	*Robinia pseudoacacia*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	617/619	OP537541
Lepturinae with mycetome	*Judolidia bangi*	Yamanashi	*Larix kaempferi*	y	mycetome/gut	y	*Scheffersomyces ergatensis* strain ATCC MYA-4655	614/615	OP537542
Lepturinae with mycetome	*Leptura annulalis*	Ibaraki	*Salix* sp.	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	593/593	OP537543
Lepturinae with mycetome	*Leptura annulalis*	Ibaraki	*Salix* sp.	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	619/619	OP537544
Lepturinae with mycetome	*Leptura annulalis*	Kagoshima	unknown	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	619/619	OP537545
Lepturinae with mycetome	*Leptura annulalis*	Yamanashi	*Sorbus commixta*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	618/619	OP537546
Lepturinae with mycetome	*Leptura annulalis*	Gifu	*Betula platyphylla*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	618/619	OP537547
Lepturinae with mycetome	*Leptura ochraceofasciata*	Shizuoka	*Chamaecyparis obtusa*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	619/619	OP537548
Lepturinae with mycetome	*Leptura ochraceofasciata*	Nara	*Carpinus tschonoskii*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	618/619	OP537549
Lepturinae with mycetome	*Leptura ochraceofasciata*	Nara	*Carpinus tschonoskii*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	618/619	OP537550
Lepturinae with mycetome	*Leptura ochraceofasciata*	Nara	*Carpinus tschonoskii*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	586/587	OP537551
Lepturinae with mycetome	*Leptura ochraceofasciata*	Nara	*Carpinus tschonoskii*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	595/596	OP537552
Lepturinae with mycetome	*Leptura ochraceofasciata*	Shizuoka	*Chamaecyparis obtusa*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	586/587	OP537553
Lepturinae with mycetome	*Leptura ochraceofasciata*	Shizuoka	*Chamaecyparis obtusa*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	618/619	OP537554
Lepturinae with mycetome	*Leptura ochraceofasciata*	Aichi	*Cryptomeria japonica*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	619/619	OP537555
Lepturinae with mycetome	*Leptura ochraceofasciata*	Aichi	*Cryptomeria japonica*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	619/619	OP537556
Lepturinae with mycetome	*Leptura ochraceofasciata*	Kagoshima Yakushima Island	*Symplocos myrtacea*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	597/612	OP537557
Lepturinae with mycetome	*Leptura ochraceofasciata*	Aichi	*Lindera triloba*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	592/593	OP537558
Lepturinae with mycetome	*Leptura ochraceofasciata*	Mie	*Cryptomeria japonica*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	616/621	OP537559
Lepturinae with mycetome	*Leptura ochraceofasciata*	Shizuoka	*Symplocos coreana*	y	mycetome	y	*Scheffersomyces coipomoensis* strain Y3	615/615	OP537560
Lepturinae with mycetome	*Leptura ochraceofasciata*	Shizuoka	*Chamaecyparis obtusa*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	619/619	OP537561
Lepturinae with mycetome	*Leptura ochraceofasciata*	Kagoshima	unknown	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	619/619	OP537562
Lepturinae with mycetome	*Leptura ochraceofasciata*	Gifu	*Hamamelis japonica*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	618/619	OP537563
Lepturinae with mycetome	*Leptura ochraceofasciata*	Gifu	*Fagus crenata*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	618/619	OP537564
Lepturinae with mycetome	*Leptura subtilis*	Yamanashi	*Betula platyphylla*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	619/619	OP537565

Lepturinae with mycetome	*Leptura subtilis*	Yamanashi	*Betula platyphylla*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	619/619	OP537566
Lepturinae with mycetome	*Pigostrangalia dulcis*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	607/623	OP537567
Lepturinae with mycetome	*Pigostrangalia dulcis*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537568
Lepturinae with mycetome	*Mimostrangalia kurosonensis*	Kagoshima Yakushima Island	*Symplocos myrtacea*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	627/641	OP537569
Lepturinae with mycetome	*Munamizoa maculata*	Yamanashi	*Chamaecyparis obtusa*	Failed to observe	gut	y	*Scheffersomyces* sp. strain Y13	588/589	OP537570
Lepturinae with mycetome	*Munamizoa maculata*	Yamanashi	*Chamaecyparis obtusa*	Failed to observe	gut	y	*Scheffersomyces* sp. strain Y6	583/583	OP537571
Lepturinae with mycetome	*Munamizoa maculata*	Yamanashi	*Chamaecyparis obtusa*	y	mycetome	y	*Schefferomyces ergatensis* strain ATCC MYA-4655	615/615	OP537572
Lepturinae with mycetome	*Munamizoa maculata*	Yamanashi	*Chamaecyparis obtusa*	y	mycetome	y	*Schefferomyces ergatensis* strain ATCC MYA-4655	615/616	OP537573
Lepturinae with mycetome	*Munamizoa maculata*	Yamanashi	*Chamaecyparis obtusa*	y	mycetome	y	*Schefferomyces ergatensis* strain ATCC MYA-4655	615/615	OP537574
Lepturinae with mycetome	*Munamizoa maculata*	Yamanashi	*Chamaecyparis obtusa*	y	mycetome	y	*Schefferomyces ergatensis* strain ATCC MYA-4655	615/615	OP537575
Lepturinae with mycetome	*Munamizoa maculata*	Yamanashi	*Chamaecyparis obtusa*	y	mycetome	y	*Scheffersomyces ergatensis* strain ATCC 22589	604/611	OP537576
Lepturinae with mycetome	*Etorofus vicarius*	Shizuoka	*Chamaecyparis obtusa*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	595/596	OP537577
Lepturinae with mycetome	*Etorofus vicarius*	Shizuoka	*Chamaecyparis obtusa*	y	mycetome	y	*Scheffersomyces* sp. strain Y13	589/593	OP537578
Lepturinae with mycetome	*Etorofus vicarius*	Yamanashi	*Abies* sp.	y	mycetome	y	*Schefferomyces ergatensis* strain ATCC MYA-4655	613/616	OP537579
Lepturinae with mycetome	*Etorofus vicarius*	Yamanashi	*Abies* sp.	y	mycetome	y	*Scheffersomyces* sp. strain Y13	586/589	OP537580
Lepturinae with mycetome	*Etorofus vicarius*	Yamanashi	*Abies* sp.	y	mycetome	y	*Schefferomyces ergatensis* strain ATCC MYA-4655	613/616	OP537581
Lepturinae with mycetome	*Etorofus vicarius*	Yamanashi	*Larix kaempferi*	Failed to observe	gut	y 1	*Schefferomyces ergatensis* strain ATCC MYA-4655	613/616	OP537582
Lepturinae with mycetome	*Etorofus vicarius*	Shizuoka	*Chamaecyparis obtusa*	y	mycetome	y	*Schefferomyces ergatensis* strain ATCC MYA-4655	612/616	OP537583
Lepturinae with mycetome	*Etorofus vicarius*	Shizuoka	*Chamaecyparis obtusa*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	619/619	OP537584
Lepturinae with mycetome	*Ohbayashia nigromarginata*	Shizuoka	*Symplocos coreana*	not observed	Frass	no yeast colony			
Lepturinae with mycetome	*Ohbayashia nigromarginata*	Shizuoka	*Symplocos coreana*	y	mycetome	y	*Scheffersomyces coipomoensis* strain Y3	615/615	OP537585
Lepturinae with mycetome	*Ohbayashia nigromarginata*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	581/595	OP537586
Lepturinae with mycetome	*Ohbayashia nigromarginata*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537587
Lepturinae with mycetome	*Ohbayashia nigromarginata*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	505/519	OP537588
Lepturinae with mycetome	*Ohbayashia nigromarginata*	Shizuoka	*Symplocos coreana*	y	mycetome	y	*Scheffersomyces coipomoensis* strain Y3	615/615	OP537589
Lepturinae with mycetome	*Ohbayashia nigromarginata*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	588/602	OP537590
Lepturinae with mycetome	*Ohbayashia nigromarginata*	Shizuoka	*Symplocos coreana*	y	mycetome	y	*Scheffersomyces coipomoensis* strain Y3 [*Candida*] *vrieseae* CBS 10829	615/615 615/629	OP537591 OP537592
Lepturinae with mycetome	*Ohbayashia nigromarginata*	Wakamaya	*Symplocos myrtacea*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829 *Scheffersomyces coipomoensis* strain Y3	614/629 615/615	OP537593 OP537594
Lepturinae with mycetome	*Ohbayashia nigromarginata*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829 *Scheffersomyces coipomoensis* strain Y3	612/634 615/615	OP537595 OP537596
Lepturinae with mycetome	*Pachytodes cometes*	Yamanashi	*Larix kaempferi*	y	mycetome	y	*Scheffersomyces ergatensis* strain ATCC MYA-4655 *Scheffersomyces* sp. strain Y13	615/615 592/592	OP537597 OP537598
Lepturinae with mycetome	*Pachytodes cometes*	Aichi	*Cryptomeria japonica*	y	mycetome	y	*Scheffersomyces ergatensis* strain ATCC MYA-4655	615/615	OP537599
Lepturinae with mycetome	*Pachytodes cometes*	Yamanashi	*Larix kaempferi*	y	mycetome	y	*Scheffersomyces ergatensis* strain ATCC MYA-4655	615/615	OP537600
Lepturinae with mycetome	*Parastrangalia lesnei*	Gifu	*Hamamelis japonica*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	582/596	OP537601
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	not observed	frass	no yeast colony			
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	not observed	frass	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537602
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	not observed	frass	y	[*Candida*] *vrieseae* CBS 10829	592/606	OP537603
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	not observed	frass	no yeast colony			
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537604
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537605
Lepturinae with mycetome	*Pyrrhona laeticolor*	Yakushima Island	*Symplocos myrtacea*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	627/641	OP537606
Lepturinae with mycetome	*Pyrrhona laeticolor*	Yakushima Island	*Symplocos myrtacea*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537607
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537608
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537609
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537610

Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537611
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537612
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	613/631	OP537613
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537614
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	*Scheffersomyces coipomoensis* strain Y3	615/615	OP537615
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537616
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537617
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537618
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537619
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537620
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537621
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	605/619	OP537622
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537623
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	613/631	OP537624
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537625
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537626
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome, gut	y	*Scheffersomyces coipomoensis* strain Y3 *Scheffersomyces ergatensis* strain ATCC MYA-4655 *Scheffersomyces paraergatensis* strain NYNU 16782 [*Candida*] *vrieseae* CBS 10829	615/615 609/615 586/587 615/629	OP537627 OP537628 OP537629 OP537630
Lepturinae with mycetome	*Pyrrhona laeticolor*	Wakamaya	*Symplocos myrtacea*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	583/597	OP537631
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	615/629	OP537632
Lepturinae with mycetome	*Pyrrhona laeticolor*	Shizuoka	*Symplocos coreana*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	613/632	OP537633
Lepturinae with mycetome	*Rhagium femorale*	Nagano	*Abies veitchii*	y	mycetome	y	*Hyphopichia rhagii* culture CBS:618	369/369	OP537634
Lepturinae with mycetome	*Rhagium femorale*	Nagano	*Abies veitchii*	y	mycetome	y	*Candida tenuis*	566/566	OP537635
Lepturinae with mycetome	*Rhagium femorale*	Tochigi	unknown	y	mycetome	y	*Hyphopichia rhagii* culture CBS:618	369/369	OP537636
Lepturinae with mycetome	*Rhagium femorale*	Yamanashi	*Abies veitchii*	y	mycetome	y	*Candida tenuis*	581/581	OP537637
Lepturinae with mycetome	*Rhagium femorale*	Yamanashi	*Abies veitchii*	y	mycetome	y	*Hyphopichia rhagii* culture CBS:618	405/405	OP537638
Lepturinae with mycetome	*Rhagium femorale*	Yamanashi	*Abies veitchii*	y	mycetome	y	*Hyphopichia rhagii* culture CBS:618	377/379	OP537639
Lepturinae with mycetome	*Rhagium femorale*	Yamanashi	*Abies veitchii*	y	mycetome	y	*Hyphopichia rhagii* culture CBS:618	405/405	OP537640
Lepturinae with mycetome	*Rhagium japonicum*	Tochigi	unknown	y	mycetome	y	*Hyphopichia rhagii* culture CBS:618	382/382	OP537641
Lepturinae with mycetome	*Rhagium japonicum*	Tochigi	unknown	y	mycetome	y	*Hyphopichia rhagii* culture CBS:618	404/405	OP537642
Lepturinae with mycetome	*Strangalia koyaensis*	Aichi	*Cryptomeria japonica*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	613/629	OP537643
Lepturinae with mycetome	*Strangalia koyaensis*	Aichi	*Cryptomeria japonica*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	612/629	OP537644
Lepturinae with mycetome	*Strangalia koyaensis*	Aichi	*Cryptomeria japonica*	y	mycetome	y	[*Candida*] *vrieseae* CBS 10829	612/630	OP537645
Lepturinae with mycetome	*Strangalomorpha tenuis*	Yamanashi	*Sorbus commixta*	y	mycetome	y	*Scheffersomyces* sp. Y1052_SICYLG3	586/586	OP537646
Lepturinae with mycetome	*Encyclops olivaceus*	Aichi	*Quercus crispula*	y	mycetome	no yeast colony	*Chrysolina oricalcia* genome assembly, chromosome: 12	186/190	OP537662
Lepturinae with mycetome	*Encyclops olivaceus*	Aichi	*Quercus crispula*	y	mycetome	no yeast colony	*Acalymma blandulum*	149/153	OP537663
Lepturinae with mycetome	*Encyclops olivaceus*	Gifu	*Quercus crispula*	y	mycetome	no yeast colony	not analyzed		
Lepturinae with mycetome	*Encyclops olivaceus*	Gifu	*Quercus crispula*	y	mycetome	no yeast colony	*Chrysolina oricalcia* genome assembly, chromosome: 12	185/190	OP537664
Lepturinae w/o mycetome	*Pargaurotes doris*	Nagano		n	gut	y	[*Candida*] *vrieseae* CBS 10829	607/636	OP537647
Lepturinae w/o mycetome	*Pargaurotes doris*	Nagano		n	gut	y	*Yamadazyma mexicana* culture CBS:2089	606/618	OP537648
Lepturinae w/o mycetome	*Pargaurotes doris*	Nagano		n	gut	y	[*Candida*] *montana* isolate yHRVM49 *Wickerhamomyces canadensis* culture CBS:5676	396/408 522/586	OP537649 OP537650
Lepturinae w/o mycetome	*Pargaurotes doris*	Yamanashi		n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pargaurotes doris*	Yamanashi		n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pargaurotes doris*	Tochigi	*Fagus crenata*	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pargaurotes doris*	Gifu	*Abies veitchii*	n	gut	y	[*Candida*] *rhagii* culture CBS:618	373/373	OP537651
Lepturinae w/o mycetome	*Pargaurotes doris*	Gifu	*Abies veitchii*	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pargaurotes doris*	Gifu	*Abies veitchii*	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia aegrota*	Nara	*Carpinus tschonoskii*	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia aegrota*	Nara	*Carpinus tschonoskii*	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia aegrota*	Nara	*Carpinus tschonoskii*	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia aegrota*	Mie	*Pieris japonica*	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia aegrota*	Shizuoka	*Carpinus* sp.	n	gut	y 3	*Candida boleticola* culture CBS:6420	612/613	OP537652

Lepturinae w/o mycetome	*Pidonia amentata?*	Nara	*Carpinus tschonoskii*	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia amentata?*	Nara	*Carpinus tschonoskii*	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia discoidalis*	Shizuoka	*Fagus crenata*	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia oyamae/masakii*	Yamanashi	unknown	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia oyamae/masakii*	Yamanashi	unknown	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia oyamae/masakii*	Yamanashi	unknown	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia oyamae/masakii*	Mie	*Styrax japonica*	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia oyamae/masakii*	Mie	*Styrax japonica*	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia oyamae/masakii*	Mie	*Carpinus tschonoskii*	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia oyamae/masakii*	Mie	*Carpinus tschonoskii*	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia oyamae/masakii*	Mie	*Carpinus tschonoskii*	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia oyamae/masakii*	Shizuoka	*Carpinus* sp.	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia sp.*	Yamanashi	unknown	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Pidonia sylvicola/himehana/maculitho*	Aichi	*Kalopanax septemlobus*	n	gut	y	[*Candida*] *montana* isolate yHRVM49	396/408	OP537653
Lepturinae w/o mycetome	*Pidonia sylvicola/himehana/maculitho*	Aichi	*Kalopanax septemlobus*	n	gut	y	*Wickerhamomyces canadensis* culture CBS:5676 [*Candida*] *montana* isolate yHRVM49	522/586 396/408	OP537654 OP589179
Lepturinae w/o mycetome	*Pidonia sylvicola/himehana/maculitho*	Aichi	*Kalopanax septemlobus*	n	gut	y	[*Candida*] *montana* isolate yHRVM49	396/408	OP537655
Lepturinae w/o mycetome	*Pidonia sylvicola/himehana/maculitho*	Yamanashi	unknown	n	gut	no yeast colony			
Lepturinae w/o mycetome	*Japanocorus caeruleipennis*	Culture		n	gut	y	[*Candida*] *gorgasii* ATCC MYA-4334 [*Candida*] *michaelii* culture CBS:9502 *Sugiyamaella smithiae* strain NS1	584/621 613/659 549/549	OP537656 OP589180 OP589181
Lepturinae w/o mycetome	*Japanocorus caeruleipennis*	Culture		n	gut	y	[*Candida*] *insectorum* *Nakazawaea* sp. isolate UFMG-CM-Y6909	625/625 609/611	OP537657 OP537658
Lepturinae w/o mycetome	*Japanocorus caeruleipennis*	Culture		n	gut	y	[*Candida*] *vrieseae* CBS 10829	587/613	OP537659
Lepturinae w/o mycetome	*Japanocorus caeruleipennis*	Culture		n	gut	no yeast colony			
Lepturinae w/o mycetome	*Japanocorus caeruleipennis*	Culture		n	gut	y	*Candida* sp. Afl10-2-1 *Pichia hampshirensis* strain ATCC 62896 *Pichia veronae* strain CECT 11800	591/591 551/566 594/596	OP537660 OP589182 OP589183
Lepturinae w/o mycetome	*Brachyta bifasciata*	Culture		n	gut	no yeast colony			
Lepturinae w/o mycetome	*Brachyta bifasciata*	Culture		n	gut	y 1	*Zygoascus meyerae* culture CBS:6360	571/572	OP537661
Necydalinae	*Necydalis formosana*	Shizuoka	*Symplocos coreana*	not observed	frass	no yeast colony			
Necydalinae	*Necydalis formosana*	Shizuoka	*Symplocos coreana*	y	mycetome	no yeast colony	Uncultured fungus clone Oc_O_AugC04	467/552	OP537665
Necydalinae	*Necydalis formosana*	Shizuoka	*Symplocos coreana*	y	mycetome	no yeast colony			
Necydalinae	*Necydalis formosana*	Shizuoka	*Symplocos coreana*	y	mycetome	no yeast colony			
Necydalinae	*Necydalis formosana*	Shizuoka	*Symplocos coreana*	y	mycetome	not done	*Phacidiales* sp. strain 19GCAS019	359/410	OP537666
Necydalinae	*Necydalis gigantea*	Nara	*Neolitsea aciculata*	not observed	frass	no yeast colony			
Necydalinae	*Necydalis gigantea*	Nara	*Neolitsea aciculata*	y		no yeast colony	*Aotearoamyces nothofagi* voucher PDD:55517	493/555	OP537667
Necydalinae	*Necydalis harmandi*	Yamanashi	*Sorbus commixta*	y		no yeast colony	*Variabilispora flava* CBS 144845	374/421	OP537668
Necydalinae	*Necydalis harmandi*	Yamanashi	*Sorbus commixta*	y	mycetome	no yeast colony	Uncultured fungus clone 3306E21	477/544	OP537669
Necydalinae	*Necydalis harmandi*	Yamanashi	*Sorbus commixta*	y	mycetome	no yeast colony	Uncultured fungus clone 3306E21	476/545	OP537670
Necydalinae	*Necydalis harmandi*	Yamanashi	*Sorbus commixta*	y	mycetome gut	no yeast colony	Uncultured fungus clone 3306E21	477/544	OP537671
Necydalinae	*Necydalis harmandi*	Yamanashi	*Sorbus commixta*	y	mycetome gut	no yeast colony	*Variabilispora flava* CBS 144845	445/509	OP537672
Spondylidinae	*Arhopalus coreanus*	Aichi	*Pinus densiflora*	y	mycetome	no yeast colony			
Spondylidinae	*Arhopalus coreanus*	Aichi	*Pinus densiflora*	y	mycetome	no yeast colony			
Spondylidinae	*Asemum striatum*	Nagano	*Larix kaempferi*	y	mycetome	y	*Candida oregonensis* CBS 5036	386/386	OP537673
Spondylidinae	*Asemum striatum*	Nagano	*Larix kaempferi*	y	mycetome	y 1	uncultured fungus/*Schwanniomyces capriottii* isolate NG_p11	574/611	OP537674
Spondylidinae	*Asemum striatum*	Nagano	*Larix kaempferi*	y	mycetome	no yeast colony	*Debaryomyces castellii* culture CBS:2923	562/599	OP537675
Spondylidinae	*Cephalallus unicolor*	Shizuoka	*Pinus thunbergii*	y	mycetome	no yeast colony	*Debaryomyces* sp. UFMG-CM-Y343	573/628	OP537676
Spondylidinae	*Cephalallus unicolor*	Shizuoka	*Pinus thunbergii*	y	mycetome	no yeast colony	*Debaryomyces* sp. UFMG-CM-Y343	572/627	OP537677
Spondylidinae	*Cephalallus unicolor*	Shizuoka	*Pinus thunbergii*	y	mycetome	no yeast colony	*Schwanniomyces vanrijiae* var. yarrowii culture CBS:6246	584/638	OP537678
Spondylidinae	*Cephalallus unicolor*	Shizuoka	*Pinus thunbergii*	y	mycetome	no yeast colony	*Schwanniomyces vanrijiae* var. yarrowii culture CBS:6246	584/638	OP537679
Spondylidinae	*Cephalallus unicolor*	Shizuoka	*Pinus thunbergii*	y	mycetome	no yeast colony	*Schwanniomyces vanrijiae* var. yarrowii culture CBS:6246	584/638	OP537680
Spondylidinae	*Megasemum quadricostulatum*	Yamanashi	*Abies veitchii*	not observed	gut	no yeast colony			

