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Error-acceptable vertical transmission fostered uncoupled phylogenetic relationships between longicorn beetles and their symbionts

Yasunori Sasakura[1], Nobuhisa Yuzawa[2], Junsuke Yamasako[3], Kazuki Mori[2], Takeo Horie[4], Masaru Nonaka[5]

1. Shimoda Marine Research Center, University of Tsukuba, Shimoda, Shizuoka 415-0025, Japan
2. Independent Researcher
3. Institute for Plant Protection, National Agriculture and Research Organization, Tsukuba, Ibaraki 305-8517, Japan
4. Laboratory for Single-cell Neurobiology, Graduate School of Frontier Biosciences, Osaka University, Suita, Osaka, 565-0871, Japan
5. Department of Biological Sciences, Graduate School of Science, Tokyo Metropolitan University, Hachioju, Tokyo 192-0397, Japan

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Corresponding to: Yasunori Sasakura

Phone: +81-558-22-6605

E-mail: sasakura@shimoda.tsukuba.ac.jp

Abstract

The coleoptera Cerambycidae (longicorn beetles) use wood under different states (living healthy, freshly snapped, completely decayed, etc.) in a species-specific manner for their larval diet. Larvae of some Cerambycidae groups have a mycetome, an accessory organ of the midgut that harbors symbiont fungal cells. The symbionts are thought to play a role in improving the nutrient conditions; however, this has not been shown experimentally. In order to elucidate the role and evolutionary mechanisms of this symbiosis, we investigated characteristics of the mycetomes in the larvae of Cerambycidae in Japan. Lepturinae, Necydalinae and Spondylidinae are the only groups that possess mycetomes, and the mycetomes and corresponding fungal cells of these three groups exhibit different characteristics. However, the phylogenetic relationship of symbiont yeasts does not coincide with those of the corresponding longicorn beetles, suggesting they did not co-evolve. We show that the vertical transmission of symbiont yeasts from female parent to child is a step that could potentially accommodate the discontinuity. Some Lepturinae species secondarily lost mycetomes. The loss is associated with their choice of diet, suggesting that better nutritional conditions could have allowed species to discard this organ. Indeed, we found that symbiont fungi are dispensable for larval growth if sufficient nutrients are given. *Aegosoma sinicum* is a longicorn beetle classified into the subfamily Prioninae, which does not possess mycetomes. However, this species does contain a restricted selection of yeast species in the larval gut, suggesting that the symbiosis between longicorn beetles and yeasts emerged prior to acquisition of the mycetomes.

Introduction

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

In the coleopteran insects, which comprise the largest animal order, the wood-eating groups are known to have important relationships with fungi, particularly yeasts (Gibson and Hunter 2010). Many yeasts have been isolated from the frass, tunnel and digestive tubes of coleopteran larvae (Suh et al., 2005; Endoh et al., 2011; Davis et al., 2015). In some groups, adults have a specific structure (the mycangium) that stores fungal cells and passes them to the next generation during oviposition (Tanahashi et al., 2010; Joseph et al., 2021). The fungi proliferate in the tunnel in which larvae dwell and/or are incorporated into the larval digestive tube. These fungi are not only consumed as part of the larval diet (Toki et al., 2012) but are also thought to contribute to the creation of a comfortable living atmosphere for the larvae and to assist in the digestion of wood. Indeed, many yeasts isolated from coleopteran larvae have cellulose-, cellobiose- and xylose-digesting activities (Davis et al., 2015).

The Cerambycidae family (longicorn beetles) is a widespread coleopteran group of wood-eating beetles. Larvae of longicorn beetles have a unique fungi-storing organ, the mycetome, near the anterior end of the midgut (Schomann 1937; Nardon and

Grenier 1989; Grünwald et al., 2010). The mycetome consists of a mass of knob-shaped sacs that attach to the outer membrane (serosa) of the gut. Mycetome sacs are filled with nutritional particles, and symbiont fungal cells consisting primarily of yeasts dwell in the lumen or in mycetocytes (Jurzitza 1960). The fungal cells are constantly transferred to the gut lumen (Grünwald et al., 2010), and are thought to assist in the digestion of wood.

Although the mycetome seems to be an essential structure for assisting the symbiosis with fungi, its function is still obscure. Indeed, the mycetome is not conserved among all longicorn beetle groups (Schomann 1937). Therefore, this structure may have been acquired in some groups that require an additive benefit from symbiont fungi to increase their survival fitness, such as the acquisition of efficient digestion assisted by fungi. However, the advantages that symbiont fungi confer to longicorn beetles have not been resolved. In order to understand the evolutionary history of the symbiosis facilitated by the mycetome, we investigated the distribution of this structure in various species from different subfamilies of longicorn beetles in Japan. Species of the subfamily Lepturinae are dominant among the species containing a mycetome; however, some Lepturinae species do not have this structure. We found that the loss of a mycetome is correlated with diet choice. The Japanese longicorn beetle groups/species that had or that lacked a mycetome in our analysis were in good accordance with those in a previous report of the presence/absence of symbiont yeasts in European species (Schomann 1937). *Aegosoma sinicum* belongs to the subfamily Prioninae, whose members do not have a mycetome. We found that its larvae exhibit symbiosis with limited yeast species classified into the genus *Schefferosomyces*, suggesting that symbiosis with yeasts preceded the acquisition of mycetomes during the

evolution of Cerambicydae.

Materials and Methods

Animals

Most of the longicorn beetle larvae were collected from dead trees. We used cultured larvae for analyses about the species whose larvae are difficult to capture from the wild. Their species names were identified by the cuticle structure at the thorax. When species could not be identified by the thoracic cuticle structure, genomic DNA were isolated from larval somatic tissues with a Wizard Genomic DNA Purification kit (Promega), followed by PCR amplification of 28s rDNA with ExTaq DNA polymerase hot start version (Takara-bio) and with the primers 5'-GAACTTTGAAGAGAGAGTT-3' and 5'-CAGGCATAGTTCACCATCTT-3'. The sequence of the PCR products was determined by the conventional Sanger method, which was used to identify species with the aid of 28s rDNA sequence isolated from adults. The larval midgut was surgically isolated with fine tweezers in phosphate buffered saline (PBS) to observe mycetomes and to collect gut materials. Frass was collected from the tunnel on wood with cotton swabs. Larvae in the tunnel were isolated together with frass to identify species.

Identification of fungi

Mycetome contents, gut materials, frass, or fluid from adult genitalia were spread on potato dextrose agar (PDA) plates including chloramphenicol and ampicillin. Eggs were immersed in PBS for a few minutes, and the supernatant was spread onto PDA plates. The plates were incubated for a few days at 25ºC or for longer duration at 4ºC. DNA

was extracted from yeast or yeast-like colonies by a microwave-based method (Izumitsu et al., 2012), and was subjected to amplification of rDNA with ExTaq DNA polymerase hot start version (Takara-bio) and with the primers 5'-CTTGGTCATTTAGAGGAAGTAA-3' and 5'-TCCGTAGGTGAACCTGCGG-3' according to the previous method (White et al., 1990; Gardes and Bruns 1993). In this analysis, DNA from at least four colonies on a single plate was extracted in bulk if the colonies were available. Sequences were determined from the amplified bands after purification with a Qiaquick Gel Extraction kit (Qiagen). When sequences could not be determined due to the low quality of the chromatographs, each colony was subjected to sequencing analysis separately. When the mycetome contents did not yield colonies because the symbiont fungi were unculturable, we isolated DNA from mycetomes with Isoplant II (Nippon-Gene) according to the manufacturer's instructions, followed by PCR amplification and sequencing of rDNA of fungal cells.

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

Phylogenetic analyses

The sequences were aligned using the M-COFFEE program (https://tcoffee.crg.eu/apps/tcoffee/do:mcoffee; Notredame et al., 2000; Wallace et al., 2006). After removing unnecessary residues, maximum likelihood trees were constructed with the MEGA software version 11 (Tamura et al., 2021). Trees were

tested with 1,000 bootstrap pseudoreplicates.

Culture of Cerambycidae larvae under an antibiotic condition

Our artificial diet was developed based on the work of Gardiner (1970). Nine grams of Insecta LFS (Nosan Corporation, Japan), 6g of dead *Symplocos coreana* powder, and 15g of cellulose (ISC powder) were kneaded in 30 or 45 ml of water, 30 or 45 ml of PDA dissolved in water, or 30 or 45 ml of PDA suspended with one of two yeasts, *Candida vrieseae* or *Schefferosomyces* sp. Y1052_SICYLG3. The yeasts were expected to improve larval growth; however, the effect was questionable and was not analyzed in detail. The ISC paste was spread in a polypropylene box and was autoclaved (20 min at 105ºC) for sterilization. Eggs or hatched larvae were placed on the autoclaved diet, and were kept at room temperature until observation. For introduction of arbitrary yeast to larvae, eggs were sterilized for more than one day by placing them on sterilized ISC paste. A wood plate of *Symplocos coreana* was sterilized by autoclaving, then soaked with PDA liquid in which yeast cells were immersed. Sterilized eggs were cultured on the yeast-soaked wood plate until hatching. Hatched larvae were collected and cultured in sterilized ISC paste for about one month until they became sufficiently large for the surgical isolation of mycetomes.

