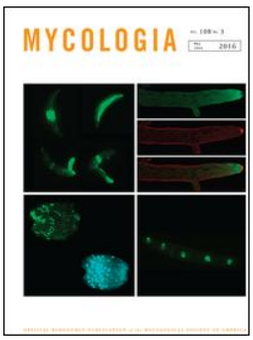

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Cultural characterization and chlamydospore function of the Ganodermataceae present in the eastern United States

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ABSTRACT

The cultural characteristics of fungi can provide useful information for studying the biology and ecology of a group of closely related species, but these features are often overlooked in the order Polyporales. Optimal temperature and growth rate data can also be of utility for strain selection of cultivated fungi such as reishi (i.e., laccate *Ganoderma* species) and potential novel management tactics (e.g., solarization) for butt rot diseases caused by *Ganoderma* species. Historically, the taxonomy of the laccate (shiny) *Ganoderma* species has been unresolved and many species have been treated together as *G. lucidum*. The cultural characteristics of *Ganoderma* species from the United States are needed to understand the biology of these unique species that have all been lumped under this name. Culture morphology, average growth rate, optimal temperatures, and resiliency to elevated temperature exposure were characterized for isolates of Ganodermataceae taxa from the eastern United States, including *Ganoderma curtisii, G. martinicense, G. meredithiae, G. ravenelii, G. sessile, G. tsugae, G. tuberculosum, G.* cf. *weberianum, G. zonatum*, and *Tomophagus colossus*. We documented differences in linear growth rates and optimal temperatures between taxa. Isolates of *G. sessile* and *T. colossus* grew the fastest, and isolates of *G. meredithiae, G. ravenelii*, and *G. tsugae* grew the slowest. Isolates of *G. sessile, G. martinicense, G.* cf. *weberianum*, and *T. colossus* constitutively produced chlamydospores on malt extract agar, and these species were the only species to survive long-term exposure (30 or 40 d) to 40 C. We hypothesize that chlamydospores function as survival structures that serve as propagules resilient to adverse temperature conditions, especially heat. Cultural characteristics of *G. martinicense, G. ravenelii, G. tuberculosum*, and *G.* cf. *weberianum* collected from the United States are described for the first time.

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Chlamydospores; Ganodermataceae; polypores; survival

INTRODUCTION

Ganoderma Karst. is a large and diverse genus, and these fungi cause white rot of the roots and lower bole of many tree species (Murrill 1902; Elliott and Broschat 2001; Schwarze and Ferner 2003). Species of *Ganoderma* are found worldwide in both urban and natural settings and are generally associated with declining and dead trees. The taxonomy of North American *Ganoderma* species is confusing (Moncalvo et al. 1995; Zhou et al. 2015) but has recently been resolved for the laccate *Ganoderma* species in the United States (Loyd et al. 2018a). In the past century, the name *G. lucidum* sensu lato has been used for any laccate (varnished or polished) *Ganoderma* species growing on hardwood trees (Gilbertson and Ryvarden 1986; Adaskaveg and Gilbertson 1988, 1989; Hapuarachchi et al. 2015). It is now recognized that *G. lucidum* sensu stricto (Curtis) Karst only occurs in Europe and possibly some parts of China (Moncalvo et al. 1995; Postnova and Skolotneva 2010; Zhou et al. 2015; Hennicke et al. 2016). Since there are many species of laccate *Ganoderma* in North America (Loyd et al. 2018a), it is likely that these species differ in their physiology, anatomy, and ecological niches.

The laccate *Ganoderma* species have nuanced differences in basidiomata morphology, host preference, and geographic limitations, and these features have been studied extensively in many parts of the world (Murrill 1902, 1908; Steyaert 1980; Gilbertson and Ryvarden 1986; Welti and Courtecuisse 2010; Loyd et al. 2018a). The in vitro cultural growth habits such as growth rate and optimal temperature ranges have been rarely reported for species of *Ganoderma* (Nobles 1965; Bazzalo and Wright 1982; Adaskaveg and Gilbertson 1989). However, the in vitro growth rates of the laccate *Ganoderma* species can be used as

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a diagnostic feature to distinguish some species of *Ganoderma*, such as *G. colossum, G. zonatum*, and *G. tsugae* (Adaskaveg and Gilbertson 1988, 1989). Similarly, previous research found that decay rates are generally proportional to linear growth rates at an optimal temperature for *Ganoderma* taxa (Adaskaveg and Gilbertson 1986a). This suggests that rapidly growing isolates would likely decay woody substrates quicker than slower growing isolates. This proxy can be used in rapid cultural experiments that estimate decay rates of common wood decay fungi.

In addition to growth rates, optimal temperature ranges can distinguish some *Ganoderma* species, such as *G. tsugae* (20–25 C) and *G. colossum* (35–40 C) (Adaskaveg and Gilbertson 1989). Furthermore, investigating the optimal temperature ranges of wood-degrading fungi can be useful for understanding geographic distributions. This approach has been used to study the limitations of *Fomes fomentarius* and *F. fasciatus* in the eastern United States (McCormick et al. 2013). Experiments that investigate optimal temperature ranges can also elucidate the etiology of pathogenic *Ganoderma* species, such as *G. zonatum*, and potentially lead to novel management strategies for landscape professionals.

Lastly, optimal temperatures and growth rates can be important in strain selection for the reishi cultivation industry to help determine the best strains, as observed with other medicinal fungi (Dresch et al. 2015). *Ganoderma* species have been used medicinally for thousands of years in Asia where they are commonly referred to as "reishi" or "lingzhi" (Stamets 2000). Reishi have been prescribed as a supplement in traditional Eastern medicine for use as an anti-inflammatory, immune-enhancing, and cancer therapy drug (Wang et al. 2012; Hennicke et al. 2016). Recently, Loyd et al. (2018d) showed that cultivated "Grow-your-own" reishi kits sold in the United States are labeled as *G. lucidum* but in fact almost always contain another species such as the Asian species *G. lingzhi*. Optimal temperature and growth rate data could be helpful for industrial cultivators or hobby growers in choosing a tenacious strain of reishi and growing it at the correct growth temperature, assuming it is labeled with the correct species.

In addition to mycelial growth, chlamydospores are produced by some species of laccate *Ganoderma* (Nobles 1965; Adaskaveg and Gilbertson 1989). Chlamydospores are asexually produced, thick-walled spores that are found in numerous groups of fungi and fungal-like organisms (e.g., *Fusarium* spp., *Phytophthora* spp., and some wood decay fungi) (Adaskaveg and Gilbertson 1986b, 1989; Erwin and Ribeiro 1996; Chang 2003; Bennett 2012). Chlamydospores are generally considered survival structures that can withstand adverse environmental conditions (e.g., dry conditions, heat, cold, flooding, etc.) (Erwin and Ribeiro 1996; Chang 2003; Bennett 2012). Chlamydospores could play an important role in the life cycle of this group of wood decay fungi, and few studies have characterized the function of chlamydospores in the Ganodermataceae (Chang 2003).