Spondylidinae	*Megasemum quadricostulatum*	Yamanashi	*Abies veitchii*	y	mycetome	no yeast colony			
Spondylidinae	*Megasemum quadricostulatum*	Yamanashi	*Abies veitchii*	y	mycetome	no yeast colony	*Debaryomyces castellii* culture CBS:2923	589/627	OP537681
Spondylidinae	*Megasemum quadricostulatum*	Yamanashi	*Abies veitchii*	y	mycetome	y 6	*Debaryomyces castellii* culture CBS:2923 (mycetome) *Scheffersomyces* sp. strain Y13 (colony) *Candida ergatensis* strain ATCC MYA-4655 (colony)	589/627 577/578 613/616	OP537682 OP537683 OP537684
Spondylidinae	*Megasemum quadricostulatum*	Yamanashi	*Abies veitchii*	y	mycetome	no yeast colony	*Debaryomyces castellii* culture CBS:2923	589/627	OP537685
Spondylidinae	*Megasemum quadricostulatum*	Yamanashi	*Larix kaempferi*	y	mycetome	no yeast colony	*Debaryomyces castellii* culture CBS:2923	589/627	OP537686
Spondylidinae	*Megasemum quadricostulatum*	Yamanashi	*Larix kaempferi*	y	mycetome	no yeast colony	Uncultured fungus clone OTU_F389 *Debaryomyces castellii* culture CBS:2923	43/43 589/627	OP537687 OP537688
Spondylidinae	*Tetropium castaneum*	Nagano	*Picea jezoensis*	y	mycetome	no yeast colony	*Debaryomyces* sp. UFMG-CM-Y343	618/660	OP537689
Spondylidinae	*Tetropium castaneum*	Nagano	*Picea jezoensis*	y	mycetome	no yeast colony			
Prioninae	*Psephactus remiger*	Shizuoka	*Fagus crenata*	n	gut	y	*Scheffersomyces* sp. strain Y13	601/601	OP537694
Prioninae	*Psephactus remiger*	Shizuoka	*Fagus crenata*	n	gut	y	*Candida boleticola*	590/590	OP537695
Prioninae	*Psephactus remiger*	Shizuoka	*Fagus crenata*	n	gut	no yeast colony			
Prioninae	*Psephactus remiger*	Shizuoka	*Fagus crenata*	n	gut	y 2	*Sugiyamaella smithiae* strain NS1	549/550	OP537696
Prioninae	*Psephactus remiger*	Yamanashi	*Abies* sp.	n	gut	y	*Scheffersomyces coipomoensis* strain Y3 *Scheffersomyces* sp. Y1052_SICYLG	614/615 619/619	OP537697 OP537698
Prioninae	*Psephactus remiger*	Mie	*Mallotus japonicus*	n	gut	y	[*Candida*] *boleticola* strain UCDFST:80-62#	590/590	OP537699
Prioninae	*Prionus insularis*	Cultured		n	gut	no yeast colony			
Prioninae	*Prionus insularis*	Cultured		n	gut	no yeast colony			
Prioninae	*Aegosoma sinicum*	Shizuoka1	*Mallotus japonicus*	n	gut	y	*Scheffersomyces anoplophorae*	591/591	OP537700
Prioninae	*Aegosoma sinicum*	Mie1	*Mallotus japonicus*	n	gut	y	*Scheffersomyces anoplophorae* *Rhodotorula mucilaginosa* strain 2018A0762M12	579/579 576/577	OP537701 OP537702
Prioninae	*Aegosoma sinicum*	Mie2	*Mallotus japonicus*	n	gut	y	*Scheffersomyces anoplophorae*	579/579	OP537703
Prioninae	*Aegosoma sinicum*	Mie3	*Mallotus japonicus*	n	gut	y	*Scheffersomyces* sp. YW28.1	613/613	OP537704
Prioninae	*Aegosoma sinicum*	Shiga	*Fagus crenata*	n	gut	y 7	*Scheffersomyces anoplophorae*	591/591	OP589184
Prioninae	*Aegosoma sinicum*	Kyoto1	*Populus* sp.	not observed	gut	no yeast colony			
Prioninae	*Aegosoma sinicum*	Kyoto2	*Populus* sp.	not observed	gut	y	*Scheffersomyces anoplophorae*	587/591	OP537705
Prioninae	*Aegosoma sinicum*	Kyoto3	*Populus* sp.	not observed	gut	y	*Scheffersomyces anoplophorae*	577/581	OP537706
Prioninae	*Aegosoma sinicum*	Ibaraki1	*Salix* sp.	n	gut	y	Uncultured Scheffersomyces clone 15y-3 Scheffersomyces sp. YW01.2	582/584 577/577	OP537707 OP537708
Prioninae	*Aegosoma sinicum*	Ibaraki2	*Salix* sp.	n	gut	y 4	*Scheffersomyces* sp. YW01.2	612/612	OP537709
Prioninae	*Aegosoma sinicum*	Ibaraki3	*Salix* sp.	n	gut	y	*Scheffersomyces anoplophorae*	591/591	OP537710
Prioninae	*Aegosoma sinicum*	Ibaraki4	*Salix* sp.	n	gut	y 3	*Scheffersomyces anoplophorae*	582/582	OP537711
Prioninae	*Aegosoma sinicum*	Ibaraki5	*Salix* sp.	n	gut	y	*Scheffersomyces anoplophorae*	590/591	OP537712
Prioninae	*Aegosoma sinicum*	Kagoshima Yakushima Island	unknown	n	frass gut	y	*Scheffersomyces* sp. YW01.2	612/612	OP537713
Prioninae	*Aegosoma sinicum*	Shizuoka2	*Fagus crenata*	n	gut	y	*Scheffersomyces* sp. YW27.1	612/612	OP537714
Prioninae	*Eurypoda batesi*	Kagoshima Yakushima Island	unknown	n	gut	y	*Scheffersomyces coipomoensis* strain Y3	615/615	OP537715
Prioninae	*Eurypoda batesi*	Kagoshima Yakushima Island	unknown	n	gut	no yeast colony			
Cerambycinae	*Xystrocera globosa*	Aichi	*Albizia julibrissin*	n	gut	y	*Ogataea naganishii* culture CBS:7259 *Kluyveromyces* sp. UFMG-CM-Y3682	711/712 657/676	OP537716 OP537717
Cerambycinae	*Xystrocera globosa*	Aichi	*Albizia julibrissin*	n	gut	y	*Ogataea naganishii* culture CBS:7259	711/711	OP537718
Cerambycinae	*Xylotrechus emaciatus*	Kyoto	*Celtis jessoensis*	n	gut	no yeast colony			
Cerambycinae	*Xylotrechus emaciatus*	Kyoto	*Celtis jessoensis*	n	gut	no yeast colony			
Cerambycinae	*Xylotrechus cuneipennis*	Shizuoka	*Acer* sp.	not observed	frass	y	*Scheffersomyces* sp. strain Y13	603/603	OP537719
Cerambycinae	*Xylotrechus cuneipennis*	Shizuoka	*Carpinus* sp.	n	frass	y	*Scheffersomyces* sp. strain Y13 *Candida khao -thaluensis* strain CBS 9930	590/590 608/622	OP537720 OP537721
Cerambycinae	*Xylotrechus cuneipennis*	Shizuoka	*Carpinus* sp.	n	frass	no yeast colony			
Cerambycinae	*Xylotrechus cuneipennis*	Shizuoka	*Fagus crenata*	n	gut	no yeast colony			
Cerambycinae	*Xylotrechus cuneipennis*	Shizuoka	*Fagus crenata*	n	gut	no yeast colony			
Cerambycinae	*Xylotrechus cuneipennis*	Shizuoka	*Fagus crenata*	n	gut	no yeast colony			
Cerambycinae	*Xylotrechus cuneipennis*	Shizuoka	*Fagus crenata*	n	gut	no yeast colony			
Cerambycinae	*Xylotrechus clarinus*	Nagano	*Betula platyphylla*	failed to observe	gut	no yeast colony			
Cerambycinae	*Xylotrechus clarinus*	Nagano	*Betula platyphylla*	n	gut	no yeast colony			
Cerambycinae	*Thranius variegatus*	Aichi	*Mallotus japonicus*	n	gut	y	*C. schatavii*	586/593	OP537722

Cerambycinae	*Thranius variegatus*	Aichi	*Mallotus japonicus*	n	gut	y	*C. schatavii*	586/593	OP537723
Cerambycinae	*Purpuricenus temminckii*	Aichi	*Phyllostachys edulis*	n	gut	y	*Candida blattae*	350/351	OP537724
Cerambycinae	*Purpuricenus temminckii*	Aichi	*Phyllostachys edulis*	n	gut	y	*Hyphopichia* sp. strain NYNU 20899	392/395	OP537725
Cerambycinae	*Phymatodes testaceus*	Nagano	*Quercus serrata*	n	gut	no yeast colony			
Cerambycinae	*Paraclytus excultus*	Yamanashi	Hardwood	n	gut	no yeast colony			
Cerambycinae	*Paraclytus excultus*	Yamanashi	Hardwood	n	gut	no yeast colony			
Cerambycinae	*Paraclytus excultus*	Yamanashi	Hardwood	n	gut	no yeast colony			
Cerambycinae	*Paraclytus excultus*	Yamanashi	Hardwood	failed to observe	gut	no yeast colony			
Cerambycinae	*Trichoferus campestris*	Yamanashi	*Prunus persica*	n	gut	y	*Hyphopichia homilentoma* culture CBS:6099	376/379	OP537726
Cerambycinae	*Trichoferus campestris*	Yamanashi	*Prunus persica*	n	gut	no yeast colony			
Cerambycinae	*Grammographus notabilis*	Yamanashi	*Pterocarya rhoifolia*	n	gut	y 4	*Hyphopichia homilentoma* culture CBS:6099 *Yamadazyma akitaensis* CBS 6701	385/386 604/611	OP537727 OP537728
Cerambycinae	*Epania dilaticornis*	Kagoshima	*Diospyros lotus*	failed to observe	frass	no yeast colony			
Cerambycinae	*Epania dilaticornis*	Kagoshima	*Diospyros lotus*	failed to observe	frass	no yeast colony			
Cerambycinae	*Epania dilaticornis*	Kagoshima	*Diospyros lotus*	failed to observe	frass	no yeast colony			
Cerambycinae	*Cyrtoclytus caproides*	Ibaraki	*Salix* sp.	n	gut	no yeast colony	not analyzed		
Cerambycinae	*Clytus auripilis*	Kyoto	*Celtis jessoensis*	n	gut	y 3	Uncultured *Candida*	608/615	OP537729
Cerambycinae	*Ceresium holophaeum*	Aichi	*Euonymus japonicus*	n	gut	y	*Hyphopichia pseudoburtonii*	400/400	OP537730
Cerambycinae	*Ceresium holophaeum*	Aichi	*Euonymus japonicus*	n	gut	y	*Meyerozyma carpophila*	568/568	OP537731
Cerambycinae	*Callidiellum rufipenne*	Nagano	*Chamaecyparis obtusa*	failed to observe	gut	no yeast colony			
Cerambycinae	*Callidiellum rufipenne*	Nagano	*Chamaecyparis obtusa*	n	gut	no yeast colony			
Cerambycinae	*Callidiellum rufipenne*	Nagano	*Chamaecyparis obtusa*	n	gut	no yeast colony			
Cerambycinae	*Aromia bungii*	Oosaka	*Cerasus* sp.	not observed	frass	y	[*Candida*] *succiphila* culture CBS:7297	623/623	OP537732
Cerambycinae	*Aromia bungii*	Oosaka	*Cerasus* sp.	not observed	frass	no yeast colony			
Cerambycinae	*Anaglyptus niponensis*	Yamanashi	*Pterocarya rhoifolia*	n	gut	y 2	*Hyphopichia homilentoma* culture CBS:6099	409/412	OP537733
Lamiinae	*Acalolepta sejuncta*	Shizuoka	*Acer* sp.	not observed	frass	no yeast colony			
Lamiinae	*Acalolepta sejuncta*	Shizuoka	*Carpinus* sp.	n	gut	no yeast colony			
Lamiinae	*Eumecocera argyrosticta*	Shizuoka	*Acer* sp.	not observed	frass	no yeast colony			
Lamiinae	*Eumecocera gleneoides*	Shizuoka	*Cornus controversa*	not observed	frass	no yeast colony			
Lamiinae	*Eutetrapha chrysochloris*	Shizuoka	*Tilia japonica*	not observed	frass	y	[*Candida*] *gorgasii* ATCC MYA-4334	607/644	OP537734
Lamiinae	*Eutetrapha chrysochloris*	Shizuoka	*Acer* sp.	not observed	frass	no yeast colony			
Lamiinae	*Eutetrapha chrysochloris*	Nagano	*Quercus serrata*	n	gut	y	*Candida* sp. (in: *Saccharomycetales*) strain NYUN 211183	435/436	OP537735
Lamiinae	*Eutetrapha chrysochloris*	Nagano	*Quercus serrata*	n	gut	y	*Yamadazyma mexicana* culture CBS:2089 *Pichia hampshirensis* strain ATCC 62896 *Candida* sp. (in: *Saccharomycetales*) strain NYUN 211183	606/618 572/587 435/436	OP537736 OP537737 OP537738
Lamiinae	*Leiopus stillatus*	Shizuoka	*Carpinus sp.*	not observed	frass	no yeast colony			
Lamiinae	*Leiopus stillatus*	Shizuoka	*Acer* sp.	not observed	frass	y	*Candida* sp. (in: *Saccharomycetales*) isolate P10-3-5	568/570	OP537739
Lamiinae	*Mecynippus pubicornis*	Nagano	*Salix* sp.	n	gut	no yeast colony			
Lamiinae	*Mecynippus pubicornis*	Nagano	*Salix* sp.	n	gut	no yeast colony			
Lamiinae	*Monochamus alternatus*	Shizuoka	*Pinus thunbergii*	n	gut	y	*Hyphopichia heimii* strain SSA1530	364/366	OP537740
Lamiinae	*Monochamus alternatus*	Shizuoka	*Pinus thunbergii*	n	gut	y	*Hyphopichia heimii* strain SSA1530	364/366	OP537741
Lamiinae	*Nanohammus rufescens*	Aichi	*Quercus crispula*	n	gut	no yeast colony			
Lamiinae	*Nanohammus rufescens*	Aichi	*Quercus crispula*	n	gut	no yeast colony			
Lamiinae	*Niphona furcata*	Aichi	*Bambuseae*	n	gut	y	*Hyphopichia burtonii* strain XWB32-4 *Hyphopichia* sp. isolate SFC101142	404/404 385/390	OP537742 OP537743
Lamiinae	*Niphona furcata*	Aichi	*Bambuseae*	n	gut	y	*Hyphopichia pseudoburtonii* isolate makgeolli	377/377	OP537744
Lamiinae	*Niphona furcata*	Aichi	*Bambuseae*	n	gut	y	*Hyphopichia burtonii* culture CBS:2452	425/428	OP537745
Lamiinae	*Oplosia suvorovi*	Aichi	*Tilia japonica*	n	gut	no yeast colony			
Lamiinae	*Palimna liturata*	Shizuoka	*Fagus crenata*	n	gut	no yeast colony			
Lamiinae	*Palimna liturata*	Shizuoka	*Fagus crenata*	n	gut	no yeast colony			
Lamiinae	*Palimna liturata*	Kagoshima	unknonw	n	gut	no yeast colony			
Lamiinae	*Parechthistatus gibber*	Shizuoka	*Fagus crenata*	n	frass	no yeast colony			
Lamiinae	*Parechthistatus gibber*	Shizuoka	*Fagus crenata*	n	frass	y	*Candida* sp. W370	539/541	OP537746
Lamiinae	*Parechthistatus gibber*	Shizuoka	*Fagus crenata*	n	gut	y	*Scheffersomyces* sp. Y1052_SICYLG3 *Sugiyamaella boreocaroliniensis* CBS 10344	596/596 555/555	OP537747 OP537748