Results

Distribution of mycetomes in Japanese Cerambycidae

In order to elucidate the evolutionary history of the mycetome in longicorn beetles, we investigated the distribution of mycetomes in the Japanese species (Fig. 1). Only three

major subfamilies, Lepturinae, Necydalinae and Spondylidinae, had mycetomes near the anterior end of the larval midgut (Fig. 1 and Table 1). There are group-specific characteristics in the shapes of mycetomes. The mycetomes of Lepturinae (Fig. 1B-C) and Spondylidinae (Fig. 1D) adhered tightly to the serosa of the midgut. By contrast, the large mycetomes of Necydalinae species were well separated from the serosa with a connection through several thin tubes (Fig. 1E). The mycetomes of Lepturinae form a single circular ring surrounding the serosa (Fig. 1B), while those of Spondylidinae larvae form two narrower rings (Fig. 1D). The characteristics of the appearance of mycetomes in Lepturinae and Spondylidinae agree with those in the previous report (Buchner 1965).

We found mycetomes in 22 out of 31 species of Lepturinae, and in all 3 of the examined Necydalinae species (Table 1). Because most of the species of Lepturinae/Necydalinae had mycetomes (Fig. 1B and Fig. 2), it is likely that the ancestor of Lepturinae/Necydalinae had a mycetome before the diversification of these subfamilies. *Paragaurotes doris*, *Japanocorus caeruleipennis*, *Brachyta bifasciata* and all *Pidonia* lacked mycetomes (Fig. 1F and Fig. 2). In all of these cases, the mycetomes were probably lost secondarily during speciation. Because these mycetome-lacking species do not form a single clade, the loss of mycetomes is suspected to have occurred several times. Interestingly, all but one of the mycetome-lacking Lepturinae are inner bark-eaters at the larval stage. *B. bifasciata* eats the roots of living *Paeonia*. Inner bark-eating Lepturinae are not always mycetome-lacking species, since two *Rhagium* species have mycetomes, although these two species are also inner bark-eaters. We found mycetomes in all examined Spondylidinae species (n=5; Table 1).

Characteristics of yeasts or yeast-like fungi in mycetomes

We found that mycetomes and fungi dwelling in them had group-specific characteristics. Each lobe of the Lepturinae mycetome includes cone-shaped fungal cells, a vesicular structure probably corresponding to that of mycetocytes, and particles much smaller than yeast cells (Fig. 3A and B, B'). The density of yeast cells in a mycetome is usually not high, and the cells do not fully occupy the lumen. When cultured on potato dextrose agar (PDA) plates, almost all of the fungi from the mycetomes of Lepturinae larvae formed colonies (Fig. 3C), and their rDNA sequences indicated that they were yeasts.

We classified yeasts isolated from the Lepturinae mycetome or gut by determining the sequence of the ITS site of mitochondria rDNA (Table 2). These rDNA sequences were used as queries of blastn searches to estimate the species of yeasts (Altschul et al., 1997). In the following section, we refer to the characterized yeasts as the species exhibiting the highest similarity to the rDNA sequences in the blastn analyses for simplicity; however, we have to note that it does not always guarantee the actual yeast species because in many cases our rDNA sequences did not exhibit a perfect match to a known rDNA sequence (Table 2, "Score" column).

In almost all cases, only a single yeast species was detected from the mycetome of a larva (87%, n=125; Table 1). However, seven larvae had more than one yeast species in the mycetome. Moreover, the rDNA sequence derived from the mycetomes of single larvae exhibited nucleotide variations in three cases. These results suggest that the yeast cells in mycetomes are not always clones. There are species-specificities between longicorn beetles and yeasts. For example, two Japanese *Rhagium* species had a yeast whose rDNA exhibited the best homology to that of *Candida rhagii*, which was

also found from a *Rhagium* species in Germany (Grünwald et al., 2010). In total, at least 10 yeast species were isolated from 22 mycetome-possessing Lepturinae species, suggesting that some yeast species were shared among different Lepturinae species (Table 1, Fig. 2 and Fig. 4). However, the Lepturinae species that share the same symbiont yeast are not always phylogenetically close to each other (Fig. 2), which was confirmed by phylogenetic analysis of the yeast species isolated in this study (Fig. 4). For example, the larvae of *Leptura orchraceofasciata* isolated from both hardwoods and conifers almost always had *Scheffersomyces* sp. Y1052_SICYLG3 (Table 2). This yeast was also found in the larvae of *Caraphia lepturoides*, although *L. orchracerofasciata* and *C. lepturoides* are phylogenetically distant. Larvae of *Pyrrhona laeticolor*, *Ohbayashia nigromarginata*, *Ephies japonicas*, *Pigostrangalia dulcis* and *Mimostrangalia kurosonensis* had the yeast related to *Candida vrieseae* in their mycetomes. All of these Lepturinae species use the hardwood group Symplocaceae as their larval hosts. This was not due to the ubiquity of *C. vrieseae* in the dead Symplocaceae tree, since larvae of the stag beetle *Aesalus asiaticus* isolated from a dead *Symplocos coreana* together with *Pyrrhona laeticolor* had a different yeast (*Schefferosomyces ergatensis*) in their digestive tube (Table 2, stagbeetle). We could assume that Lepturinae longicorn beetles require *C. vrieseae* for efficient digestion of dead Symplocaceae trees. However, the relationships between yeast species and host plants of longicorn beetles are not expected to be rigid, since *Anastrangalia scotodes* and *Strangalia koyaensis*, which use several conifers as hosts, also possessed *Candida vrieseae* (Table 2 and Fig. 4).

The characteristics of the mycetome contents of *Encyclops olivaceus* and the three examined Necydalinae species were quite different from those of Lepturinae.

Encyclops and Necydalinae mycetomes were fully occupied by fungal cells and we could not find a vesicular structure corresponding to mycetocytes (Fig. 3). The elongated fungal cells in these Necydalinae mycetomes were obviously larger than the yeast cells of Lepturinae, and none of the Necydalinae symbionts formed a colony on PDA plates. The unculturable symbionts of *Encyclops* are similar in shape but smaller than those of Necydalinae species (Fig. 3E). All analyzed larvae of Necydalinae species are dependent on dead hardwood trees. To further determine whether the symbiont characteristics are shared among Necydalinae species, we examined the symbiont of *N. sachalinensis*, which is dependent on the dead part of living conifers. Because its larvae were unavailable, we isolated fungal cells from the oviducts of *N. sachalinensis* females. The fungal cells had the same characteristics as those of the fungi isolated from the other Necydalinae larvae (Fig. 3H' and Table 3).

We attempted to characterize the fungi from *Encyclops olivaceus* and Necydalinae mycetomes by directly isolating DNA from their mycetomes followed by PCR amplification of their ITS sequences. The ITS sequences did not show a sufficient similarity to those of any known fungi (Table 2), suggesting that the symbiont fungi are distinct from yeasts. The shared characteristics of the symbionts among *Encyclops* and Necydalinae might suggest that they are phylogenetically related to each other.

The internal structure of Spondylidinae mycetomes was similar to those of typical Lepturinae in that it contained many vesicles and the density of fungal cells was not high (Fig. 3J). The shape of Spondylidinae fungal cells was rod-like (Fig. 3K-K'), and none of these fungi made a colony on PDA plates (Fig. 3L). Unlike Necydalinae, Spondylidinae fungal cells are classified into the yeast group *Debaryomyces*/*Schwanniomyces* (Table 2 and Fig. 4). These yeasts derived from

Spondylidinae form a single clade in the phylogenetic tree of the yeasts isolated from mycetome-positive Lepturinae/Spondylidinae (Fig. 4). Therefore, there is broad group-specificity in the choice of symbiont fungi when Lepturinae, Necydalinae and Spondylidinae are compared. When viewed from the species level, however, the phylogenetic relationships of Lepturinae longicorn beetles are not reflected in those of the symbiont yeasts (Fig. 2). This asymmetry is our next target to address.

Imperfect delivery of yeasts across generations

It has been suggested that the symbiont fungi in the larval mycetome are provided by the maternal parent (Schomann 1937; Douglas 1989). We characterized yeasts isolated from eight species of Lepturinae adult females. Although not perfectly matched, the adult females had the yeast species that were isolated from the larvae of the same species (Table 3), confirming the vertical transfer of symbionts. We found that yeasts in the female genitalia could be easily excreted by gently pushing the oviduct, suggesting that yeasts are "sprayed" around the eggs during spawning, as previous studies described (Schomann 1937; Douglas 1989). Hatched larvae may take yeasts together with egg shells and/or nearby wood to incorporate yeasts in their gut. Indeed, 1st instar larvae of *Ohbayashia nigromarginata* and *Necydalis formosana* at a few days after hatching already had mycetome-like knobs at the anterior midgut, and fungal cells in the structures (Fig. 5).