Due to the ambiguous use of the name *G. lucidum* in North America (where the name has been historically used for many laccate *Ganoderma* species), the cultural growth habits published in the literature for this taxon in North America are questionable and should be reassessed (Atkinson 1908; Haddow 1931; Nobles 1948; Overholts 1953; Gilbertson and Ryvarden 1986; Adaskaveg and Gilbertson 1989). The objectives of this research are to (i) characterize the cultural morphology, (ii) determine the optimal temperature ranges and average growth rates, and (iii) determine the resiliency following elevated temperature (40 C) exposure of Ganodermataceae taxa collected in the eastern United States. It is likely that the physiology and general biology of these species are different. Determining differences in their physiology is needed to better understand the role they play in tree decline and death.

MATERIALS AND METHODS

Isolate collection and colony morphology.—Thirty-two dikaryotic isolates of laccate *Ganoderma* species from the eastern United States (TABLE 1) were made from basidiomata by excising small pieces (<1 cm^3) of context tissue with a sterile scalpel and placing them onto medium made with a base of malt extract agar (MEA) (Difco Laboratories, Franklin Lakes, New Jersey) according to the manufacturer's instructions with the addition of streptomycin sulfate (100 mg/L) (Fisher Scientific, Waltham, Massachusetts), benomyl 95% (4 mg/L) (Benlate, Sigma-Aldrich, St. Louis, Missouri), and lactic acid (1 mL/L) (Fisher Scientific). Isolates were subcultured to obtain pure cultures and then maintained on MEA without antibiotics. All isolates were maintained for long-term storage on colonized pieces of MEA medium submerged in sterile deionized water (diH_2O). Culture morphology was assessed visually on MEA after 8 d of growth at each temperature for each taxon. Colony morphology was described using the guidelines of Stalpers (1978), with colors based on Ridgway (1912). Each isolate was examined microscopically for the presence/absence of chlamydospores. Chlamydospores were examined by making slide mounts of fresh mycelium from the center of an 8-d-old culture in a drop of 5% KOH and then visualizing using a Nikon Eclipse 55i light

Table 1. Isolates of the laccate *Ganoderma* species used in these experiments, with metadata including isolate names, locations, ITS accession numbers, and host substrates.

Isolate	Taxon	Location	Associated substrate[a]	Culture collection[b]	Accession no.
WD2085	*G. boninense*	Japan	Unknown	Unknown	KJ143906
WD2028	*G. boninense*	Japan	Unknown	Unknown	KJ143905
102NC	***G. curtisii***	**North Carolina, USA**	**Hardwood**	**CFMR**	**MG654073**
UMNGA1	***G. curtisii***	**South Carolina, USA**	**Hardwood**	**CFMR**	**MG654117**
UMNFL28	***G. curtisii***	**Florida, USA**	**Hardwood**	**CFMR**	**MG654097**
UMNFL60	***G. curtisii***	**Florida, USA**	**Hardwood**	**CFMR**	**MG654105**
CBS100131	*G. curtisii*	North Carolina, USA	Unknown	Unknown	JQ781848
CBS100132	*G. curtisii*	North Carolina, USA	Unknown	Unknown	JQ781849
Cui9166	*G. lingzhi*	Shandong, China	Unknown	Unknown	KJ143907
Dai12479	*G. lingzhi*	Anhui, China	Unknown	Unknown	JQ781864
MT26/10	*G. lucidum*	Czech Republic	Unknown	Unknown	KJ143912
Rivoire4195	*G. lucidum*	France	Unknown	Unknown	KJ143909
231NC	***G. martinicense***	**North Carolina, USA**	**Hardwood**	**CFMR**	**MG654182**
246TX	***G. martinicense***	**Texas, USA**	**Hardwood**	**CFMR**	**MG654185**
UMNSC7	***G. martinicense***	**South Carolina, USA**	**Hardwood**	**CFMR**	**MG654177**
UMNTN1	***G. martinicense***	**Tennessee, USA**	**Cactus**	**CFMR**	**MG654178**
LIPSW-Mart08-55	*G. martinicense*	French West Indies	Unknown	Unknown	KF963256
LIPSW-Mart08-44	*G. martinicense*	French West Indies	Unknown	Unknown	KF963257
124FL	***G. meredithiae***	**Florida, USA**	**Conifer**	**CFMR**	**MG654188**
UMNFL50	***G. meredithiae***	**Florida, USA**	**Conifer**	**CFMR**	**MG654103**
UMNFL64	***G. meredithiae***	**Florida, USA**	**Conifer**	**CFMR**	**MG654106**
CWN04670	*G. multipileum*	Taiwan, China	Unknown	Unknown	KJ143913
Dai9447	*G. multipileum*	Hainan, China	Unknown	Unknown	KJ143914
151FL	***G. ravenelii***	**Florida, USA**	**Hardwood**	**CFMR**	**MG654208**
MS187FL	***G. ravenelii***	**Florida, USA**	**Hardwood**	**CFMR**	**MG654211**
CBS194.76	*G. resinaceum*	Netherlands	Unknown	Unknown	KJ143916
Rivoire4150	*G. resinaceum*	France	Unknown	Unknown	KJ143915
113FL	***G. sessile***	**Florida, USA**	**Hardwood**	**CFMR**	**MG654307**
114FL	***G. sessile***	**Florida, USA**	**Hardwood**	**CFMR**	**MG654308**
117TX	***G. sessile***	**Texas, USA**	**Hardwood**	**CFMR**	**MG654309**
UMNFL22	***G. sessile***	**Florida, USA**	**Hardwood**	**CFMR**	**MG654232**
UMNFL10	***G. sessile***	**Florida, USA**	**Hardwood**	**CFMR**	**MG654227**
JV1209/27	*G. sessile*	Arizona, USA	Unknown	Unknown	KF605630
NY00985711	*G. sessile*	New York, USA	Unknown	Unknown	KJ143918
UMNMI30	***G. tsugae***	**Michigan, USA**	**Conifer**	**CFMR**	**MG654326**
UMNNC4	***G. tsugae***	**North Carolina, USA**	**Conifer**	**CFMR**	**MG654329**
UMNWI1	***G. tsugae***	**Wisconsin, USA**	**Conifer**	**CFMR**	**MG654333**
Dai12760	*G. tsugae*	Connecticut, USA	Unknown	Unknown	KJ143920
UMNFL82	***G. tuberculosum***	**Florida, USA**	**Hardwood**	**CFMR**	**KY646215**
PLM540	***G. tuberculosum***	**Florida, USA**	**Hardwood**	**CFMR**	**MG654368**
PLM684	***G. tuberculosum***	**Florida, USA**	**Hardwood**	**CFMR**	**MG654369**
LIPSW-Mart08-45	*G. tuberculosum*	French West Indies	Unknown	Unknown	KF96325
261FL	*G.* cf. *weberianum*	Florida, USA	Hardwood	Unknown	MG654370
UMNFL100	***G.* cf. *weberianum***	**Florida, USA**	**Hardwood**	**CFMR**	**MG654373**
123FL	***G. zonatum***	**Florida, USA**	**Palm**	**CFMR**	**MG654416**
UMNFL85	***G. zonatum***	**Florida, USA**	**Palm**	**CFMR**	**MG654402**
UMNFL59	***G. zonatum***	**Florida, USA**	**Palm**	**CFMR**	**MG654395**
UMNFL54	***G. zonatum***	**Florida, USA**	**Palm**	**CFMR**	**MG654393**
PLM639	***G. zonatum***	**Florida, USA**	**Palm**	**CFMR**	-
FL-02	*G. zonatum*	Florida, USA	Palm	Unknown	KJ143921
TC-02	*T. cattienensis*	Vietnam	Unknown	Unknown	KJ143923
255FL	***T. colossus***	**Florida, USA**	**Cycad**	**CFMR**	**MG654427**
UMNFL110	***T. colossus***	**Florida, USA**	Unknown	**CFMR**	**MG654429**