Lamiinae	*Pseudocalamobius montanus*	Gifu	*Ribes japonicum*	n	gut	no yeast colony			
Lamiinae	*Pseudocalamobius montanus*	Gifu	*Ribes japonicum*	n	gut	no yeast colony			
Lamiinae	*Saperdini sp.*	Aichi	*Tilia japonica*	n	gut	no yeast colony			
Disteniidae	*Distenia gracilis*	Mie	*Carpinus tschonoskii*	n	gut	y	[*Candida*] *boleticola* strain UCDFST:80-62#	613/613	OP537690
Disteniidae	*Distenia gracilis*	Ibaraki	*Salix* sp.	n	gut	y 8	[*Candida*] *intermedia* strain DL6Y	374/374	OP537691
Disteniidae	*Distenia gracilis*	Shizuoka	*Fagus crenata*	n	gut	no colony			
Disteniidae	*Distenia gracilis*	Shizuoka	*Carpinus* sp.	n	gut	y 2	*Candida rhagii* culture CBS:618	376/415	OP537692
Disteniidae	*Distenia gracilis*	Shizuoka	*Carpinus* sp.	n	gut	y 2	*Candida* sp. (in: *Saccharomycetales*) strain NYUN 211183	435/437	OP537693
Stag beetle	*Aesalus asiaticus*	Shizuoka	*Symplocos coreana*	n	gut	y	*Scheffersomyces ergatensis* strain ATCC MYA-4655	609/615	OP537749
Stag beetle	*Aesalus asiaticus*	Shizuoka	*Symplocos coreana*	n	gut	y	*Scheffersomyces ergatensis* strain ATCC MYA-4655	609/615	OP537750
Stag beetle	*Aesalus asiaticus*	Shizuoka	*Symplocos coreana*	n	gut	y 1	not analyzed		
Stag beetle	*Aesalus asiaticus*	Shizuoka	*Symplocos coreana*	n	gut	y	*Scheffersomyces ergatensis* strain ATCC MYA-4655	609/615	OP537751

*The digits at the columns of "*Aegosoma sinicum* " correspond to those in Figure 9.
**The numbers of colonies on the plates are indicated when a few colonies appeared.

Table S2. Alignment of nucleotide sequences used for phylogenetic tree construction