This process of yeast delivery does not guarantee accurate transmission of maternal yeasts to their descendants, because larvae that accidentally move from the spawned area before yeast uptake may have a chance to incorporate a different yeast species from the environment. We did observe multiple examples of imperfect yeast

transmission, and we will here describe several of them. Larvae of *L. orchraceofasciata* almost always have *Scheffersomyces* sp. Y1052_SICYLG3; however, we found a single larva that had the yeast *Candida vrieseae* (Table 2). This individual was found in a dead tree of Symplocaceae. As we stated above, Lepturinae species that depend on this tree group usually have *Candida vrieseae*, and the larva of *L. orchraceofasciata* is suspected to have accidentally incorporated *Candida vrieseae* supplied by another Lepturinae species. We also found two larvae of *L. orchraceofasciata* and *P. laeticolor* that had the yeast *Scheffersomyces coipomoensis* (Table 2). These larvae were isolated from one dead tree of *Symplocos coreana* at Mt. Amagi. We isolated *Scheffersomyces coipomoensis* from yellowish dead trees of *Symplocos coreana* on the same mountain, suggesting that this yeast dwells in the environment and could have been accidentally incorporated into the larvae. These exceptions could be easily explained if longicorn beetles adopt a somewhat imperfect manner of yeast transmission between generations. Moreover, this accidental "error" could disturb the phylogenetic relationship between symbiont yeasts and longicorn beetle species.

If the above hypothesis of yeast delivery system is correct, it would be possible to generate yeast-free larvae, or larvae having a yeast species different from that dominantly possessed by larvae in the wild populations, by removing maternally supplied yeasts. In order to show this, we developed a way to culture larvae under an antibiotic condition. After oviposition, we placed eggs on an artificial diet including antibiotics for sterilization so that the hatched larvae did not come into contact with external yeasts during and after birth. Larvae of *Leptura orchraceofasciata* and *Stictoleptura dichroa* grew well under this condition (Fig. 6A). They had brownish mycetomes near the anterior end of the midgut (Fig. 6B and D). The position of their

mycetomes was identical to those of larvae from the wild. Microscopic observations showed that these brown mycetomes did not include yeasts (Fig. 6C and E), which was confirmed by culturing of mycetome contents on PDA plates (Fig. 6C, inset), suggesting that the yeasts needed to be supplied externally in order for larvae to acquire yeasts in their mycetomes. This experiment also suggests that mycetomes can be formed without yeasts.

In the above culture experiments, some of the larvae had yeasts that were not found in the mycetomes of larvae isolated from wild populations. In the experiment with *L. orchraceofasciata*, we found that a few larvae (12.5%, n=16) had *Meyerozyma caribbica*. *Meyerozyma* is a spontaneous yeast group arising in our laboratory. In the experiment using *S. dichroa*, a portion of eggs hatched on the wood onto which their mothers had laid eggs prior to sterilization. We cultured the pre-hatched eggs and 1st instar larvae in the same container. After 1 month, we observed that the larvae were divided into three groups: larvae with yeast-free mycetomes (n=5; Fig. 6D and E), larvae with mycetomes that included yeast cells with a low density (n=4; Fig. 6F and G), and larvae with relatively yeast-rich mycetomes (n=9; Fig. 6H and I). The yeast species isolated from the yeast-poor mycetomes was different from that isolated from yeast-rich mycetomes: the former was *Meyerozyma caribbica* while the latter was *Schefferosomyces ergatensis*. *S. ergatensis* is the default species of this longicorn beetle in the wild (Table 2). We suspected that the larvae with *S. ergatensis* were the larvae hatched on the egg-laid wood, and that these larvae had a chance to incorporate maternally gifted yeast.

To investigate the above hypothesis about external yeast transfer, we next tested whether we could deliver a specific yeast species into mycetomes. In this

experiment, sterilized eggs were placed on a wood plate to which a yeast species was applied. After birth, we transferred the larvae to a plate with the artificial diet to avoid further yeast incorporation during growth until the larvae reached a sufficient size for the isolation of mycetomes. We examined 33 larvae in total, and 22 had the given yeast species (Table 4). These yeasts were never detected in the control groups to which we did not give the yeasts (0%, n=50), suggesting that this experiment successfully replaced the symbiont yeast with the arbitrary yeast. From these results, we concluded that Lepturinae longicorn beetles externally acquire yeasts after hatching. Although the yeasts are usually maternally supplied, a different yeast could be acquired accidentally.

Yeast transmission between generations in the longicorn beetles without mycetomes

There is an association between mycetome-possessing Lepturinae longicorn beetles and yeast species. Our next question is how their symbiosis was started, and particularly how mycetomes contributed to the evolution of the symbiosis. To gain insight into this evolutionary narrative, we investigated yeasts in the digestive tubes and/or frass of mycetome-lacking longicorn beetles.

We observed a total of 135 larvae of mycetome-lacking Longicorn beetles (this number includes Lepturinae) that correspond to 46 species from five families/subfamilies (Table 1). Yeasts were detected from 46% of larvae. This score is low compared to that of mycetome having Lepturinae (92%), suggesting that mycetomes have a role to stabilize symbiosis with yeasts. We isolated in total 46 yeast species from 46 mycetome-lacking longicorn beetle species. This score is two times more than that of mycetome-having Lepturinae species (10 out of 21 excluding

Encyclops olivaceus), suggesting that yeasts habiting in the mycetome-lacking species are more variable than mycetome-possessing species. These wobbles could be explained if mycetome-lacking longicorn beetles do not usually possess a way to limit the species of symbiont yeasts, such as delivery system between generation.

We found an exceptional mycetome-lacking species that could transmit specific yeasts between generations. *Aegosoma sinicum* is a kind of Prioninae which does not have mycetomes (Fig. 1G). Because both Prioninae and its sister group Cerambycinae do not possess mycetomes, *A. sinicum* was suggested to have been evolved from a mycetome-lacking ancestor. Two yeast groups belonging to the genus *Scheffersomyces* were repeatedly isolated from larvae of *A. sinicum* collected in different locations and from different host trees (Table 2 and Fig. 4), suggesting that there is a strong correlation between *A. sinicum* and the yeasts. Individual *A. sinicum* larvae exclusively possessed either of the two *Scheffersomyces* groups.

Among the possible explanations for the relationship between *A. sinicum* and *Scheffersomyces*, two stand out. The first is that *A. sinicum* females might lay eggs on the host trees in which the yeasts habit. The second possibility is that the yeasts were transferred from adult females to their progeny. We isolated the colonies of the same *Scheffersomyces* yeasts from the genitalia of females and the egg surfaces of *A. sinicum* (Table 3). Therefore, it is likely that *A. sinicum* vertically transfer yeasts between generations without mycetomes, although we cannot rule out the first possibility.

Discussion

Many dead wood-eating beetles are dependent on yeasts or related fungi at their larval

stage (Davis 2015). Although the role of this symbiosis has not been fully understood, its suspected function is to increase efficiency in the nutrient absorption from dead woods that are 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). Establishing symbiotic relationships between Cerambycidae through mycetomes would have plausible advantages for fungi. Our study and previous studies have shown that the mycetomes of some species include fungi that cannot proliferate on a PDA plate, although such a plate is sufficient for many yeasts to grow (Müller 1934). From this result, one of the functions of the mycetome is thought to be providing fungi with nutrients that are necessary for their proliferation. Moreover, we here showed that the mycetomes of larval Lepturinae possessed yeast cells even when the larvae ate an artificial diet including antibiotics, which can inactivate yeast cells by overnight incubation, suggesting that another role of the mycetome is to protect fungi from antibiotic chemicals that could be included in plants or secreted from competitive fungi. Unlike Lepturinae, *Necydalis formosana* larvae lost symbionts from their mycetomes when the larvae were cultured with a diet including antibiotics, suggesting that the mycetomes of Lepturinae and Necydalinae are functionally and structurally different. In most cases, a single yeast species occupies the mycetome of a larva, even though the larvae have a chance to incorporate external yeasts or yeast-like fungi that are spontaneously present in dead trees. Therefore, the mycetome can protect symbiotic fungi from competition with other fungi.