Note. Isolates in boldface were produced in this study, and the rest were used as reference sequences for each species.
[a]Associated substrates were based on collection information if available. If it was not available, then it is labeled as "unknown."
[b]Culture collections are noted for where cultures are stored if known. "CFMR" is the fungal culture collection at the US Forest Service Wood Products Laboratory in Madison, Wisconsin. If collection is not known, isolates are labeled as "unknown."

microscope (Melville, New York). Measurements were made for at least 20 chlamydospores per isolate using micrographs and the photo analysis software ImageJ (www.imagej.net). The measurements from each isolate were averaged for each taxon. Cultures are archived and managed at the Center for Forest Mycology Research (CFMR) Culture Collection and Herbarium, United States Department of Agriculture (USDA) Forest Service, Madison, Wisconsin, maintained by the Northern Research Station and housed in the Forest Products Laboratory. In addition, representative basidiomata are archived at the CFMR herbarium as well as duplicates at the University of Florida Mycological Herbarium (FLAS) in Gainesville, Florida.

DNA extraction, PCR, and sequencing.—Samples were identified using the macro- and micromorphological features of basidiomata based from published descriptions (Loyd et al. 2018a), and these identifications were confirmed through

sequencing and analysis of the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) (Murrill 1902, 1908; Overholts 1953; Steyaert 1972; Steyaert 1980; Gilbertson and Ryvarden 1986; Welti and Courtecuisse 2010; Loyd et al. 2018a). DNA was extracted from fresh mycelium of each isolate with the Extract-N-Amp rapid DNA kit (Sigma-Aldrich) per the manufacturer's recommendations. Amplification of the ITS region was performed with the primers ITS1f and ITS4 on a MJ Mini thermocycler (Bio-Rad, Hercules, California) with thermocycling conditions of an initial cycle of 94 C for 4 min and followed with 37 cycles of 94 C for 50 s, 55 C for 50 s, and 72 C for 1 min that produced an amplicon of ~700–800 nucleotides (White et al. 1990; Gardes and Bruns 1993). Amplicons were cleaned with Exo-SAP-IT (ThermoFisher, Waltham, Massachusetts) according to the manufacturer's instructions. Sanger sequencing was performed using both forward and reverse primers at the Interdisciplinary Center for Biotechnology Research (ICBR) at the University of Florida. Forward and reverse sequences were aligned and edited using Geneious 10 (Aukland, New Zealand). ITS sequences have been deposited to the National Center for Biotechnology Information (NCBI) GenBank database (TABLE 1).

Phylogenetic analysis.—The ITS sequences were queried against reliable, reference sequences from recent phylogenetic studies using the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1997; Zhou et al. 2015; Loyd et al. 2018a). In order to verify the phylogenetic placement and relatedness of our isolates, 51 sequences (including the sequences generated in this study and reliable reference sequences) were used in a phylogenetic analysis. Sequences were aligned using the MAFFT (Katoh et al. 2002) plugin in Geneious 10. The alignment was visually edited to remove ambiguities and minimize differences that could have resulted from sequencing error. The alignment contained 460 nucleotide characters and was used for phylogenetic analysis based on maximum likelihood with the RAxML (Stamatakis 2014) plugin in Geneious 10. The RAxML analysis used a general time reversible (GTR) evolutionary model with rapid bootstrapping and 1000 bootstrap replications. The analysis was rooted with *Tomophagus* species that belong to Ganodermataceae but are placed outside the genus *Ganoderma*. The alignment has been submitted to TreeBASE under the submission number 23202 (http://purl.org/phylo/treebase/phylows/study/TB2:S23202).

Optimal temperature and growth rate.—To determine the optimal temperature and linear growth rates, one to five isolates of each taxon (TABLE 1) were cultured onto 20 mL of MEA in Petri dishes and placed in incubators set at 15, 25, 30, and 40 C or kept at ambient temperature (~22 C). Additional temperatures (45, 50, and 55 C) were included to test the temperature range of *T. colossus*. Colony diameter (cm) was measured in two directions daily for 8 d for each isolate. An average of the two diameters (mm) was used to calculate the average linear growth rate (mm/d) for each taxon by averaging the linear growth rates of each isolate of a given taxon for each day. This was repeated three times, and the incubators were randomly assigned temperatures independently in each repetition.

Survival at elevated temperatures over time.—To test the resiliency to elevated temperature exposure over time, one to five isolates of Ganodermataceae taxa were cultured onto MEA for 7 d at 28 C and then used to colonize sterilized rye grain in half-filled 237-mL glass jars incubated at 28 C until fully colonized (approximately 3 wk). Commercially available birch dowels (Fungi Perfecti, Olympia, Washington) (0.8 × 2.54 cm) were soaked in diH_2O for 24 h and then sterilized by autoclaving for 45 min in 237-mL glass jars at 121 C at 103 kPa. Hydrated, sterile wooden dowels were infested with grain inoculum of each respective isolate and were incubated at 28 C for 6 wk. After the dowels were fully colonized, they were placed into glass test tubes and put into an incubator set at 40 C. At each time point and temperature, there were three replicates per isolate of each taxon, except *G.* cf. *weberianum*, which had six replicates because there was only one isolate of this taxon. At 5, 12, 20, and 30 or 40 d, individual dowels of each replicate of each representative isolate were plated onto MEA, where they were incubated at 28 C for 5 d. After incubation, all replicates of each isolate were scored as alive or dead, and percent survival was calculated for each taxon by averaging the mean survival of each replicate per isolate of each taxon. In addition, colony growth area (cm^2) was measured for each colony by subtracting the area of the colonized wooden dowel from the total area of the fungal colony. Colony growth area was measured by analyzing photographs calibrated to size with Assess 2.0 software (APS, Minneapolis, Minnesota). Colony growth areas of all replicates of each isolate for a given taxon were averaged. The experiment was repeated twice.