Corresponding Figure	Indications in the tree	DNA sequence with gaps
	Judolidia bangi	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGC--CG--TCGGCGTTGGATGG-TCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCT--TTCTTCGAACCGACGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Munamizoa maculata	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGC--CG--TCGGCGTTGGATGG-TCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCT--TTCTTCGAACCGACGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Pachytodes cometes	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGAC--CG--TCGGCGTCGGATGG-TCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCT--TCCTTCGAACCGGCGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Aredolpona succedanea	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGCA--G--TCGGCGTCGGATGGATAGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCT--TCCTTCGAACCGGCGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Pyrrhona laeticolor	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGCC--G--TCGGCGTTGGATGG-TCGAGTGACGGACGGCG-T-A-CTCGTCG-TCCGCGCCTTCCT--TTCTTCGAACCGACGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Nakanea vicaria	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGC--CG--TCGGCGTTGGATGG-TCGAGTGACGGATGGCGGT-C-TACGTCG-TCCGCGCCTTCCT--TCCTTCGAACCGGCGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Ohbayashia nigromarginata	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGCC--G--TCGGCGTTGGATGG-TCGAGTGACGGACGGCG-T-A-CTCGTCG-TCCGCGCCTTCCT--TTCTTCGAACCGACGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Leptura ochraceofasciata	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGCC--G--TCGGCGTTGGATGG-TCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCT--TCTTTCGAACCGACGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Leptura modicenotata	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGCC--G--TTGGCGTTGGATGG-TCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCT--TCTTTCGAACCGACGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Strangalomorpha tenuis	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGCTCTG--TCGGCGTTGGATGG-TCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCTCGTCCTTCGAACCGACGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Anastrangalia scotodes	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGCA--G--TCGGCGTTGGATG-GTCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCT--TCCTTCGAACCGGCGTCGAACGCGTGCACTTTTCGCCCTAGTAGGACGTC
	Eustrangalis anticereducta	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGC--CG--TCGGCGTCGGATGG-TCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCT--TCCTTCGARCCGACGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Caraphia lepturoides	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGC--CG--TCGGCGTAGGATGG-TCGTGCGACGGACGGCG-TTCGCGCGTCG-TCCGCGTCCTCTT--TCCTTCGAACCGGCGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
Fig. 2	*Rhagium femorale*	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGC--CG--TCGGCGTTGGATGG-TCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCT--TCTTTCGAACCGGCGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Ephies japonicus	AGTACGTGAAACCGTTTAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGTCT--G--TCGGCGTTGGACGGGTCGACTGACGGACGACG-T-A-CGCGTCG-TCCGCTTCTTCCT--TTCTTCGAACCGACGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Mimostrangalia kurosonensi	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGCTTTG--TTGGCGTCGAATGG-TCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCT--TTCTTCGAACCGACGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Strangalia koyaensis	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGCTTCG--TCGGCGTTGGATGG-TCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCT--TTCTTCGAACCGGCGCCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Mimostrangalia dulcis	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGCTTTGTGTCGGCGTCGAATGG-TCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCT--TTCTTCGAACCGACGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Parastrangalia lesnei	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGC--CG--TCGGCGTCGGATGG-TCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCT--TCCTTCGAACCGGCGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Stenocorus caeruleipennis	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGC--CG--TCGGCGTGGGATGG-TGGAGTGACGGACGGCG-TTC-TGCGTCG-TCCGCGCCTTCTT--TCCTTCGTACCGGCGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Rhagium japonicum	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGC--CG--TCGGCGTTGGATGG-TCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCT--TCCTTCGAACCGGCGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Pidonia aegrota	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGC--TG--CCGGCGTTGGATGG-TCGAGTGACGGACGGCG-T-A-CGCGTCG-ACCGCGCCTTCCT--TTCTTCGAACCGGCGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Pidonia oyamae	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGC--TG--CCGGCGTTGGATGG-TCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCTT--TCCTTCGAACCGGCGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Gaurotes doris	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGC--CG--TCGGCGTCGGTAGG-TCGAGTGACGGATGGCG-T-A-CGCGTCG-TCCGCGCCTTCCT--TCCTTCGATCCGGCGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Encyclops olivacea	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGGC--CG--TCGGCGTCGGATGG-TCGAGTGACGGATGGCG-T-T-CGCGTCG-TCCGCGCCTTCCT--TCCTTCGAACCGGCGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Necydalis gigantea	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTTTCGAT--CG--CTGGCGTGGGACGGGTCGTGCGACGGACGACG-T-A-CGCGTCG-TTCGCGCCTTCCT--TCTTTCGAACCGCGATCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Necydalis formosana	AGTACGTGAAACCGTTCAGGGGTAAACCTGAGAAACCCGAAAGGTCGAAAGGGGAAATTCATTCGCGTGTCGAC--GC--GCGGCGAGGGTCGGATCGGGCGACGGTCGGCG-T-A-CGCGCCGTTCCGCGCCTTCCT--TTCTTCGAACCGTCGTCGAACGCGTGCACTTTTCCCCTAGTAGGACGTC
	Anastrangalia scotodes Cv OP537521	CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTTACACACTGTGTTTTTGTTTTT-TT---GAAACATTGCTTTGGCTTGGCTT------AATTGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTTT-TTAATTTGTTTGTC----AATTTTGTTTGATTAATTTC
	Anastrangalia scotodes Se OP537527	TTACCTGCGCTTAATTGCGCGGTAGACA------AA-CT-TACACACAGTGTTTTTCTTTAT-T---GAAACTATTGCTTTGGTCTGACTT-AGAAATA-AGCTGGGCCAGAGGTTTAA---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAAATTC
	Stictoleptura dichroa Se OP537530	TTACCTGCGCTTAATTGCGCGGTAGACA------AA-CT-TACACACAGTGTTTTTCTTTAT-T---GAAACTATTGCTTTGGTCTGACTT-AGAAATA-AGCTGGGCCAGAGGTTTAA---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAAATTC
	Caraphia lepturoides Ssp OP537535	TTGCCAGCGCTTAACTGCGCGGCGAAAA------AACCT-TACACACAGTGTTTT-CTTTATTA---GAAACTATTGCTTTGGTCTGGCTT-AGAAATA-AGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATTTTATTTAATTTGTC----AATTTG--TTGATTAAATTC
	Ephies japonicus Cv OP537537	CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTTACACACTGTGTTTTTGTTTTT-TT---GAAACATTGCTTTGGCTTGGCTT------TATTGTCGGGCCAGAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTT-TTAATTTATTTGTC----AATTTTGTTTGATTAATTTC
	Eustrangalis anticereducta Ssp OP537539	TTGCCGGCGCTTAACTGCGCGGCGAAAA------AACCT-TACACACAGTGTTTT-CTTTATTA---GAAACTATTGCTTTGGTCTGGCTT-AGAAATA-AGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATTTTATTTAATTTGTC----AATTTG--TTGATTAAATTC
	Judolidia bangi Se OP537542	TTACCTGCGCTTAATTGCGCGGTAGACA------AA-CT-TACACACAGTGTTTTTCTTTAT-T---GAAACTATTGCTTTGGTCTGACTT-AGAAATA-AGCTGGGCCAGAGGTTTAA---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAAATTC
	Leptura annulalis Ssp OP537543	TTGCCAGCGCTTAACTGCGCGGCGAAAA------AACCT-TACACACAGTGTTTT-CTTTATTA---GAAACTATTGCTTTGGTCTGGCTT-AGAAATA-AGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATTTTATTTAATTTGTC----AATTTG--TTGATTAAATTC
	Leptura ochraceofasciata Ssp OP537548	TTGCCAGCGCTTAACTGCGCGGCGAAAA------AACCT-TACACACAGTGTTTT-CTTTATTA---GAAACTATTGCTTTGGTCTGGCTT-AGAAATA-AGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATTTTATTTAATTTGTC----AATTTG--TTGATTAAATTC
	Leptura ochraceofasciata Cv OP537557	CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTTACACACTGTGTTTTTGTTTTT-TT---GAAACATTGCTTTGGCTTGGCTT------AATTGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTTT-TTAATTTGTTTGTC----AATTTTGTTTGATTAATTTC
	Leptura ochraceofasciata Sc OP537560	TTACCTGCGCTTAATTGCGCGGTAAACA------AATCT-TACACACAGTGTTTTTCTTTAT-T--AGAAACTTTTGCTTTGGTTTGGCTT-AGAAATA-AGTTGGGCCAGAGGTTTAT---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAATTTC
	Pigostrangalia dulcis Cv OP537567	CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTTACACACTGTGTTTTTGTTTTT-TT---GAAACATTGCTTTGGCTTGGCTT------AATTGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTT-TTAATTTGTTTGTC----AATTTTGTTTGATTAATTTC
	Mimostrangalia kurosonensis Cv OP537569	CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTTACACACTATGTTTTTGTTTTT-TT---GAAACATTGCTTTGGCTTGGCTT------TATTGTCGGGCCAGAGACTACACTAAACTTCAGATTTTTATTTGAATTGTT---TTAATTTGTTTGTC----AATTTTGTTTGATTAATTTC
	Munamizoa maculata Se OP537572	TTACCTGCGCTTAATTGCGCGGTAGACA------AA-CT-TACACACAGTGTTTTTCTTTAT-T---GAAACTATTGCTTTGGTCTGACTT-AGAAATA-AGCTGGGCCAGAGGTTTAA---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAAATTC
	Munamizoa maculata Ssp OP537570	TTACCTGCGCTTAATTGCGCGGTAGACA------AA-CT-TACACACAGTGTTTTTCTTTAT-T---GAAACTATTGCTTTGGTCTGACTT-AGAAATA-AGCTGGGCCAGAGGTTTAA---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAAATTC
	Munamizoa maculata Ssp OP537571	TTACCTGCGCTTAATTGCGCGGTAGACA------AA-CT-TACACACAGTGTTTTTCTTTAT-T---GAAACTATTGCTTTGGTCTGACTT-AGAAATA-AGCTGGGCCAGAGGTTTAA---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAAATTC
	Etorofus vicarius Ssp OP537580	TTACCTGCGCTTAATTGCGCGGTAAACA------AA-CT-TACACACAGTGTTTTTCTTTAT-T---GAAACTATTGCTTTGGTCTGACTT-AGAAATA-AGCTGGGCCAGAGGTTTAA---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAAATTC
	Etorofus vicarius Ssp OP537584	TTGCCAGCGCTTAACTGCGCGGCGAAAA------AACCT-TACACACAGTGTTTT-CTTTATTA---GAAACTATTGCTTTGGTCTGGCTT-AGAAATA-AGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATTTTATTTAATTTGTC----AATTTG--TTGATTAAATTC
	Etorofus vicarius Se OP537581	TTACCTGCGCTTAATTGCGCGGTAAACA------AA-CT-TACACACAGTGTTTTTCTTTAT-T---GAAACTATTGCTTTGGTCTGACTT-AGAAATA-AGCTGGGCCAGAGGTTTAA---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAAATTC
Fig. 4	*Ohbayashia nigromarginata* Sc OP537585	TTACCTGCGCTTAATTGCGCGGTAAACA------AATCT-TACACACAGTGTTTTTCTTTAT-T--AGAAACTTTTGCTTTGGTTTGGCTT-AGAAATA-AGTTGGGCCAGAGGTTTAT---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAATTTC
	Ohbayashia nigromarginata Cv OP537587	CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTTACACACTGTGTTTTTGTTTTT-TT---GAAACATTGCTTTGGCTTGGCTT------AATTGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTTT-TTAATTTGTTTGTC----AATTTTGTTTGATTAATTTC
	Pachytodes cometes Se OP537597	TTACCTGCGCTTAATTGCGCGGTAGACA------AA-CT-TACACACAGTGTTTTTCTTTAT-T---GAAACTATTGCTTTGGTCTGACTT-AGAAATA-AGCTGGGCCAGAGGTTTAA---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAAATTC
	Pachytodes cometes Ssp OP537598	TTACCTGCGCTTAATTGCGCGGTAGACA------AA-CT-TACACACAGTGTTTTTCTTTAT-T---GAAACTATTGCTTTGGTCTGACTT-AGAAATA-AGCTGGGCCAGAGGTTTAA---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAAATTC
	Parastrangalia lesnei Cv OP537601	CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTTACACACTGTGTTTTTGTTTTT-TT---GAAACATTGCTTTGGCTTGGCTT------AATTGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTTT-TTAATTTGTTTGTC----AATTTTGTTTGATTAATTTC
	Pyrrhona laeticolor Cv OP537602	CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTTACACACTGTGTTTTTGTTTTT-TT---GAAACATTGCTTTGGCTTGGCTT------AATTGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTTT-TTAATTTGTTTGTC----AATTTTGTTTGATTAATTTC
	Pyrrhona laeticolor Sc OP537615	TTACCTGCGCTTAATTGCGCGGTAAACA------AATCT-TACACACAGTGTTTTTCTTTAT-T--AGAAACTTTTGCTTTGGTTTGGCTT-AGAAATA-AGTTGGGCCAGAGGTTTAT---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAATTTC
	Pyrrhona laeticolor Se OP537628	TTACCTGCGCTTAATTGCGCGGTAGACA------AA-CT-TACACACAGTGTTTT-CTTTAT-T---GAAACTATTGCTTTGGTCTGATTT-AGAAATA-AGTTGGGCCAGAGGTTTAA---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAAATTC
	Pyrrhona laeticolor Sp OP537629	TTACCTGCGCTTAATTGCGCGGTAGACA------AA-CT-TACACACAGTGTTTT-CTTTAT-T---GAAACTATTGCTTTGGTCTGATTT-AGAAATA-AGTTGGGCCAGAGGTTTAA---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAAATTC
	Rhagium femorale Ct OP537635	TTGCCAGCGCTTAACTGCGCGGCGAAAAC----TAATCCTTACACACTGTGTTTTTGTTAT-TACAAGAACCATTGCTTTGGCTTGGCCG------CAAGGTTGGGCCAGAGGACACAC-AAACTTCAATTTT--ATT-GAATTGTTAATTTTAATTATTTGTC----AATTT-GTTTGATTAATTTC
	Strangalia koyaensis Cv OP537643	CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTTACACACTGTGTTTTTGTTTTT-TT---GAAACATTGCTTTGGCTTGGCTT------CATTGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTTT-TTAATTTGTTTGTC----AATTTTGTTTGATTAATTTC
	Strangalomorpha tenuis Ssp OP537646	TTGCCAGCGCTTAACTGCGCGGCGAAAA------AACCT-TACACACAGTGTTTT-CTTTATTA---GAAACTATTGCTTTGGTCTGGCTT-AGAAATA-AGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATTTTATTTAATTTGTC----AATTTG--TTGATTAAATTC
	Leptura subtilis Ssp OP537566	TTGCCAGCGCTTAACTGCGCGGCGAAAA------AACCT-TACACACAGTGTTTT-CTTTATTA---GAAACTATTGCTTTGGTCTGGCTT-AGAAATA-AGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATTTTATTTAATTTGTC----AATTTG--TTGATTAAATTC
	Megasemum quatricostulatum Dc OP537681	TTGCCAGCGCTTAATTGCGCGGCGAAAA------AACCT-TACACACAGTGTTTTTCTTATTTA---GAAACTATTGCTTTGGTCTGGCTCTAGAAATAGAGTTGGGCCAGAGGTCAAACCAAACTTCAATTTT-TAATTGAATTGTTATT-TAATTATAA----TGTCAATTTG--TTGATTAAATTC
	Megasemum quatricostulatum Ssp OP537683	TTACCTGCGCTTAATTGCGCGGTAAACA------AA-CT-TACACACAGTGTTTTTCTTTAT-T---GAAACTATTGCTTTGGTCTGACTT-AGAAATA-AGCTGGGCCAGAGGTTTAA---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAAATTC
	Megasemum quatricostulatum Se OP537684	TTACCTGCGCTTAATTGCGCGGTAAACA------AA-CT-TACACACAGTGTTTTTCTTTAT-T---GAAACTATTGCTTTGGTCTGACTT-AGAAATA-AGCTGGGCCAGAGGTTTAA---AACTTCAATTTT-TAATTGAATTGTTTATTTATTTAATT-GTC----AATTTG--TTGATTAAATTC
	Asemum striatum Sc OP537674	TTGCCAGCGCTTAATTGCGCGGCGAAAA------AACCT-TACACACAGTGTTTTTCTTATT-A---AAAACTATTGCTTTGGTCTGGCTCTAGAAATAGAGTTGGGCCAGAGGTCAAACCAAATTTCAATTTT-TAATTGAATTGTTATT-TAATTA-ATTGTC----AATTTG--TTGATTAAATTC
	Asemum striatum Dc OP537675	TTGCCAGCGCTTAATTGCGCGGCGAAAA------AACCT-TACACACAGTGTTTTTCTTATT-A---AAAACTATTGCTTTGGTCTGGCTCTAGAAATAGAGTTGGGCCAGAGGTCAAACCAAATTTCAATTTT-TAATTGAATTGTTATT-TAATTA-ATTGTC----AATTTG--TTGATTAAATTC
	Cephalallus unicolor Sv OP537677	TTGCCAGCGCTTAATTGCGCGGTAAAAA------AACCTTTACACACAGTGTTTTTCTTATTTA---GAAACTATTGCTTTGGTTTGGCTCTAGAAATAGAGTTGGGCCAGAGGTCAAACCAAACTTCAATTTT-TAATTGAATTGTTATT-TAATTATATAATGTATCAACTT----TGTTTAAATTC