In contrast to the advantages for fungi, the benefits conferred to the longicorn beetles themselves remain obscure. It is clear that longicorn beetles initiatively evolved

this symbiosis because the mycetome is a longicorn beetle-derived organ and fungi are not necessary for its formation. This suggests that the symbiosis with fungi is advantageous for longicorn beetles. It has been proposed that symbiont fungi are nutritionally beneficial for host beetles, as in the case of many wood-eating insects and their symbiotic gut microorganisms (Gibson and Hunter 2010). Our study provided a line of evidence suggesting the symbiosis is related to nutrition. Some Lepturinae species do not have mycetomes, and the loss of this organ occurred over several rounds of speciation (Fig. 2). The loss of the mycetome is strongly associated with the choice of diet. The mycetome-lacking Lepturinae species eat inner bark or living roots, while most Lepturinae larvae having mycetomes depend on brown putrefied wood. Because inner bark and living roots contain more nutrients than wood (Schowalter 1992), the change of diet preference may have enabled the Lepturinae species to be free from mycetome-dwelling yeasts due to the improved nutrient uptake. We also showed that mycetome-possessing Lepturinae larvae can grow without symbiont yeasts when fed an artificial diet. For this experiment, we used an artificial diet based on the silkworm diet, which is much richer in nutrients than dead woods. Therefore, the species having mycetomes do not require symbiont yeasts under such improved nutritional conditions, which paradoxically supports the idea that the benefits from symbiont fungi are associated with nutrients. To confirm this more conclusively, we need to compare the growth rate between larvae having mycetomes with or those having mycetomes without yeast when fed a diet having low nutritional value. Our method of obtaining larvae with yeast-free mycetomes will assist in such future experiments.

Among the Cerambycidae group, many Lamiinae and Cerambycinae species are known to preferentially eat inner bark or live trees. These species do not have

mycetomes, probably because their diets are more nutritive than those of mycetome-possessing groups. Lamiinae is the sister group of Spondylidinae, which is dominant in mycetome-possessing species. Unlike Lamiinae, Spondylidinae longicorn beetles are usually dependent on brown putrefied woods in Japan. The strategy of symbiosis with fungi in these two groups may have differed in relation to their different diet choices after the groups split. We have to note that the tendency of diet choice is not always supported, since several mycetome-lacking species eat decayed wood or outer bark. These species may have another way to strength their nutritional condition which includes establishing symbiosis with another microorganism group such as bacteria, as was reported previously (Grünwald et al., 2010).

In our experiments, mycetome-lacking larvae of longicorn beetles tended not to have yeast in their gut, suggesting that these species do not transfer yeasts between generations. When yeasts were found in these larvae, the assortment of yeast species usually varied among individuals. A possible explanation is that mycetome-lacking larvae uptake yeasts from their environment, and yeast species that can withstand larval digestive systems proliferate in the gut. Such "accidental" dwelling of yeast is likely to be the ancestral form of the symbiosis between longicorn beetles and yeasts, as was suggested previously (Douglas 1989), and the external delivery of yeasts observed in mycetome-possessing species is probably the remnant of this ancestral symbiosis. Because the mycetome-lacking longicorn beetle *A. sinicum* can share a limited number of yeast species between generations, the emergence of mycetomes is not requisite for longicorn beetles to initiate symbiosis with specific yeasts.

Because mycetome-possessing longicorn beetles tend to have a single preferred yeast or fungal species/group as their symbiosis partner, mycetomes are thought to have

been developed to capture a specific symbiont. Mycetomes and symbiont fungi have characteristics specific to longicorn beetle groups. These group-specific characteristics suggest the presence of a strong constraint associated with group-specific diet choice. During the speciation of longicorn beetles, however, the error-acceptable transmission of symbiont fungi allowed diversification of symbiont yeast species, and such diversification is not always associated with longicorn beetle speciation. 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 asymmetric evolution is shared among Coleopteran groups. Such asymmetric 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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Figure legends

Figure 1.

Distribution of mycetomes in Cerambycidae of Japan. (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*. Anterior is toward the top. Scale bar, 500 μm. (C) Mycetomes of *Encyclops olivaceus*. (D) Mycetomes of the Spondylidinae *Asemum striatum*. The mycetomes of this group form two rings surrounding the serosa, which are shown by two arrows. (E) Giant mycetomes (My) of *Necydalis gigantea*. MG, midgut; Th, thorax. (F) Mycetome-lacking midgut of the Lepturinae *Paragaurotes doris*. (G) Anterior midgut of the Prioninae *Aegosoma sinicum*. The mycetome is absent.

Figure 2.

Distribution of mycetomes in the Tribes Lepturinae and Necydalinae. This phylogenetic tree was generated by the maximum likelihood method based on the alignment of 28s rDNA sequences. Crossed circles indicate mycetome-lacking species. Gray circles indicate Lepturinae species that preferentially include the yeast *Schefferosomyces* sp. Y1052_SICYLG3 in their mycetomes. Black circles indicate Lepturinae species that preferentially include the yeast *Schefferosomyces ergatensis*. Squares indicate Lepturinae species that preferentially include the yeast *Candida vrieseae* with the information of their host trees. These three yeasts were frequently isolated from various species. Note that this tree does not include all species examined in this study for simplicity. The numbers beside branches indicate the percentage of times that a node

was supported in 1,000 bootstrap pseudoreplications. The scores that are equal to or exceed 50% are shown. Species names are based on Danilevsky (2020). The accession numbers are shown at the right side of the species names.

Figure 3.

Fungal cells dwelling in the mycetomes. (A-C) Results for typical Lepturinae species. The names of the examined longicorn beetles are shown at the bottom of each panel. (A) Mycetome contents and yeast cells. Vesicular structures that are suspected to be mycetocytes are shown by arrows. An arrowhead indicates an example of symbiont yeast cells. Yeast cells are shown in higher magnified images in (B). Scale bar, 50 μm in (A) and 10 μm in (B). (C) Yeast colonies on a PDA plate. (D-F) The results for Lepturinae *Encyclops olivaceus*. In (F), no colony was detected. (G-I) The results for Necydalinae species. The fungal cell in (H') was isolated from a female oviduct. (J-L) The results for Spondylidinae species. Arrows indicate the vesicular structures suspected to be mycetocytes.

Figure 4.

Phylogenetic relationships of yeasts isolated from longicorn beetle larvae in this study. This phylogenetic tree was generated by the maximum likelihood method based on the alignment of ITS rDNA sequences. To simplify the results, the yeasts isolated from mycetome-possessing species and *Aegosoma sinicum* were analyzed. The species names of yeasts are abbreviated. For example, "Judolidia bangi Se" indicates "*Schefferosomyces ergatensis* isolated from *Judolidia bangi*". The yeasts isolated from larvae of *Aegosoma sinicum* are indicated by the sampling location and identification

numbers corresponding to those in Table 2. The number noted at a given branch indicates the percentage of times that a node was supported in 1,000 bootstrap pseudoreplications. The scores that are equal to or exceed 30% are shown.

Figure 5.

Fungal cells seen in the 1st instar larvae of Lepturinae/Necydalinae beetles. The observation was carried out over the first few days after hatching. (A-A') Anterior midgut of *Ohbayashia nigromarginata* and yeast cells isolated from the gut. The bracket indicates mycetomes. Bar, 50 μm for each panel. (B-B') Anterior midgut of *Necydalis formosana* and a fungal cell isolated from the gut. Mycetome-like knobbed structures are shown by the bracket.

Figure 6.

Yeast-free mycetomes in Lepturinae larvae. (A) *Leptura orchraceofasciata* larvae cultured with an artificial diet. The images were obtained approximately one month after hatching. (B) Yeast-free mycetomes (knobs shown by the bracket). (C) A magnified image of the contents of yeast-free mycetomes. The inset shows the results of culturing of the contents on a PDA plate. No colony appeared. (D-I) The results for *Stictoleptura dichroa*. (D-E) Yeast-free mycetomes. (F-G) Yeast-poor mycetomes. Note that the color of the mycetomes is somewhat whitish. Four examples of yeast cells are shown by arrowheads. The yeast *Meyerozyma caribbica* was identified from the mycetomes. (H-I) Yeast-rich mycetomes. The yeast *Schefferosomyces ergatensis* was identified from these mycetomes.