RESULTS

Isolate collection and identification.—Based on a survey of the laccate *Ganoderma* species in the eastern United States conducted between 2013 and 2017 (Loyd et al. 2018a), 10 described taxa have been collected and identified: *G. curtisii* (Berk.) Murrill, *G. martinicense* Welti & Court., *G. meredithiae* Adask. & Gilb., *G. ravenelii* Steyaert, *G. sessile* Murrill, *G. tsugae* Murrill, *G. tuberculosum* Murrill, *G.* c.f. *weberianum* (Bres. & Henn. Ex Sacc.) Steyaert, *G. zonatum* Murrill, and *Tomophagus colossus* (Fr.) Murrill (syn. *G. colossus*). For this study, 31 isolates were selected as representatives of *G. curtisii* (n = 4), *G. martinicense* (n = 4), *G. meredithiae* (n = 3), *G. ravenelii* (n = 2), *G. sessile* (n = 5), *G. tsugae* (n = 3), *G. tuberculosum* (n = 3), *G.* cf. *weberianum* (n = 1), *G. zonatum* (n = 4), and *T. colossus* (n = 2) (TABLE 1). This represents all of the known species of laccate *Ganoderma* in the eastern United States (Loyd et al. 2018a). Based on phylogenetic analysis of ITS sequences (TABLE 1), the 10 morphologically identified Ganodermataceae taxa clustered well with reference sequences, with the exception of *G. meredithiae* (FIG. 1). This result is consistent with the findings of Loyd et al. (2018a), who proposed that *G. meredithiae* was conspecific with *G. curtisii* based on a multilocus phylogenetic analysis and morphology. However, Loyd et al. (2018a) also recognized that *G. meredithiae* is physiologically different in that it grows on pines in the southeastern United States and has a slow in vitro growth rate on MEA. Accordingly, Loyd et al. (2018a) proposed the informal classification of *G. curtisii* f. sp. *meredithiae* to differentiate isolates of *G. curtisii* from pine that have slow in vitro growth on MEA.

Colony morphology.—*Ganoderma* isolates generally produced white colonies with yellow, orange, or beige pigmentation toward the center of the colony after 8 d of growth at each temperature optimum (SUPPLEMENTARY FIG. 1). Colony pigments were more pronounced when isolates were grown at temperatures above the temperature optimum for each taxon. Isolates of *Tomophagus colossus* were woolly and had brown pigments after 8 d of growth on MEA. Colony morphologies were similar for all isolates of a given taxon. Descriptions are summarized in TABLE 2.

Chlamydospore production.—In 8-d-old cultures grown on MEA at 30 C, chlamydospores were produced by *G. martinicense, G. sessile, G.* cf. *weberianum*, and *Tomophagus colossus* (FIG. 2). Chlamydospores of these four taxa were readily produced in 8-d-old MEA cultures; adverse conditions were not required for their production. Chlamydospores of *G. martinicense* and *T. colossus* were pigmented and ornamented, whereas those of *G. sessile* and *G.* cf. *weberianum* were hyaline and smooth. Chlamydospore morphology and measurements are described in TABLE 2.

Optimal temperature and growth rate.—All isolates of each *Ganoderma* taxon grew at 15, 22, 25, 30, and 35 C, whereas no growth was observed at 40 C, with the exception of some negligible growth (<1 mm/d) in a few isolates of *G. sessile* and *G. martinicense* (FIG. 3). Isolates of *T. colossus* grew at 22, 25, 30, 35, 40, and 45 C, but no growth was observed at 50 or 55 C, and negligible growth at 15 C (<1 mm/d) (FIG. 3). Isolates of *G. tsugae* grew optimally at the coolest temperature range with no difference in growth between 22 and 25 C. Most *Ganoderma* species grew in the optimal temperature range of 25–30 C, including isolates of *G. curtisii, G. meredithiae, G. ravenelii, G. sessile, G. tuberculosum, G.* cf. *weberianum*, and *G. zonatum. Ganoderma martinicense* grew optimally at a slightly higher temperature range of 30–35 C, and *T. colossus* grew optimally between 35 and 40 C (TABLE 2).

At the respective optimal temperature range for each taxon, there were differences in linear growth rates between some of the taxa. Isolates of *T. colossus* grew the fastest at its optimal temperature of 40 C, growing on average at 15.3 ± 0.8 mm/d, whereas isolates of *G. ravenelii* had the slowest average linear growth rate of 1.6 ± 0.2 mm/d at its optimal temperature of 25 C (FIG. 3). At each respective optimal temperature, isolates of each taxon were grouped as growing slow, moderate, or fast. Isolates of *G. meredithiae, G. ravenelii*, and *G. tsugae* grew slowly (1.4–3.4 mm/d). Isolates of *G. curtisii, G. martinicense, G. tuberculosum, G.* cf. *weberianum*, and *G. zonatum* grew moderately fast (4.6–8.5 mm/d). Lastly, isolates of *G. sessile* and *T. colossus* grew fast (9.3–16.1 mm/d) (TABLE 2).

Survival at elevated temperatures over time.—There were differences in survival of *Ganoderma* species when exposed to 40 C over different periods of time. After 12 d of exposure, only the chlamydospore-producing taxa survived (*G. martinicense, G. sessile, G.* cf. *weberianum*, and *T. colossus*) (TABLE 3). However, there were differences in the vigor and resilience of the chlamydospore-producing taxa. Isolates of *T. colossus* were the most vigorous; colony growth area after 30 d of exposure to 40 C was 92% of the colony growth area after 5 d of exposure to 40 C. However, its optimal temperature range was between 35 and 40 C. *Gandoderma sessile* was