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Cephalallus unicolor Dsp OP537676      TTGCCAGCGCTTAATTGCGCGGTAAAAA------AACCTTTACACACAGTGTTTTTCTTATTTA---GAAACTATTGCTTTGGTTTGGCTCTAGAAATAGAGTTGGGCCAGAGGTCAAACCAAACTTCAATTTT-TAATTGAATTGTTATT-TAATTATATAATGTATCAACTT----TGTTTAAATTC
Tetropium castaneum Dsp OP537689       TTGCCAGCGCTTAGTTGCGCGGCGAAAA------AACCT-TACACACAGTGTTTTTCTTATT-A---AAAACTATTGCTTTGGTCTGGCTCTAGAAGTAGAGTTGGGTCAGAGGTCAAACCAAACTTCAATTTT-TAATTGAATTGTTATT-TAATTATAATGTC----AATTTG--TTGATTAAATTC
Strangalomorpha tenuis Ad OP537752     ACA----G-TATTCT----TTTTGCCAGCGCTTAACTGCGCGGCG-------AAAAAACCTTACACACAGTG-TTTTCTTTATTAGAAACTATTGCTTTGGTCTGGCTTAGAAATAAGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATTTTATTTAATTTGTCAATTT-G-
Strangalomorpha tenuis Ad OP537753     ACA----G-TATTCT----TTTTGCCAGCGCTTAACTGCGCGGCG-------AAAAAACCTTACACACAGTG-TTTTCTTTATTAGAAACTATTGCTTTGGTCTGGCTTAGAAATAAGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATTTTATTTAATTTGTCAATTT-G-
Strangalomorpha tenuis Ad OP537754     ACA----G-TATTCT----TTTTGCCAGCGCTTAACTGCGCGGCG-------AAAAAACCTTACACACAGTG-TTTTCTTTATTAGAAACTATTGCTTTGGTCTGGCTTAGAAATAAGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATTTTATTTAATTTGTCAATTT-G-
Pyrrhona laeticolor Ad OP537758        ACA----G-TATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------AACAAATCTTACACACAGTGTTTTTCTTTATTAGAAACTTTTGCTTTGGTTTGGCTTAGAAATAAGTTGGGCCAGAGGTTTA---TAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Pyrrhona laeticolor Ad OP537759        ACAGTTAG-TTTATGTTCTCTCAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTTACACACTGTGTTTTTGTTTTTTTGAAAC-ATTGCTTTGGCTTGGCTTAA----TTGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTTTTTAATTTGTT-TGTCAATTTTGT
Pachytodes cometes Ad OP537760         ATT----G-TATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------GACAAA-CTTACACACAGTGTTTTTCTTTATT-GAAACTATTGCTTTGGTCTGACTTAGAAATAAGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Pachytodes cometes Ad OP537761         ATT----G-TATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------GACAAA-CTTACACACAGTGTTTTTCTTTATT-GAAACTATTGCTTTGGTCTGACTTAGAAATAAGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Pachytodes cometes Ad OP537762         ATT----G-TATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------GACAAA-CTTACACACAGTGTTTTTCTTTATT-GAAACTATTGCTTTGGTCTGACTTAGAAATAAGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Pachytodes cometes Ad OP537763         ATT----G-TATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------GACAAA-CTTACACACAGTGTTTTTCTTTATT-GAAACTATTGCTTTGGTCTGACTTAGAAATAAGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Pachytodes cometes Ad OP537764         ACA----G-TATTCT----TTTTGCCAGCGCTTAACTGCGCGGCG-------AAAAAACCTTACACACAGTG-TTTTCTTTATTAGAAACTATTGCTTTGGTCTGGCTTAGAAATAAGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATTTTATTTAATTTGTCAATTT-G-
Ohbayashia nigromarginata Ad OP537766  ACA----G-TATTC-----TTTTGCCAGCGCTTAACTGCGCGGCG-------AA-AAACCTTACACACAGTG-TCTTTTTGATACAGAACTCTTGCTTTGGTTTGGCCTAGAGATAGGTTGGGCCAGAGGTTTA---AC-----AAAACA-CAATTTAATTATTTTTACAGTTA----GTCAAATT-T-
Ohbayashia nigromarginata Ad OP589185  ACAGTTAG-TTTATGTTCTCTCAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTTACACACTGTGTTTTTGTTTTTTTGAAAC-ATTGCTTTGGCTTGGCTTAA----TTGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTTTTTAATTTGTT-TGTCAATTTTGT
Ohbayashia nigromarginata Ad OP537767  ACA----G-TATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------AACAAATCTTACACACAGTGTTTTTCTTTATTAGAAACTTTTGCTTTGGTTTGGCTTAGAAATAAGTTGGGCCAGAGGTTTA---TAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Ohbayashia nigromarginata Ad OP537768  ACA----G-TATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------AACAAATCTTACACACAGTGTTTTTCTTTATTAGAAACTTTTGCTTTGGTTTGGCTTAGAAATAAGTTGGGCCAGAGGTTTA---TAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Etorofus vicarius Ad OP537769          AAT----GTTATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------AACAAA-CTTACACACAGTGTTTTTCTTTATT-GAAACTATTGCTTTGGTCTGACTTAGAAATAAGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Etorofus vicarius Ad OP537770          AAT----GTTATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------AACAAA-CTTACACACAGTGTTTTTCTTTATT-GAAACTATTGCTTTGGTCTGACTTAGAAATAAGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Etorofus vicarius Ad OP537771          ATT----G-TATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------GACAAA-CTTACACACAGTGTTTTTCTTTATT-GAAACTATTGCTTTGGTCTGACTTAGAAATAAGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Strangalomorpha tenuis Lv OP537646    ACA----G-TATTCT----TTTTGCCAGCGCTTAACTGCGCGGCG-------AAAAAACCTTACACACAGTG-TTTTCTTTATTAGAAACTATTGCTTTGGTCTGGCTTAGAAATAAGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATTTTATTTAATTTGTCAATTT-G-
Pyrrhona laeticolor Lv OP537602        ACAGTTAG-TTTATGTTCTCTCAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTTACACACTGTGTTTTTGTTTTTTTGAAAC-ATTGCTTTGGCTTGGCTTAA----TTGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTTTTTAATTTGTT-TGTCAATTTTGT
Pyrrhona laeticolor Lv OP537615        ACA----G-TATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------AACAAATCTTACACACAGTGTTTTTCTTTATTAGAAACTTTTGCTTTGGTTTGGCTTAGAAATAAGTTGGGCCAGAGGTTTA---TAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Pyrrhona laeticolor Lv OP537628        ACA----G-TATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------GACAAA-CTTACACACAGTG-TTTTCTTTATT-GAAACTATTGCTTTGGTCTGATTTAGAAATAAGTTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Pyrrhona laeticolor Lv OP537629        ACA----G-TATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------GACAAA-CTTACACACAGTG-TTTTCTTTATT-GAAACTATTGCTTTGGTCTGATTTAGAAATAAGTTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Pachytodes cometes Lv OP537597         ATT----G-TATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------GACAAA-CTTACACACAGTGTTTTTCTTTATT-GAAACTATTGCTTTGGTCTGACTTAGAAATAAGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Pachytodes cometes Lv OP537598         ATT----G-TATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------GACAAA-CTTACACACAGTGTTTTTCTTTATT-GAAACTATTGCTTTGGTCTGACTTAGAAATAAGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Ohbayashia nigromarginata Lv OP537585  ACA----G-TATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------AACAAATCTTACACACAGTGTTTTTCTTTATTAGAAACTTTTGCTTTGGTTTGGCTTAGAAATAAGTTGGGCCAGAGGTTTA---TAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Ohbayashia nigromarginata Lv OP537587  ACAGTTAG-TTTATGTTCTCTCAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTTACACACTGTGTTTTTGTTTTTTTGAAAC-ATTGCTTTGGCTTGGCTTAA----TTGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTTTTTAATTTGTT-TGTCAATTTTGT
Etorofus vicarius Lv OP537580          AAT----GTTATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------AACAAA-CTTACACACAGTGTTTTTCTTTATT-GAAACTATTGCTTTGGTCTGACTTAGAAATAAGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Etorofus vicarius Lv OP537581          AAT----GTTATTAG----TTTTACCTGCGCTTAATTGCGCGGTA-------AACAAA-CTTACACACAGTGTTTTTCTTTATT-GAAACTATTGCTTTGGTCTGACTTAGAAATAAGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTATTTATTTAAT-TGTCAATTT-G-
Etorofus vicarius Lv OP537584          ACA----G-TATTCT----TTTTGCCAGCGCTTAACTGCGCGGCG-------AAAAAACCTTACACACAGTG-TTTTCTTTATTAGAAACTATTGCTTTGGTCTGGCTTAGAAATAAGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATTTTATTTAATTTGTCAATTT-G-
Anastrangalia scotodes Cv OP537521     CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTT-ACACACTGTGTTTTTGTTTTTT---TGAAACATTGCTTTGGCTTGGCT-TAA---T--TGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTT-T--TTAATTTGTTTGTCAATTTTGTTTGATTAATTTCAA
Anastrangalia scotodes Se OP537527     TTACCTGCGCTTAATTGCGCGGTAGACAA------A-CT-T-ACACACAGTGTTTTTC--TTTATT-GAAACTATTGCTTTGGTCTGACT-TAGAAATA-AGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAAATTCAA
Stictoleptura dichroa Se OP537530      TTACCTGCGCTTAATTGCGCGGTAGACAA------A-CT-T-ACACACAGTGTTTTTC--TTTATT-GAAACTATTGCTTTGGTCTGACT-TAGAAATA-AGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAAATTCAA
Caraphia lepturoides Ssp OP537535      TTGCCAGCGCTTAACTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTT-C--TTTATTAGAAACTATTGCTTTGGTCTGGCT-TAGAAATA-AGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATT--TTATTTAATTTGTCAATTT--GTTGATTAAATTCAA
Ephies japonicus Cv OP537537           CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTT-ACACACTGTGTTTTTGTTTTTT---TGAAACATTGCTTTGGCTTGGCT-TTA---T--TGTCGGGCCAGAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTT-T--TTAATTTATTTGTCAATTTTGTTTGATTAATTTCAA
Eustrangalis anticereducta Ssp OP537539 TTGCCGGCGCTTAACTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTT-C--TTTATTAGAAACTATTGCTTTGGTCTGGCT-TAGAAATA-AGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATT--TTATTTAATTTGTCAATTT--GTTGATTAAATTCAA
Judolidia bangi Se OP537542            TTACCTGCGCTTAATTGCGCGGTAGACAA------A-CT-T-ACACACAGTGTTTTTC--TTTATT-GAAACTATTGCTTTGGTCTGACT-TAGAAATA-AGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAAATTCAA
Leptura annulalis Ssp OP537543         TTGCCAGCGCTTAACTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTT-C--TTTATTAGAAACTATTGCTTTGGTCTGGCT-TAGAAATA-AGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATT--TTATTTAATTTGTCAATTT--GTTGATTAAATTCAA
Leptura ochraceofasciata Ssp OP537548  TTGCCAGCGCTTAACTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTT-C--TTTATTAGAAACTATTGCTTTGGTCTGGCT-TAGAAATA-AGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATT--TTATTTAATTTGTCAATTT--GTTGATTAAATTCAA
Leptura ochraceofasciata Cv OP537557   CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTT-ACACACTGTGTTTTTGTTTTTT---TGAAACATTGCTTTGGCTTGGCT-TAA---T--TGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTT-T--TTAATTTGTTTGTCAATTTTGTTTGATTAATTTCAA
Leptura ochraceofasciata Sc OP537560   TTACCTGCGCTTAATTGCGCGGTAAACAA------ATCT-T-ACACACAGTGTTTTTC--TTTATTAGAAACTTTTGCTTTGGTTTGGCT-TAGAAATA-AGTTGGGCCAGAGGTTTA---TAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAATTTCAA
Pigostrangalia dulcis Cv OP537567      CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTT-ACACACTGTGTTTTTGTTTTTT---TGAAACATTGCTTTGGCTTGGCT-TAA---T--TGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTT-T--TTAATTTGTTTGTCAATTTTGTTTGATTAATTTCAA
Mimostrangalia kurosonensis Cv OP537569 CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTT-ACACACTATGTTTTTGTTTTTT---TGAAACATTGCTTTGGCTTGGCT-TTA---T--TGTCGGGCCAGAGACTACACTAAACTTCAGATTTTTATTTGAATTGTT-----TTAATTTGTTTGTCAATTTTGTTTGATTAATTTCAA
Munamizoa maculata Se OP537572         TTACCTGCGCTTAATTGCGCGGTAGACAA------A-CT-T-ACACACAGTGTTTTTC--TTTATT-GAAACTATTGCTTTGGTCTGACT-TAGAAATA-AGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAAATTCAA
Munamizoa maculata Ssp OP537570        TTACCTGCGCTTAATTGCGCGGTAGACAA------A-CT-T-ACACACAGTGTTTTTC--TTTATT-GAAACTATTGCTTTGGTCTGACT-TAGAAATA-AGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAAATTCAA
Munamizoa maculata Ssp OP537571        TTACCTGCGCTTAATTGCGCGGTAGACAA------A-CT-T-ACACACAGTGTTTTTC--TTTATT-GAAACTATTGCTTTGGTCTGACT-TAGAAATA-AGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAAATTCAA
Etorofus vicarius Ssp OP537580         TTACCTGCGCTTAATTGCGCGGTAAACAA------A-CT-T-ACACACAGTGTTTTTC--TTTATT-GAAACTATTGCTTTGGTCTGACT-TAGAAATA-AGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAAATTCAA
Etorofus vicarius Ssp OP537584         TTGCCAGCGCTTAACTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTT-C--TTTATTAGAAACTATTGCTTTGGTCTGGCT-TAGAAATA-AGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATT--TTATTTAATTTGTCAATTT--GTTGATTAAATTCAA
Etorofus vicarius Se OP537581          TTACCTGCGCTTAATTGCGCGGTAAACAA------A-CT-T-ACACACAGTGTTTTTC--TTTATT-GAAACTATTGCTTTGGTCTGACT-TAGAAATA-AGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAAATTCAA
Ohbayashia nigromarginata Sc OP537585  TTACCTGCGCTTAATTGCGCGGTAAACAA------ATCT-T-ACACACAGTGTTTTTC--TTTATTAGAAACTTTTGCTTTGGTTTGGCT-TAGAAATA-AGTTGGGCCAGAGGTTTA---TAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAATTTCAA
Ohbayashia nigromarginata Cv OP537587  CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTT-ACACACTGTGTTTTTGTTTTTT---TGAAACATTGCTTTGGCTTGGCT-TAA---T--TGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTT-T--TTAATTTGTTTGTCAATTTTGTTTGATTAATTTCAA
Pachytodes cometes Se OP537597         TTACCTGCGCTTAATTGCGCGGTAGACAA------A-CT-T-ACACACAGTGTTTTTC--TTTATT-GAAACTATTGCTTTGGTCTGACT-TAGAAATA-AGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAAATTCAA
Pachytodes cometes Ssp OP537598        TTACCTGCGCTTAATTGCGCGGTAGACAA------A-CT-T-ACACACAGTGTTTTTC--TTTATT-GAAACTATTGCTTTGGTCTGACT-TAGAAATA-AGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAAATTCAA
Parastrangalia lesnei Cv OP537601      CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTT-ACACACTGTGTTTTTGTTTTTT---TGAAACATTGCTTTGGCTTGGCT-TAA---T--TGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTT-T--TTAATTTGTTTGTCAATTTTGTTTGATTAATTTCAA
Pyrrhona laeticolor Cv OP537602        CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTT-ACACACTGTGTTTTTGTTTTTT---TGAAACATTGCTTTGGCTTGGCT-TAA---T--TGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTT-T--TTAATTTGTTTGTCAATTTTGTTTGATTAATTTCAA
Pyrrhona laeticolor Sc OP537615        TTACCTGCGCTTAATTGCGCGGTAAACAA------ATCT-T-ACACACAGTGTTTTTC--TTTATTAGAAACTTTTGCTTTGGTTTGGCT-TAGAAATA-AGTTGGGCCAGAGGTTTA---TAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAATTTCAA
Pyrrhona laeticolor Se OP537628        TTACCTGCGCTTAATTGCGCGGTAGACAA------A-CT-T-ACACACAGTGTTTT-C--TTTATT-GAAACTATTGCTTTGGTCTGATT-TAGAAATA-AGTTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAAATTCAA
Pyrrhona laeticolor Sp OP537629        TTACCTGCGCTTAATTGCGCGGTAGACAA------A-CT-T-ACACACAGTGTTTT-C--TTTATT-GAAACTATTGCTTTGGTCTGATT-TAGAAATA-AGTTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAAATTCAA
Rhagium femorale Ct OP537635           TTGCCAGCGCTTAACTGCGCGGCGAAAAC----TAATCCTT-ACACACTGTGTTTTTTGTTATTACAAGAACCATTGCTTTGGCTTGGCC-GCA---A--GGTTGGGCCAGAGGACACAC-AAACTTCAATTT--TATT-GAATTGTTAAT--TTTAATTATTTGTCAATTT-GTTTGATTAATTTCAA
Strangalia koyaensis Cv OP537643       CAGCCTGCGCTTAATTGCGCGGCGAGGAACAGAC-AACCTT-ACACACTGTGTTTTTGTTTTTT---TGAAACATTGCTTTGGCTTGGCT-TCA---T--TGTCGGGCCAAAGACTACACTAAACTTCAGATTTTTATTTGAATTGTTT-T--TTAATTTGTTTGTCAATTTTGTTTGATTAATTTCAA
Strangalomorpha tenuis Ssp OP537646   TTGCCAGCGCTTAACTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTT-C--TTTATTAGAAACTATTGCTTTGGTCTGGCT-TAGAAATA-AGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATT--TTATTTAATTTGTCAATTT--GTTGATTAAATTCAA
Leptura subtilis Ssp OP537566          TTGCCAGCGCTTAACTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTT-C--TTTATTAGAAACTATTGCTTTGGTCTGGCT-TAGAAATA-AGTTGGGCCAGAGGTTTAACTAAACTTCAATTTTATTATTGAATTGTTATT--TTATTTAATTTGTCAATTT--GTTGATTAAATTCAA
Megasemum quatricostulatum Dc OP537681 TTGCCAGCGCTTAATTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTTTC--TTATTTAGAAACTATTGCTTTGGTCTGGCTCTAGAAATAGAGTTGGGCCAGAGGTCAAACCAAACTTCAATTTT-TAATTGAATTGTTATT---TAATTATAATGTCAATTTG--TTGATTAAATTCAA
Megasemum quatricostulatum Ssp OP537683 TTACCTGCGCTTAATTGCGCGGTAAACAA------A-CT-T-ACACACAGTGTTTTTC--TTTATT-GAAACTATTGCTTTGGTCTGACT-TAGAAATA-AGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAAATTCAA
Megasemum quatricostulatum Se OP537684 TTACCTGCGCTTAATTGCGCGGTAAACAA------A-CT-T-ACACACAGTGTTTTTC--TTTATT-GAAACTATTGCTTTGGTCTGACT-TAGAAATA-AGCTGGGCCAGAGGTTTA---AAACTTCAATTTT-TAATTGAATTGTTTAT--TTATTTAATT-GTCAATTT--GTTGATTAAATTCAA
Asemum striatum Sc OP537674            TTGCCAGCGCTTAATTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTTTC--TTATT-AAAAACTATTGCTTTGGTCTGGCTCTAGAAATAGAGTTGGGCCAGAGGTCAAACCAAATTTCAATTTT-TAATTGAATTGTTATT---TAATTAA-TTGTCAATTTG--TTGATTAAATTCAA
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Fig. 5