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

Groups	Number of observed species	Number of observed larvae	Number of yeast colonies yielding larvae	Number of larvae which had more than one yeast species	Number 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-possessing groups	30	156	119	8	18
Mycetome-lacking groups	46	135	63	13	46

Table 2. Symbiont fungi isolated from larvae of Longicorn beetles in Japan

Group	Species	Collected location*	Host	Mycetome	Analyzed region	Colonies on the 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 orchraceofasciata*	Kagoshima Yakushima Island	Symplocos myrtacea	y	mycetome	y	[Candida] vrieseae CBS 10829	597/612	OP537557
Lepturinae with mycetome	*Leptura orchraceofasciata*	Aichi	Lindera triloba	y	mycetome	y	Scheffersomyces sp. Y1052_SICYLG3	592/593	OP537558
Lepturinae with mycetome	*Leptura orchraceofasciata*	Mie	Cryptomeria japonica	y	mycetome	y	Scheffersomyces sp. Y1052_SICYLG3	616/621	OP537559
Lepturinae with mycetome	*Leptura orchraceofasciata*	Shizuoka	Symplocos coreana	y	mycetome	y	Scheffersomyces coipomoensis strain Y3	615/615	OP537560
Lepturinae with mycetome	*Leptura orchraceofasciata*	Shizuoka	Chamaecyparis obtusa	y	mycetome	y	Scheffersomyces sp. Y1052_SICYLG3	619/619	OP537561
Lepturinae with mycetome	*Leptura orchraceofasciata*	Kagoshima	unknown	y	mycetome	y	Scheffersomyces sp. Y1052_SICYLG3	619/619	OP537562
Lepturinae with mycetome	*Leptura orchraceofasciata*	Gifu	Hamamelis japonica	y	mycetome	y	Scheffersomyces sp. Y1052_SICYLG3	618/619	OP537563
Lepturinae with mycetome	*Leptura orchraceofasciata*	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 obs	gut	y	Scheffersomyces sp. strain Y13	588/589	OP537570
Lepturinae with mycetome	*Munamizoa maculata*	Yamanashi	Chamaecyparis obtusa	Failed to obs	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 obs	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	396/408	OP537649
							Wickerhamomyces canadensis culture CBS:5676	522/586	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/maculith*	Aichi	Kalopanax septemlobus	n	gut	y	[Candida] montana isolate yHRVM49	396/408	OP537653
Lepturinae w/o mycetome	*Pidonia sylvicola/himehana/maculith*	Aichi	Kalopanax septemlobus	n	gut	y	Wickerhamomyces canadensis culture CBS:5676	522/586	OP537654
							[Candida] montana isolate yHRVM49	396/408	OP589179
Lepturinae w/o mycetome	*Pidonia sylvicola/himehana/maculith*	Aichi	Kalopanax septemlobus	n	gut	y	[Candida] montana isolate yHRVM49	396/408	OP537655
Lepturinae w/o mycetome	*Pidonia sylvicola/himehana/maculith*	Yamanashi	unknown	n	gut	no yeast colony			
							[Candida] gorgasii ATCC MYA-4334	584/621	OP537656
Lepturinae w/o mycetome	*Japanocorus caeruleipennis*	Culture		n	gut	y	[Candida] michaelii culture CBS:9502	613/659	OP589180
							Sugiyamaella smithiae strain NS1	549/549	OP589181
							[Candida] insectorum	625/625	OP537657
Lepturinae w/o mycetome	*Japanocorus caeruleipennis*	Culture		n	gut	y	Nakazawaea sp. isolate UFMG-CM-Y6909	609/611	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			
							Candida sp. Af110-2-1	591/591	OP537660
Lepturinae w/o mycetome	*Japanocorus caeruleipennis*	Culture		n	gut	y	Pichia hampshirensis strain ATCC 62896	551/566	OP589182
							Pichia veronae strain CECT 11800	594/596	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
							Debaryomyces castellii culture CBS:2923 (mycetome)	589/627	OP537682
Spondylidinae	*Megasemum quadricostulatum*	Yamanashi	Abies veitchii	y	mycetome	y 6	Scheffersomyces sp. strain Y13 (colony)	577/578	OP537683
							Candida ergatensis strain ATCC MYA-4655 (colony)	613/616	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	43/43	OP537687
							Debaryomyces castellii culture CBS:2923	589/627	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	614/615	OP537697
							Scheffersomyces sp. Y1052_SICYLG	619/619	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	579/579	OP537701
							Rhodotorula mucilaginosa strain 2018A0762M12	576/577	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	did not obse	gut	no yeast colony			
Prioninae	*Aegosoma sinicum*	Kyoto2	Populus sp	did not obse	gut	y	Scheffersomyces anoplophorae	587/591	OP537705
Prioninae	*Aegosoma sinicum*	Kyoto3	Populus sp	did not obse	gut	y	Scheffersomyces anoplophorae	577/581	OP537706
Prioninae	*Aegosoma sinicum*	Ibaraki1	Salix sp.	n	gut	y	Uncultured Scheffersomyces clone 15y-3	582/584	OP537707
							Scheffersomyces sp. YW01.2	577/577	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 obs	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 obs	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 obs	frass	no yeast colony			
Cerambycinae	*Epania dilaticornis*	Kagoshima	Diospyros lotus	failed to obs	frass	no yeast colony			
Cerambycinae	*Epania dilaticornis*	Kagoshima	Diospyros lotus	failed to obs	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 obs	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

							Yamadazyma mexicana culture CBS:2089	606/618	OP537736
Lamiinae	*Eutetrapha chrysochloris*	Nagano	Quercus serrata	n	gut	y	Pichia hampshirensis strain ATCC 62896	572/587	OP537737
							Candida sp. (in: Saccharomycetales) strain NYUN 211183	435/436	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	404/404	OP537742
							Hyphopichia sp. isolate SFC101142	385/390	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	596/596	OP537747
							Sugiyamaella boreocaroliniensis CBS 10344	555/555	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 numbers in the colum labeled "Aegosoma sinicum" correspond to those in Figure 4.

**The numbers of colones are shown for plates on which serveral or more colonies appeared.

Table 3. Symbiont fungi isolated from female adults of Longicorn beetles in Japan

Group	Species	Collected location	Analyzed region	Colonies on the 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 [Candida] vrieseae CBS 10829	OP537766 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	

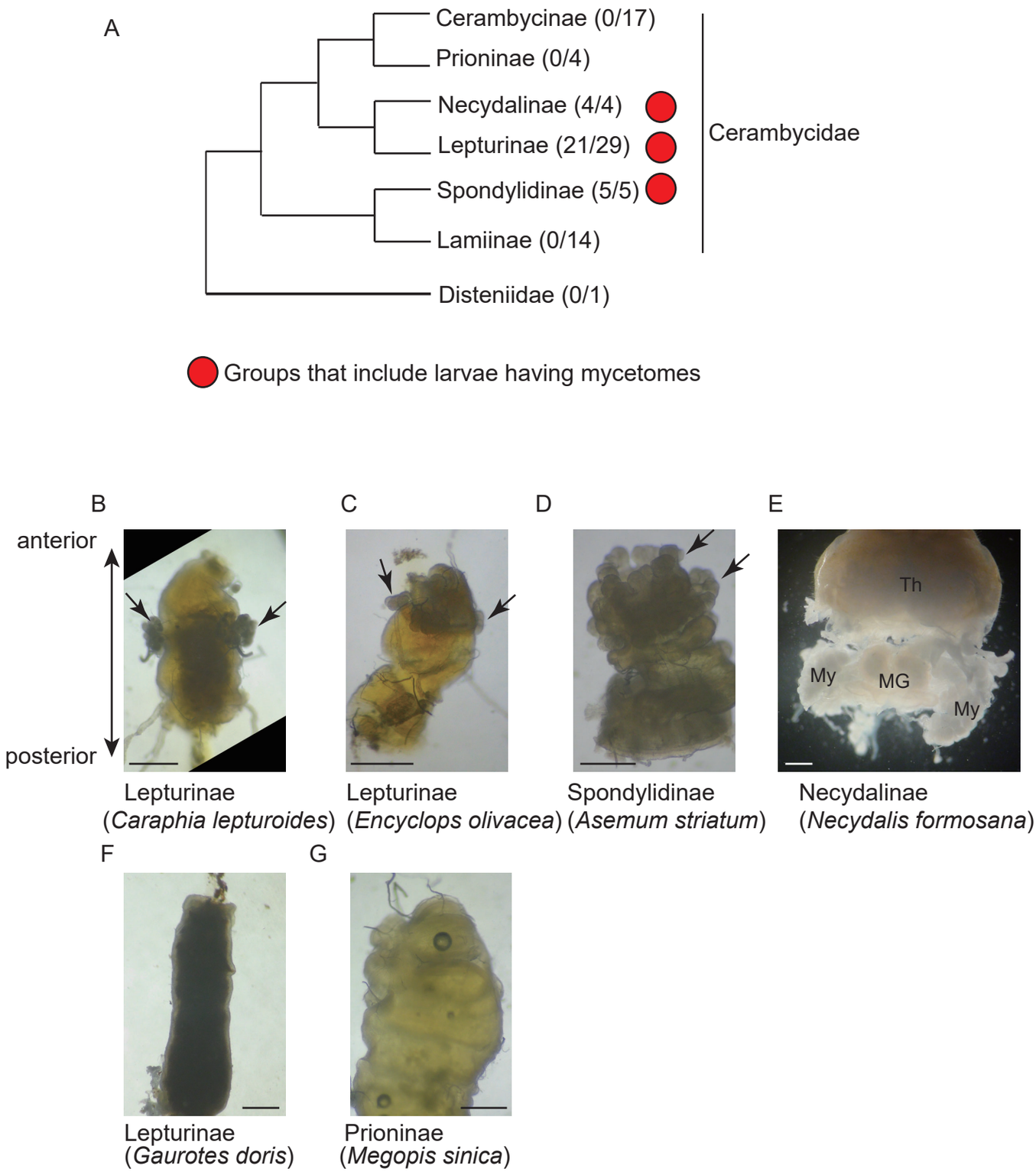
Lepturinae	*Leptura ochraceofasciata*	Shizuoka	Female oviduct	y	Scheffersomyces sp. Y1052_SICYLG3	
Lepturinae	*Encyclops olivaceus*	Yamanashi	Female oviduct	no yeast colony	Chrysolina oricalcia genome assembly, chromosome: 12	OP537772
Lepturinae	*Encyclops olivaceus*	Yamanashi	Female oviduct	no yeast colony	Chrysolina oricalcia genome assembly, chromosome: 12	OP537773
Necydalinae	*Necydalis sacharinensis*	Nagano	Female oviduct	no yeast colony	Chrysolina oricalcia genome assembly, chromosome: 12	OP537774
					Uncultured fungus isolate FICUS 144	OP537775
Necydalinae	*Necydalis sacharinensis*	Nagano	Female oviduct	no yeast colony	Chrysolina oricalcia genome assembly, chromosome: 12	OP537776
					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
Prioninae	*Aegosoma sinicum*	Shizuoka	Egg surface	y	Scheffersomyces anoplophorae	OP537781
					Meyerozyma guilliermondii strain CBS 2030	OP537782
					Meyerozyma carpophila strain CBS 5256	OP537783
Prioninae	*Aegosoma sinicum*	Osaka	Egg surface	y	Scheffersomyces anoplophorae	OP537784
					Kodamaea ohmeri strain DM9Y	OP537785
					Kodamaea ohmeri strain SM14UFAM	OP537786
					Debaryomyces hansenii IFM 55872	OP537787
					[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 4. Artificial transfer of yeasts to larvae