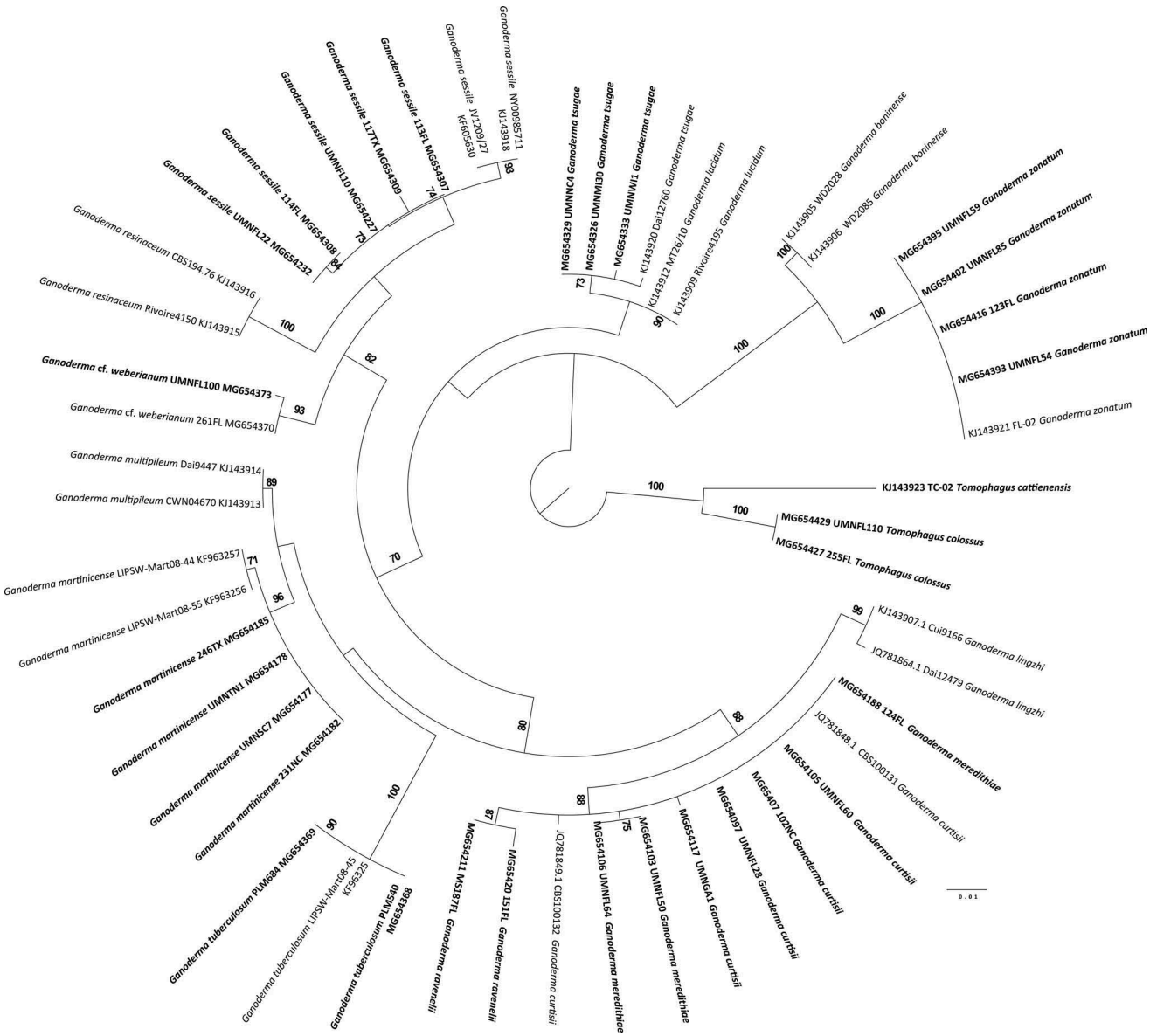

Figure 1. Tree topology derived from a RAxML phylogenetic analysis of an alignment of 460 characters derived from ITS sequences of *Ganoderma* species from this study or reference sequences. Statistical values shown are ML bootstrap values above 70%. Samples in boldface were generated in this study. *Tomophagus* species were used to root the tree.

the most vigorous of the other three chlamydospore-producing *Ganoderma* species. The colony growth area of isolates after 40 d exposure to 40 C was on average 72% of the colony growth area after 5 d of exposure to 40 C. Isolates of *G. martinicense* and *G.* cf. *weberianum* survived 30 d of exposure to 40 C, but the vigor of colony growth was strongly reduced after this exposure (FIG. 4).

DISCUSSION

Cultural characteristics such as colony morphology, chlamydospore production, and average growth rate are often overlooked during of studies of the Polyporales. These characteristics can be useful diagnostic features, distinguish physiological differences, and explain geographic distribution limits among similar taxa (Nobles 1965; Adaskaveg and Gilbertson 1986b, 1989; McCormick et al. 2013). There were differences in colony morphology, chlamydospore production, linear growth rates, and optimal temperatures for isolates of the 10 Ganodermataceae taxa investigated here (TABLE 2).

Optimal temperature data supported the geographic distribution of *Ganoderma tsugae*, which had an optimal temperature between 22 and 25 C. This species is typically found in northern latitudes following the geographic distribution of *Tsuga canadensis* (Gilbertson

Table 2. Summary of cultural characterization for 10 taxa in the Ganodermataceae in the eastern United States.

		Chlamydospores			Cultural characteristics		
Taxon	Authority	Presence/absence[a]	Shape	Size[b]	Optimal temperature range (°C)	Average linear growth rate (mm/d)[c]	Colony morphology
Ganoderma curtisii	(Berk.) Murrill 1902	Absent	—	—	25–30	5.6 ± 1.0	Plumose, felty colony margin and appressed to the medium; densely white, and with age developed yellow to orange pigments
Ganoderma martinicense	Welti & Court. 2010	Present	Hyaline to pigmented, ovate to spherical or irregularly shaped with protruding appendages	17.1 (13.5–21.1) × 12.2 (9.2–17.3)	30–35	7.9 ± 0.6	Fringed or even margin appressed to the medium; felty in texture, and at maturity lacked aerial mycelia; densely white, and with age developed yellowish pigments
Ganoderma meredithiae	Adask. & Gilb. 1988	Absent	—	—	25–30	2.6 ± 0.6	Plumose, felty colony margin and appressed to the medium; densely white, and with age developed yellow to orange pigments
Ganoderma ravenelii	Steyaert 1980	Absent	—	—	25–30	1.6 ± 0.2	Plumose, felty colony margin and appressed to the medium; densely white, and with age developed yellow to orange pigments
Ganoderma sessile	Murrill 1902	Present	Hyaline, elliptical to obpyriform to ovate, and smooth	16.0 (12.0–26.0) × 11.0 (9.5–12.0)	25–30	11.0 ± 1.7	Fringed or even margin appressed to the medium; felty in texture, and at maturity lacked aerial mycelia; densely white, and farinaceous (mealy) in texture
Ganoderma tsugae	Murrill 1902	Absent	–	–	20–25	3.0 ± 0.4	Slightly fringed or even margin appressed to the medium, and were floccose to felty in texture; densely white, developing yellowish pigments toward the center of the colony
Ganoderma tuberculosum	Murrill 1908	Absent	—	—	25–30	6.5 ± 1.2	Slightly fringed or even margin appressed to the medium, and were cottony but becoming felty over time; densely white, and with age produced bright yellow pigments
Ganoderma cf. *weberianum*	(Bres. & Henn. Ex. Sacc.) Steyaert 1972	Present	Hyaline, elliptical to obpyriform to ovate, and smooth	17.1 (14.1–20.1) × 12.0 (9.6–14.1)	25–30	6.7 ± 1.2	Fringed or even margin appressed to the medium; felty in texture, and at maturity lacked aerial mycelia; densely white, and farinaceous (mealy) in texture
Ganoderma zonatum	Murrill 1902	Absent	—	—	25–30	7.3 ± 0.9	Fringed to even margin that were floccose becoming felty over time, with aerial mycelium common in young cultures but rare in mature cultures; yellowish to ochraceous to cream colored pigments in the center of the colony that were lacunose (sunken or depressed) and crustose
Tomophagus colossus	(Fr.) Murrill 1905	Present	Pigmented, globose, with protruding appendages	16.1 (15.1–17.6)	35–40	15.3 ± 0.8	Even margin that was cottony to woolly with aerial hphae; white aging to tan or brown throughout the entire colony

[a]Presence/absence of chlamydospores was noted on malt extract agar (MEA) following 8 d of growth in the dark at each taxon's optimal temperature.
[b]At least 20 chlamydospores were measured for *G. martinicense* (n = 4), *G. sessile* (n = 5), *G.* cf. *weberianum* (n = 1), and *T. colossus* (n = 2).
cAverage linear growth rate was based on growth on MEA at each respective optimal peak temperature for each taxon. The standard deviation is also given.