Fig. 9

Asemum striatum Dc OP537675	TTGCCAGCGCTTAATTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTTTC--TTATT-AAAAACTATTGCTTTGGTCTGGCTCTAGAAATAGAGTTGGGCCAGAGGTCAAACCAAATTTCAATTTT-TAATTGAATTGTTATT---TAATTAA-TTGTCAATTTG--TTGATTAAATTCAA
Cephalallus unicolor Sv OP537677	TTGCCAGCGCTTAATTGCGCGGTAAAAAA------ACCT-TTACACACAGTGTTTTTC--TTATTTAGAAACTATTGCTTTGGTTTGGCTCTAGAAATAGAGTTGGGCCAGAGGTCAAACCAAACTTCAATTTT-TAATTGAATTGTTATT---TAATTATATAATGTATCAACTTTGTTTAAATTCAA
Cephalallus unicolor Dsp OP537676	TTGCCAGCGCTTAATTGCGCGGTAAAAAA------ACCT-TTACACACAGTGTTTTTC--TTATTTAGAAACTATTGCTTTGGTTTGGCTCTAGAAATAGAGTTGGGCCAGAGGTCAAACCAAACTTCAATTTT-TAATTGAATTGTTATT---TAATTATATAATGTATCAACTTTGTTTAAATTCAA
Tetropium castaneum Dsp OP537689	TTGCCAGCGCTTAGTTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTTTC--TTATT-AAAAACTATTGCTTTGGTCTGGCTCTAGAAGTAGAGTTGGGTCAGAGGTCAAACCAAACTTCAATTTT-TAATTGAATTGTTATT---TAATTATAATGTCAATTTG--TTGATTAAATTCAA
As Shizuoka1 OP537700	TTGCCTGCGCTTAATTGCGCGGCGAGAAA------ACCT-T-ACACACAATGTTTTTGTTTTTATTAAGAAACTTTGCTTTGGTTTGGCG-TAGAGATA-CGTTGGGCCAGAGGTTTA-CCAAACTTCAATTTT-TAATTGAATTGTTTTTAACTATTTTATTTGTCAATTGT-GATTTTATAATCAAT
As Mie1 OP537701	TTGCCTGCGCTTAATTGCGCGGCGAGAAA------ACCT-T-ACACACAATGTTTTTGTTTTTATTAAGAAACTTTGCTTTGGTTTGGCG-TAGAGATA-CGTTGGGCCAGAGGTTTA-CCAAACTTCAATTTT-TAATTGAATTGTTTTTAACTATTTTATTTGTCAATTGT-GATTTTATAATCAAT
As Mie2 OP537703	TTGCCTGCGCTTAATTGCGCGGCGAGAAA------ACCT-T-ACACACAATGTTTTTGTTTTTATTAAGAAACTTTGCTTTGGTTTGGCG-TAGAGATA-CGTTGGGCCAGAGGTTTA-CCAAACTTCAATTTT-TAATTGAATTGTTTTTAACTATTTTATTTGTCAATTGT-GATTTTATAATCAAT
As Shiga1 OP589184	TTGCCTGCGCTTAATTGCGCGGCGAGAAA------ACCT-T-ACACACAATGTTTTTGTTTTTATTAAGAAACTTTGCTTTGGTTTGGCG-TAGAGATA-CGTTGGGCCAGAGGTTTA-CCAAACTTCAATTTT-TAATTGAATTGTTTTTAACTATTTTATTTGTCAATTGT-GATTTTATAATCAAT
As Mie3 OP537704	TTGCCAGCGCTTAACTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTT-C--TTTATTAGAAACTATTGCTTTGGTCTGGCT-CAGAAATG-AGTTGGGCCAGAGGTTTA-CCAAACTTCAATTTTA---TTGAATTGTTATT--TTATT-ATTTTGTCAATTT--GTTGATTAAATTCAA
As Kyoto2 OP537705	TTGCCTGCGCTTAATTGCGCGGCGAAAAA------ACCT-T-ACACACAATGTTTTTGTTTTTATTAAGAAACTTTGCTTTGGTTTGGCG-TAGAAATA-CGTTGGGCCAGAGGTTTA-CCAAACTTCAATTTT-TAATTGAATTGTTTT-TACTATTTTATTTGTCAATTGT-GATTTTATAATCAAT
As Kyoto3 OP537706	TTGCCTGCGCTTAATTGCGCGGCGAAAAA------ACCT-T-ACACACAATGTTTTTGTTTTTATTAAGAAACTTTGCTTTGGTTTGGCG-TAGAAATA-CGTTGGGCCAGAGGTTTA-CCAAACTTCAATTTT-TAATTGAATTGTTTT-TACTATTTTATTTGTCAATTGT-GATTTTATAATCAAT
As Ibaraki1 OP537708	TTGCCAGCGCTTAACTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTT-C--TTTATTAGAAACTATTGCTTTGGTCTGGCT-CAGAAATG-AGTTGGGCCAGAGGTTTA-CCAAACTTCAATTTTA---TTGAATTGTTATT--TTATT-AATTTGTCAATTT--GTTGATTAAATTCAA
As Ibaraki2 OP537709	TTGCCAGCGCTTAACTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTT-C--TTTATTAGAAACTATTGCTTTGGTCTGGCT-CAGAAATG-AGTTGGGCCAGAGGTTTA-CCAAACTTCAATTTTA---TTGAATTGTTATT--TTATT-AATTTGTCAATTT--GTTGATTAAATTCAA
As Ibaraki3 OP537710	TTGCCTGCGCTTAATTGCGCGGCGAGAAA------ACCT-T-ACACACAATGTTTTTGTTTTTATTAAGAAACTTTGCTTTGGTTTGGCG-TAGAGATA-CGTTGGGCCAGAGGTTTA-CCAAACTTCAATTTT-TAATTGAATTGTTTTTAACTATTTTATTTGTCAATTGT-GATTTTATAATCAAT
As Ibaraki4 OP537711	TTGCCTGCGCTTAATTGCGCGGCGAGAAA------ACCT-T-ACACACAATGTTTTTGTTTTTATTAAGAAACTTTGCTTTGGTTTGGCG-TAGAGATA-CGTTGGGCCAGAGGTTTA-CCAAACTTCAATTTT-TAATTGAATTGTTTTTAACTATTTTATTTGTCAATTGT-GATTTTATAATCAAT
As Ibaraki5 OP537712	TTGCCTGCGCTTAATTGCGCGGCGAGAAA------ACCT-T-ACACACAATGTTTTTGTTTTTATTAAGAAACTTTGCTTTGGTTTGGCG-TAGAGATA-CGTTGGGCCAGAGGTTTA-CCAAACTTCAATTTT-TAATTGAATTGTTTTTAACTATTTTATTTGTCAATTGT-GATTTTATAATCAAT
As Yakushima OP537713	TTGCCAGCGCTTAACTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTT-C--TTTATTAGAAACTATTGCTTTGGTCTGGCT-CAGAAATG-AGTTGGGCCAGAGGTTTA-CCAAACTTCAATTTTA---TTGAATTGTTATT--TTATT-AATTTGTCAATTT--GTTGATTAAATTCAA
As Shizuoka2 OP537714	TTGCCAGCGCTTAACTGCGCGGCGAAAAA------ACCT-T-ACACACAGTGTTTT-C--TTTATTAGAAACTATTGCTTTGGTTTGGCT-CAGAAATG-AGTTGGGCCAGAGGTTTA-CTAAACTTCAATTTTA---TTGAATTGTTATT--TTATT-AATTTGTCAATTT--GTTGATTAAATTCAA