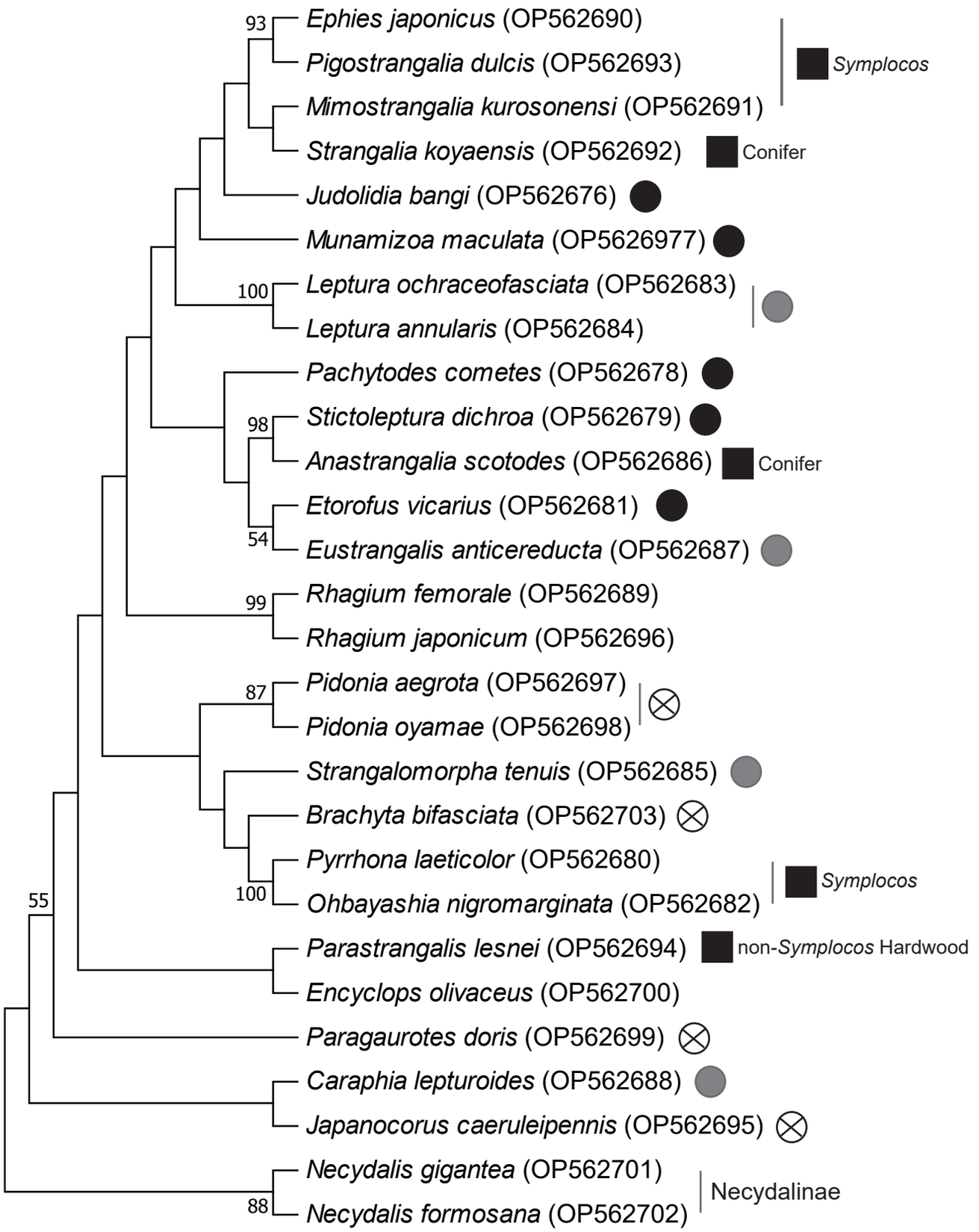
Species and biological replicate	Sterilization	Given yeast	Detected yeast
Pyrrhona laeticolor Exp 1	No	Not given	Meyerozyma guilliermondii strain CBS 2030
	No	Not given	[Candida] vrieseae CBS 10829
	No	Not given	[Candida] vrieseae CBS 10829
	No	Not given	Meyerozyma guilliermondii strain CBS 2030
	No	Not given	Meyerozyma guilliermondii strain CBS 2030
	No	Not given	No yeast detected
	No	Not given	No yeast detected
	No	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Scheffersomyces sp. Y1052_SICYLG3	Scheffersomyces sp. Y1052_SICYLG3
	Yes	Scheffersomyces sp. Y1052_SICYLG3	Scheffersomyces sp. Y1052_SICYLG3
	Yes	Scheffersomyces sp. Y1052_SICYLG3	No yeast detected
	Yes	Scheffersomyces sp. Y1052_SICYLG3	Scheffersomyces sp. Y1052_SICYLG3
	Yes	Scheffersomyces coipomoensis strain Y3	Scheffersomyces coipomoensis strain Y3
	Yes	Scheffersomyces coipomoensis strain Y3	Scheffersomyces coipomoensis strain Y3
	Yes	Scheffersomyces coipomoensis strain Y3	Scheffersomyces coipomoensis strain Y3
	Yes	Scheffersomyces coipomoensis strain Y3	Scheffersomyces coipomoensis strain Y3
	Yes	Scheffersomyces coipomoensis strain Y3	Scheffersomyces coipomoensis strain Y3
	Yes	Scheffersomyces coipomoensis strain Y3	Scheffersomyces coipomoensis strain Y3
Pyrrhona laeticolor Exp 2	No	Not given	Meyerozyma guilliermondii isolate E22316_ITS
	No	Not given	Meyerozyma guilliermondii
	Yes	Not given	Meyerozyma guilliermondii strain CBS 2030
	Yes	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Scheffersomyces sp. Y1052_SICYLG3	Scheffersomyces sp. Y1052_SICYLG3
	Yes	Scheffersomyces sp. Y1052_SICYLG3	Scheffersomyces sp. Y1052_SICYLG3
	Yes	Scheffersomyces sp. Y1052_SICYLG3	Scheffersomyces sp. Y1052_SICYLG3
	Yes	Scheffersomyces coipomoensis strain Y3	Scheffersomyces coipomoensis strain Y3
	Yes	Scheffersomyces coipomoensis strain Y3	No yeast detected
Ohbayashia nigromarginata	No	Not given	No yeast detected
	No	Not given	[Candida] vrieseae CBS 10829
	No	Not given	[Candida] vrieseae CBS 10829
	Yes	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Scheffersomyces sp. Y1052_SICYLG3	No yeast detected
	Yes	Scheffersomyces sp. Y1052_SICYLG3	No yeast detected
	Yes	Scheffersomyces sp. Y1052_SICYLG3	No yeast detected
	Yes	Scheffersomyces coipomoensis strain Y3	No yeast detected
	Yes	Scheffersomyces coipomoensis strain Y3	Scheffersomyces coipomoensis strain Y3
	Yes	Scheffersomyces coipomoensis strain Y3	No yeast detected
Leptura orchraceofasciata Exp 1	No	Not given	Scheffersomyces sp. Y1052_SICYLG3
	No	Not given	Scheffersomyces sp. Y1052_SICYLG3
	No	Not given	Scheffersomyces sp. Y1052_SICYLG3
	No	Not given	Scheffersomyces sp. Y1052_SICYLG3
	No	Not given	Scheffersomyces sp. Y1052_SICYLG3
	No	Not given	Scheffersomyces sp. Y1052_SICYLG3
	No	Not given	Scheffersomyces sp. Y1052_SICYLG3
	No	Not given	Scheffersomyces sp. Y1052_SICYLG3
	No	Not given	Scheffersomyces sp. Y1052_SICYLG3
	No	Not given	Scheffersomyces sp. Y1052_SICYLG3
	Yes	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Not given	Meyerozyma guilliermondii strain CBS 2030
	Yes	Scheffersomyces coipomoensis strain Y3	Scheffersomyces coipomoensis strain Y3
	Yes	Scheffersomyces coipomoensis strain Y3	Scheffersomyces coipomoensis strain Y3