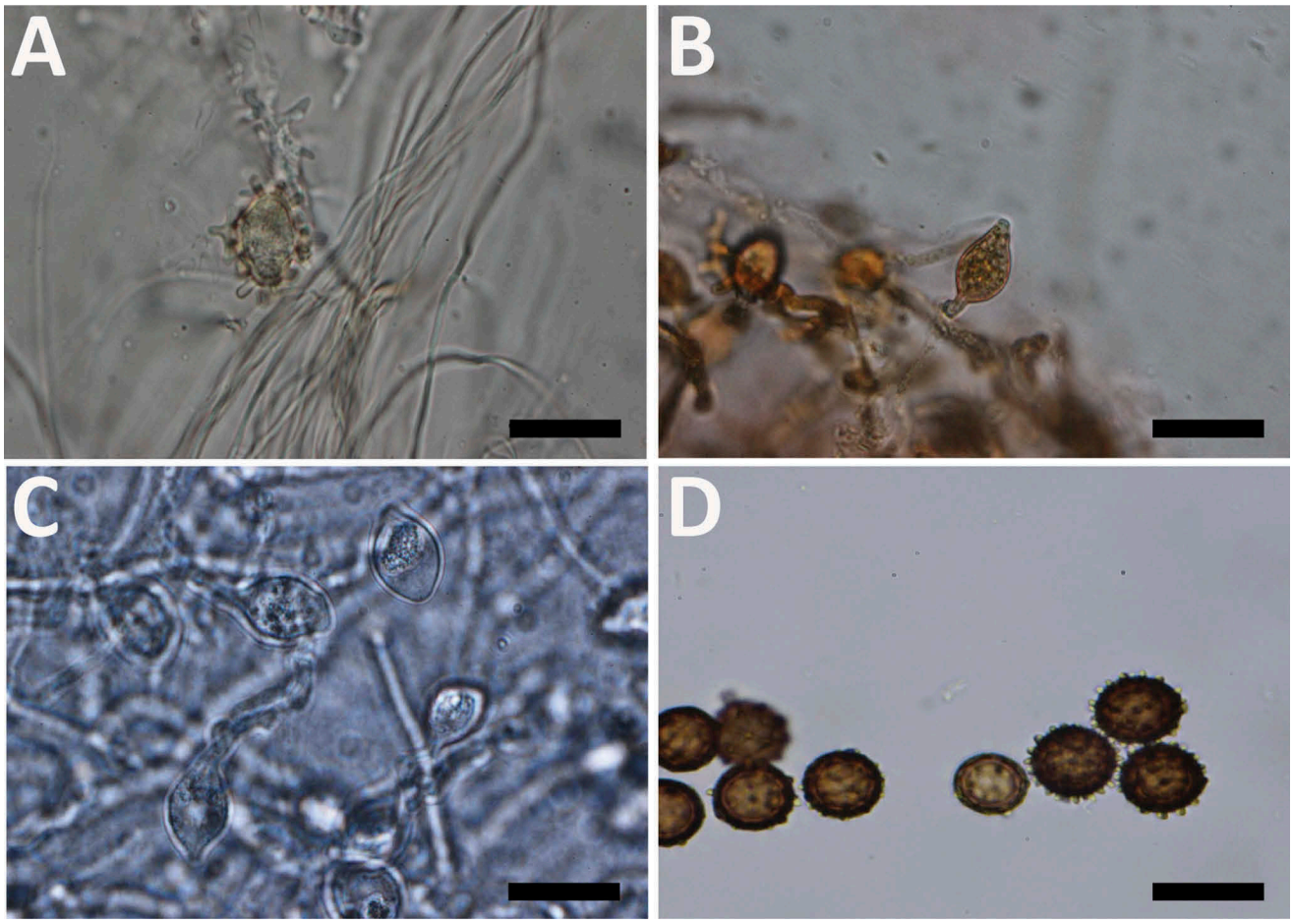

Figure 2. Chlamydospores produced by four taxa of the Ganodermataceae. A. Ornamented and pigmented double-walled intercalary ovate chlamydospore produced by *G. martinicense* (231NC). B. Dextrinoid, terminal chlaymospore produced by *G. sessile* (113FL) stained in Melzer's reagent. C. Ovate to obpyriform chlamydospores produced by *G.* c.f. *weberianum* (UMNFL100). D. Mature glogose, double-walled pigmented and ornamented chlamydospores of *T. colossus* (UMNFL110). Bars = 20 µm.

and Ryvarden 1986; Loyd et al. 2018a). Similarly, *T. colossus* has a limited geographic distribution in tropical locales and has only been reported within the United States in Florida. *Tomophagus colossus* has an optimal temperature range between 35 and 40 C, which is consistent with its distribution in areas with constant warm temperatures and rare freezing (Gilbertson and Ryvarden 1986; Adaskaveg and Gilbertson 1989). Lastly, *G. sessile* has the largest geographic distribution in the eastern United States and is found in most states east of the Rocky Mountains (Gilbertson and Ryvarden 1986; Loyd et al. 2018a). Relative to other *Ganoderma* species, isolates of *G. sessile* had fast linear growth at the optimal temperature of 30 C but also had fast linear growth from 15 to 30 C (FIG. 3). The fast linear growth across the wide temperature range is consistent with the large geographic distribution of this species from tropical, subtropical, and temperate regions of the eastern United States. We hypothesize that the fast growth rate and chlamydospore production of *G. sessile* has aided in its survival across this wide geographic range.