Table S2. Symbiont fungi isolated from female adults of Longicorn beetles in Japan

Group	Species	Collected location	Analyzed region	Colonies on plate	Blastn Best Hit	Accession Number
Lepturinae	*Strangalomorpha tenuis*	Yamanashi	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	OP537752
Lepturinae	*Strangalomorpha tenuis*	Yamanashi	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	OP537753
Lepturinae	*Strangalomorpha tenuis*	Yamanashi	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	OP537754
Lepturinae	*Rhagium femorale*	Yamanashi	Female oviduct	no yeast colony		
Lepturinae	*Rhagium femorale*	Yamanashi	Female oviduct	y	[*Candida*] *rhagii* culture CBS:618	OP537755
Lepturinae	*Rhagium femorale*	Yamanashi	Female oviduct	y	[*Candida*] *rhagii* culture CBS:618	OP537756
Lepturinae	*Rhagium femorale*	Yamanashi	Female oviduct	y	[*Candida*] *rhagii* culture CBS:618	OP537757
Lepturinae	*Pyrrhona laeticolor*	Shizuoka	Female oviduct	y	*Scheffersomyces coipomoensis* strain Y3	OP537758
Lepturinae	*Pyrrhona laeticolor*	Shizuoka	Female oviduct	y	[*Candida*] *vrieseae* CBS 10829	OP537759
Lepturinae	*Pachytodes cometes*	Gifu	Female oviduct	y	*Candida ergatensis* strain ATCC MYA-4655	OP537760
Lepturinae	*Pachytodes cometes*	Gifu	Female oviduct	y	*Candida ergatensis* strain ATCC MYA-4655	OP537761
Lepturinae	*Pachytodes cometes*	Gifu	Female oviduct	y	*Candida ergatensis* strain ATCC MYA-4655	OP537762
Lepturinae	*Pachytodes cometes*	Gifu	Female oviduct	y	*Scheffersomyces* sp. strain Y13	OP537763
Lepturinae	*Pachytodes cometes*	Yamanashi	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	OP537764
Lepturinae	*Pachypidonia bodemeyeri*	Shizuoka	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	OP537765
Lepturinae	*Ohbayashia nigromarginata*	Wakamaya	Female oviduct	y	*Meyerozyma guilliermondii* isolate NTOU5003	OP537766
					[*Candida*] *vrieseae* CBS 10829	OP589185
Lepturinae	*Ohbayashia nigromarginata*	Shizuoka	Female oviduct	y	*Scheffersomyces coipomoensis* strain Y3	OP537767
Lepturinae	*Ohbayashia nigromarginata*	Shizuoka	Female oviduct	y	*Scheffersomyces coipomoensis* strain Y3	OP537768
Lepturinae	*Etorofus vicarius*	Nagano	Female oviduct	no yeast colony		
Lepturinae	*Etorofus vicarius*	Nagano	Female oviduct	y	*Candida ergatensis* strain ATCC MYA-4655	OP537769
Lepturinae	*Etorofus vicarius*	Nagano	Female oviduct	y	*Candida ergatensis* strain ATCC MYA-4655	OP537770
Lepturinae	*Etorofus vicarius*	Yamanashi	Female oviduct	y	*Candida ergatensis* strain ATCC MYA-4655	OP537771
Lepturinae	*Leptura ochraceofasciata*	Shizuoka	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Shizuoka	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Shizuoka	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Shizuoka	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Shizuoka	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Shizuoka	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Shizuoka	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Shizuoka	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Shizuoka	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Yamanashi	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Yamanashi	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Yamanashi	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Shizuoka	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Nagano	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Nagano	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Nagano	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Nagano	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Leptura ochraceofasciata*	Shizuoka	Female oviduct	y	*Scheffersomyces* sp. Y1052_SICYLG3	
Lepturinae	*Encyclops olivaceus*	Yamanashi	Female oviduct	no yeast colony	*Chrysolina oricalcia* genome assembly, chromosome: 12	OP537772
Lepturinae	*Encyclops olivaceus*	Yamanashi	Female oviduct	no yeast colony	*Chrysolina oricalcia* genome assembly, chromosome: 12	OP537773
					Chrysolina oricalcia genome assembly, chromosome: 12	OP537774
Necydalinae	*Necydalis sacharinensis*	Nagano	Female oviduct	no yeast colony	Uncultured fungus isolate FICUS 144	OP537775
					Chrysolina oricalcia genome assembly, chromosome: 12	OP537776
Necydalinae	*Necydalis sacharinensis*	Nagano	Female oviduct	no yeast colony	Uncultured fungus isolate FICUS 144	OP537777
Prioninae	*Aegosoma sinicum*	Shizuoka	Female oviduct	y	*Scheffersomyces anoplophorae*	OP537778
Prioninae	*Aegosoma sinicum*	Shizuoka	Female oviduct	y	*Scheffersomyces* sp. YW27.1	OP537779
Prioninae	*Aegosoma sinicum*	Shizuoka	Female oviduct	y	*Scheffersomyces anoplophorae*	OP537780
					Scheffersomyces anoplophorae	OP537781
Prioninae	*Aegosoma sinicum*	Shizuoka	Egg surface	y	*Meyerozyma guilliermondii* strain CBS 2030	OP537782
					Meyerozyma carpophila strain CBS 5256	OP537783
					Scheffersomyces anoplophorae	OP537784
					Kodamaea ohmeri strain DM9Y	OP537785
					Kodamaea ohmeri strain SM14UFAM	OP537786
					Debaryomyces hansenii IFM 55872	OP537787
Prioninae	*Aegosoma sinicum*	Osaka	Egg surface	y	[*Candida*] *railenensis* isolate R97308_ITS	OP537788
					Meyerozyma caribbica strain CBS 9966	OP537789
					Meyerozyma guilliermondii isolate NTOU5003	OP537790
					[*Candida*] *oleophila* isolate B2-15b	OP537791
					Wickerhamomyces sydowiorum culture CBS:7276	OP537792

Table S4. Artificial transfer of yeasts to larvae

Species and biological replicate	Sterilization	Given yeast	Detected yeast	Number of larvae	Total
Pyrrhona laeticolor	No	Not given	Other yeast	5	10
Pyrrhona laeticolor	No	Not given	Symbiont yeast	2	
Pyrrhona laeticolor	No	Not given	No yeast detected	3	
Pyrrhona laeticolor	Yes	Not given	No yeast detected	13	28
Pyrrhona laeticolor	Yes	*Scheffersomyces* sp. Y1052_SICYLG3	Given yeast	12	
Pyrrhona laeticolor	Yes	*Scheffersomyces* sp. Y1052_SICYLG3	No yeast detected	2	
Pyrrhona laeticolor	Yes	Not given	Other yeast	1	
Ohbayashia nigromarginata	No	Not given	No yeast detected	1	3
Ohbayashia nigromarginata	No	Not given	Symbiont yeast	2	
Ohbayashia nigromarginata	Yes	Not given	No yeast detected	3	9
Ohbayashia nigromarginata	Yes	*Scheffersomyces* sp. Y1052_SICYLG3	No yeast detected	5	
Ohbayashia nigromarginata	Yes	*Scheffersomyces coipomoensis* strain Y3	Given yeast	1	
Leptura ochraceofasciata Exp 1	No	Not given	Symbiont yeast	15	15
Leptura ochraceofasciata Exp 1	Yes	Not given	No yeast detected	6	19
Leptura ochraceofasciata Exp 1	Yes	Not given	Other yeast	1	
Leptura ochraceofasciata Exp 1	Yes	*Scheffersomyces coipomoensis* strain Y3	Given yeast	8	
Leptura ochraceofasciata Exp 2	Yes	*Scheffersomyces coipomoensis* strain Y3	No yeast detected	3	
Leptura ochraceofasciata Exp 2	Yes	*Candida vrieseae*	Other yeast	1	

Table S5. Possession of symbiont cells in the mycetome after long culture

Species name	Number of examined larva	Number of larva possessing symbiont cells in the mycetomes	Weight at collection (mg)	Weight at observation (mg)	Date of collection	Date of observation
Etorofus vicarius	1	1	356	420	20-Aug-2020	03-July-2021
Leptura ochraceofasciata	1	1	40	199	20-Aug-2020	03-July-2021
Necydalis formosana	2	0	68 129	267 352	20-Aug-2020	03-July-2021