	Yes	Scheffersomyces coipomoensis strain Y3	Scheffersomyces coipomoensis strain Y3
	Yes	Candida vrieseae	Candida vrieseae
	Yes	Candida vrieseae	Candida vrieseae
	Yes	Candida vrieseae	Candida vrieseae
	No	Not given	Scheffersomyces sp. Y1052_SICYLG3
	No	Not given	Scheffersomyces sp. Y1052_SICYLG3
	No	Not given	Scheffersomyces sp. Y1052_SICYLG3
	No	Not given	Scheffersomyces sp. Y1052_SICYLG3
	No	Not given	Scheffersomyces sp. Y1052_SICYLG3
Leptura orchraceofasciata Exp 2	Yes	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Not given	No yeast detected
	Yes	Scheffersomyces coipomoensis strain Y3	Scheffersomyces coipomoensis strain Y3
	Yes	Scheffersomyces coipomoensis strain Y3	Scheffersomyces coipomoensis strain Y3
	Yes	Scheffersomyces coipomoensis strain Y3	No yeast detected
	Yes	Candida vrieseae	No yeast detected
	Yes	Candida vrieseae	Candida sp. W377
	Yes	Candida vrieseae	No yeast detected

Sasakura et al., Figure 1.



Sasakura et al., Figure 2



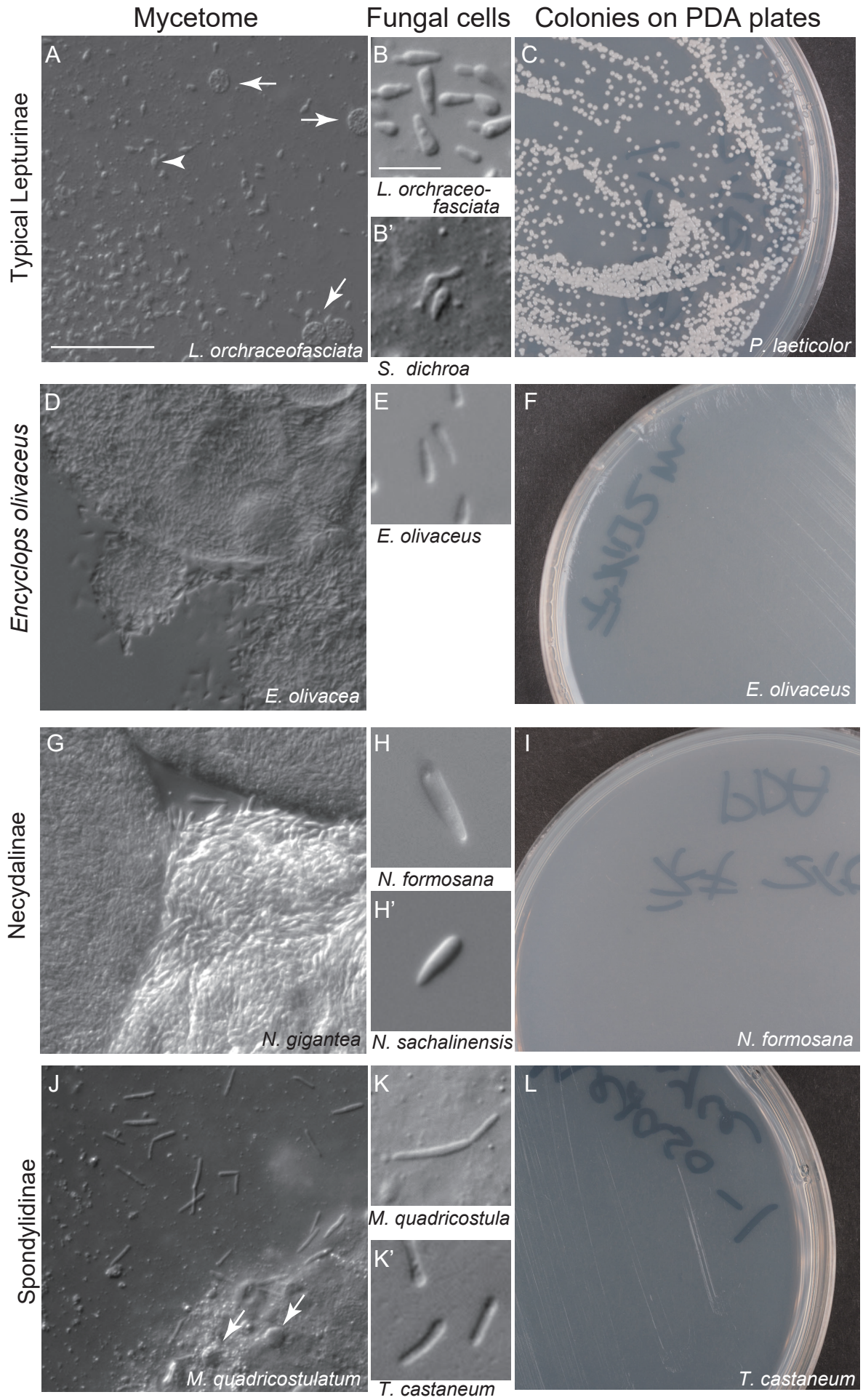
Mycetome-lacking species/groups

Species or group that possessed *Schefferosomyces* sp. Y1052_SICYLG3

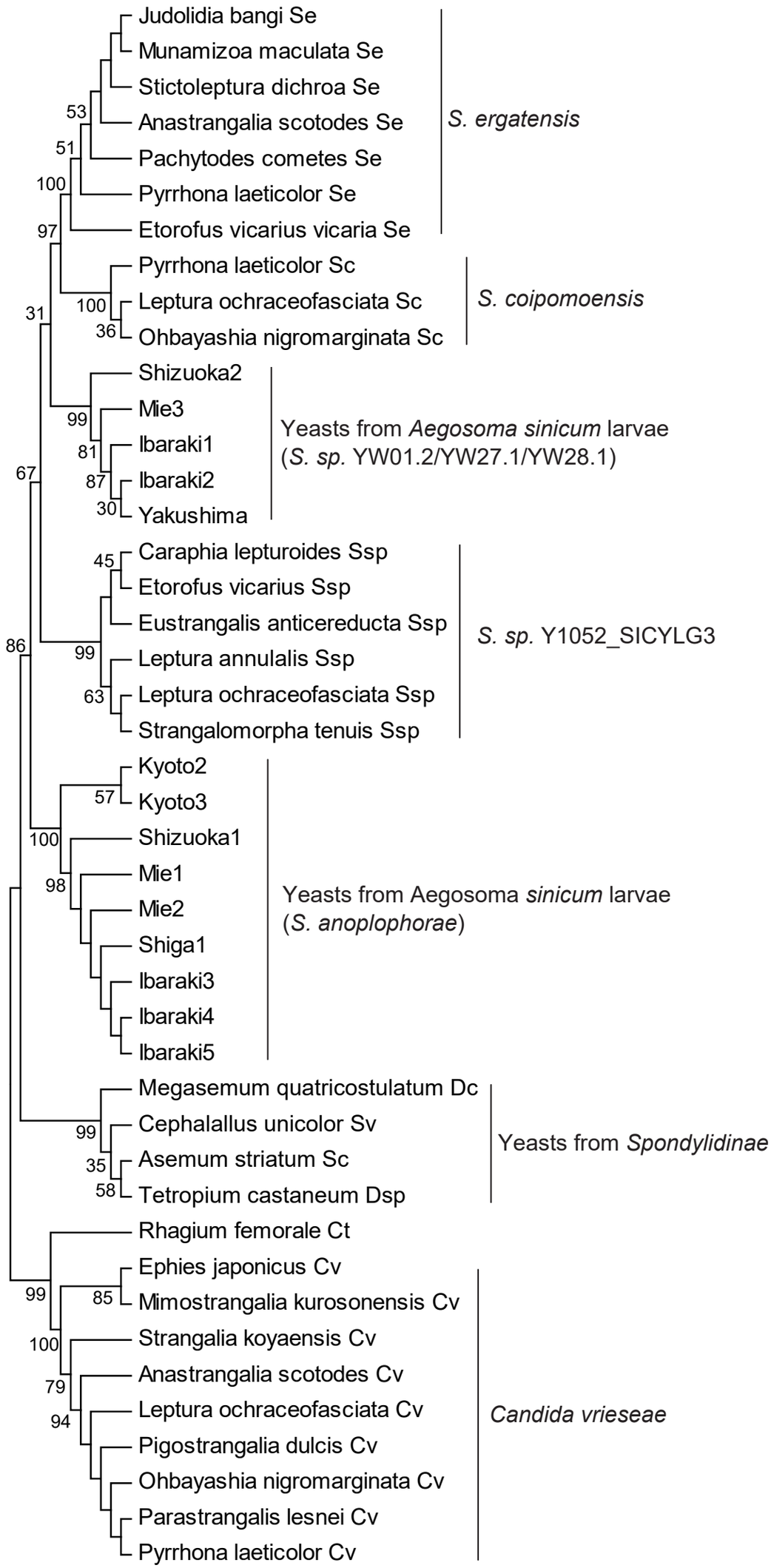
Species or group that possessed *Schefferosomyces ergatensis*