Linear growth rates have been hypothesized to be proportional to decay rates (Adaskaveg and Gilbertson 1986a). These data suggest that dikaryotic isolates of *G. sessile* and *T. colossus* would have a faster decay rate relative to the other taxa based on their fast linear growth rates. On the contrary, isolates of *G. ravenelii, G. meredithiae*, and *G. tsugae* had the slowest linear growth rates at each temperature optimum. Based on this previous hypothesis, these slow-growing taxa would decay wood slower relative to the faster growing taxa. Of the isolates with slow linear growth rates, *G. meredithiae* and *G. tsugae* predominately decay coniferous wood. Coniferous sapwood is generally more decay-resistant than the sapwood of hardwoods due to the production of resins and terpenes (Baietto and Wilson 2010). The slow in vitro growth could be due to a lack of nutrition in the artificial medium, or the

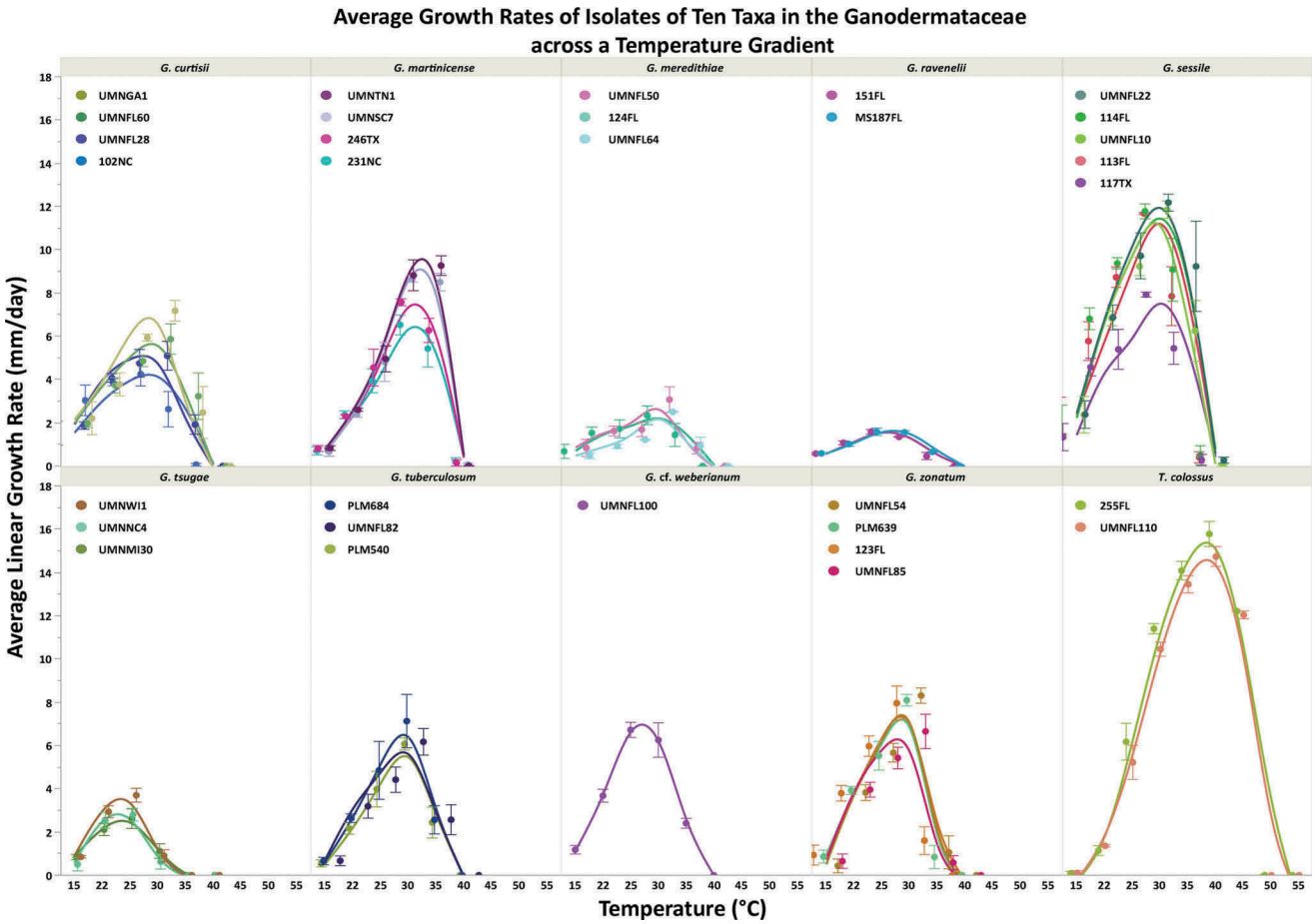

Figure 3. Average optimal temperatures and growth rates of 31 isolates representing 10 taxa within the Ganodermataceae in the eastern United States. Each isolate is labeled with a different color in each taxon box. The averages and standard error bars are based on three replications.

Table 3. Percent survival of Ganodermataceae taxa exposed to 40 C over time.

		Percentage of isolate survival at elevated temperature[a]				
Taxon	n[b]	5[c]	12[c]	21[c]	30[c]	40[c]
G. curtisii	4	14	0	0	nt	0
G. martinicense	4	100	100	75	58	nt
G. meredithiae	3	50	0	0	nt	0
G. ravenelii	2	0	0	0	0	nt
G. sessile	4	100	100	100	nt	100
G. tsugae	2	0	0	0	0	nt
G. tuberculosum	3	80	33	0	nt	0
G. cf. *weberianum*	1	100	100	33	83	nt
G. zonatum	5	0	0	0	nt	0
T. colossus	2	100	100	100	100	nt

Note. nt = not tested.
[a]Scored isolate survival after 5 d of incubation at 28 C on malt extract agar with 3 replicates per isolate
[b]Number of unique isolates for each species.
[c]Days exposed at 40 C.

result of decay specialization on conifers because linear growth can also be affected by water-soluble sapwood extracts from pine (Loyd et al. 2018b). More studies are required to elucidate these differences in physiology. These results also confirm the slow growth rate of *G. meredithiae* as compared with *G. curtissi*, as previously reported (Adaskaveg and Gilbertson 1988; Loyd et al. 2018a). This finding of no phylogenetic differences between *G. meredithiae* and *G. curtissi* but with *G. meredithiae* apparently specialized for growth on conifers supports the renaming of *G. meredithiae* to *G. curtissi* f. sp. *meredithiae*. Recent studies of ecological transitions in wood decay fungi suggest that most white rot fungi, including species of Ganodermataceae and other Polyporales, are specialized for growth on angiosperms with relatively few taxa specialized on gymnosperms (Krah et al. 2018). Although Krah et al. (2018) suggest that there are likely many ecological transitions between angiosperm specialization, gymnosperm specialization, and generalism, our findings suggest that in the case of Ganodermataceae, it is likely that *G. tsugae* and *G. curtissi* f. sp. *meredithiae* may represent evolutionarily recent switches to decay on conifers.