Species or group that possessed *Candida vrieseae*

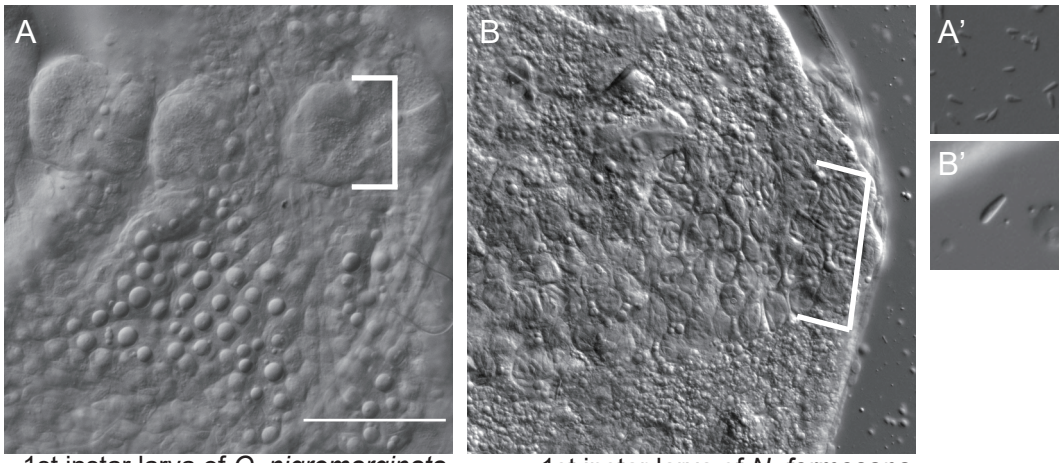
Sasakura et al., Figure 3



Sasakura et al., Figure 4



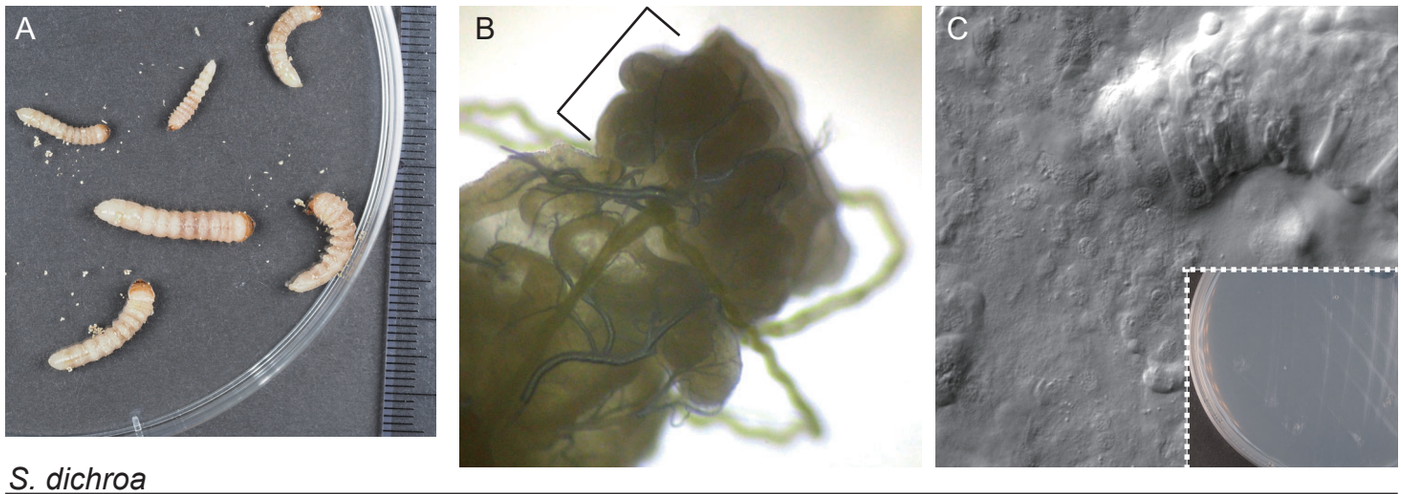
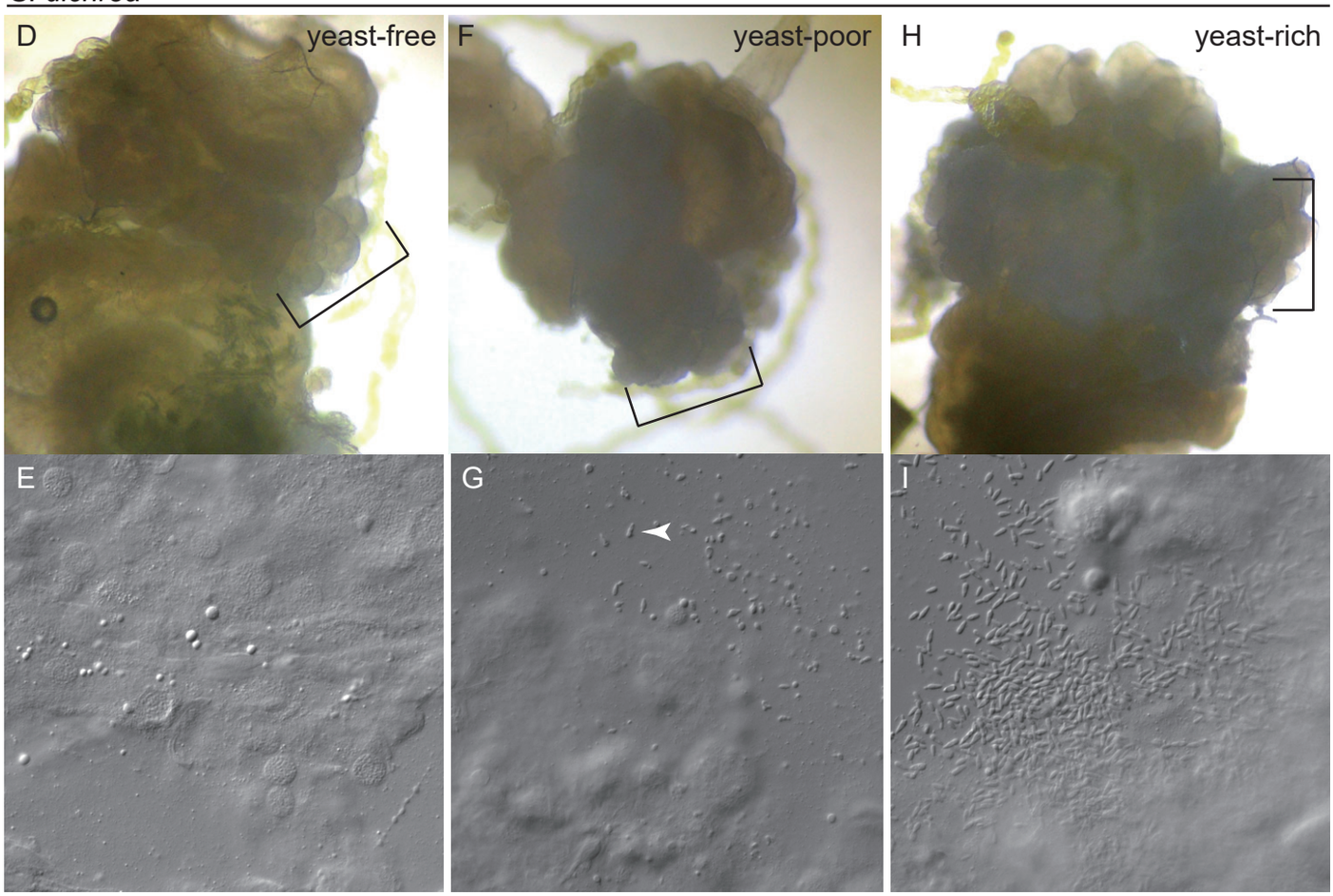
Sasakura et al., Figure 5



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

Sasakura et al., Figure 6

L. orchraceofasciata



S. dichroa