In addition to understanding differences in physiology, these optimal temperature and growth data could

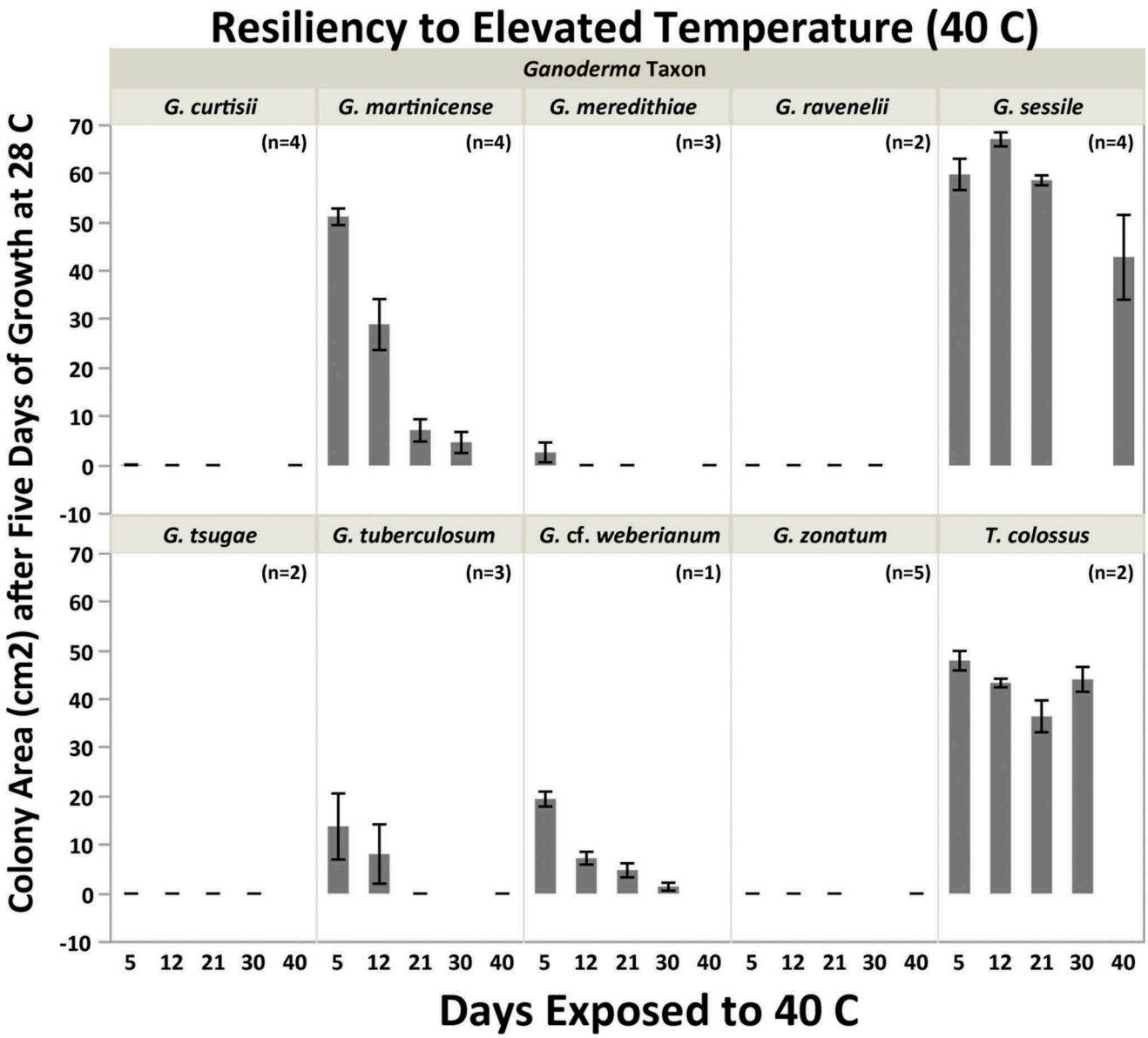

Figure 4. Effects of exposure to 40 C (elevated temperature) over time on colony growth area of Ganodermataceae taxa, where no growth indicates mortality or no growth. Number of isolates used in this experiment are indicated in each taxon box as "n=x."

also be utilitarian and benefit reishi cultivators who may choose to select fast-growing species and specific optimal growth temperatures for producing spawn of a given taxon. In addition, chlamydospore-producing species would likely have a longer shelf life across adverse temperature conditions. During strain selection, this morphological feature could help reishi spawn producers decide on strains they wish to cultivate and sell.

Another utilitarian use of this data is designing novel management tactics for Ganoderma butt rot of palms caused by *Ganoderma zonatum*. Ganoderma butt rot is the most important disease of palms in Florida (Elliott and Broschat 2001). Isolates of *G. zonatum* had the greatest decrease (−6.7 mm/d) in average growth rate when the temperature was increased from 30 to 35 C. Isolates of *G. zonatum* also failed to grow when exposed to 40 C for only 5 d. Isolates of *G. zonatum* are not tolerant to high temperatures, which could explain why pathogenicity tests conducted previously in Florida were unsuccessful (Elliott and Broschat 2001; Loyd et al. 2018c). Based on these data, we hypothesize that the spread of inoculum, presumably by basidiospores, is likely most successful in the cooler months. Furthermore, due to the phasing out of fumigants such as dazomet, soil removal and replacement is recommended for replanting palms where palms have been removed due to Ganoderma butt rot (Elliott and Broschat 2001). Based on the low tolerance of high temperatures, future research should focus on the

potential use of soil solarization and steam sterilization to eliminate *G. zonatum* inoculum. Our results suggest that these alternative practices may be useful for managing this important palm pathogen.

Chlamydospore-producing taxa were the only ones able to survive long-term exposures to 40 C. The double-walled chlamydospores produced by *G. martinicense, G. sessile, G.* c.f. *weberianum*, and *T. colossus* function as survival structures and can persist in wood despite the fact that the fungi are not actively growing. In a previous study, Loyd (2018) found that chlamydospores produced by *G. sessile* are constitutively produced and begin maturing after only 3 d of growth at the optimal temperature on MEA. Similarly, Loyd (2018) found that young 2-d-old cultures of *G. sessile* that lacked chlamydospores were not able to survive 8 d at 40 C. In contrast, mature 7-d-old cultures with abundant chlamydospores survived well under the same conditions.

Based on these survival data, we suspect that the chlamydospores of the other Ganodermataceae taxa are also regularly produced during regular growth. With the exception of *T. colossus*, the other three chlamydospore-producing *Ganoderma* species do not actively grow at 40 C. This suggests that the chlamydospores, not the hyphae, allow these fungi to revive following exposure to elevated temperatures. In addition to the function of chlamydospores, there were morphological differences distinguishing chlamydospores produced by *G. sessile* and *G.* cf. *weberianum* as compared with those produced by *G. martinicense* and *T. colossus*. The evolutionary history of chlamydospore production in the Ganodermataceae is still unresolved and requires further investigation of additional taxa. However, species in the *G. resinaceum* clade all produce morphologically similar chlamydospores (Hong and Jung 2004; Loyd et al. 2018a) that likely have similar survival functions.

Overall, there were differences in colony morphologies, chlamydospore production, average linear growth rates, optimal temperature ranges, and resiliency to elevated temperatures of the Ganodermataceae taxa investigated. This work demonstrates that cultural characteristics can be useful for understanding the basic biology of Ganodermataceae taxa and can be used to infer information about the general ecology, physiology, and utility for the laccate *Ganoderma* species. Continued research focusing on the laccate *Ganoderma* taxa in the United States is needed to better understand this cosmopolitan genus of wood decay and medicinal fungi.

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