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Leaf blister of *Quercus phillyraeoides* caused by *Taphrina caerulescens*

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An ascomycete fungus causing an undescribed disease was found on an oak, *Quercus phillyraeoides*. Naked hymenia appeared on the lower side of leaves, causing slightly swollen spots or blisters that exhibited chlorosis. Ascogenous cells were thick-walled, oval and convex. Asci were cylindric or clavate, without a stalk cell. Ascospores were rarely observed, and numerous blastospores were formed in asci. Mycelium habit was intercellular. Blastospores grew well on PDA and formed pinkish yeast-like colonies. Morphological aspects of the fungus matched well with those of *Taphrina caerulescens*. *Quercus phillyraeoides* is recorded as a new host of *T. caerulescens*, and the disease of *Q. phillyraeoides* caused by this fungus is termed "leaf blister."

Key Words——anamorph; Ascomycetes; leaf blister; *Quercus phillyraeoides*; *Taphrina caerulescens*.

One of the authors (K) found a leaf blister on *Quercus phillyraeoides* A. Gray in Yamaguchi from the middle to the end of April, 1997. Small, round, yellowish spots were observed on newly developed leaves. These spots enlarged up to 3–12 × 3–7 mm, slightly swelled upwards, and became whitish and pulverulent at their lower surface. Finally, convex-concave, swollen, and yellowish blisters were formed (Fig. 1). These showed chlorosis and turned light yellowish brown, then sometimes developed into a single blister. New spots formed by secondary infection were observed from the middle to the end of May. The blisters turned yellowish brown or blackish brown from the end of May into early June. We confirmed that the causal fungus of the disease is *Taphrina caerulescens* (Mont. et Desm.) Tul. and obtained cultures of it by single-spore isolation. Here, we describe the morphology of its teleomorph and cultural characteristics of its anamorph.

Materials and Methods

Fresh, infected leaves of *Q. phillyraeoides* were used. Blastospores from two blisters were sown onto acidified PDA, and all four cultures were obtained from a single blastospore isolation. Diazonium blue B (DBB) reaction (van der Walt and Hopsu-Havu, 1976), extracellular DNase activity (Sen and Komagata, 1979), and urease activity (Seeliger, 1956) were tested with four isolates of *T. caerulescens*.

Histology

Mycelium intercellular forms (Mix, 1949): between the interior cells of leaves. In infected tissue, the epidermal cells beneath the asci layer were larger (three to five times) than those of normal tissue; hypertrophy. Witches' brooms were not observed in the infected branches.

Taxonomy

Ascogenous cells thick-walled, oval, convex, formed on the epidermis (Fig. 5), elongated to asci (Fig. 6). Naked hymenium with number of asci observed on the lower surface of spots or blisters (Figs. 3, 4). Asci hypophyllous, cylindric or clavate, rounded at the apex (Figs. 7a, b), torn vertically or ruptured, 47–60(–70) × 17–23(–30) μm (mean 53.9 × 20.8 μm, n = 20), at the base blunt, rounded, or truncate with rhizoidal appendages (Figs. 7d, 8–10), and seated or slightly inserted on the epidermis. Stalk cells absent. Eight ascospores rarely observed in an ascus, nearly globose (Fig. 12) and 5.5 μm in diam, but observed ascospores frequently budding in asci filling them with small blastospores, which were ovate or elliptic and 3–7(–8) × 1–1.5(–2) μm (Figs. 7c, 11).

Morphology of the naked asci formed on the leaf blister lead us to place this fungus in the genus *Taphrina* Fr. Five *Taphrina* species have been recorded on Fagaceae. Of these, *T. entomospora* Thaxt., having stalked asci and found on *Nothofagus* in Patagonia, and *T. castanopsidis* Jenkins, having long cylindrical asci and found on *Castanopsis* in California, are apparently different from our fungus. *Taphrina kusanoi* Ikeno, recorded on *Castanopsis* in Japan, is distinguishable from our fungus by its host plant, symptom, and frequently expanded shape of ascus base. *Taphrina kruchii* (Vuill.) Sacc. and *T. caerulescens* are both known to be parasitic on *Quercus*, and have asci of similar shape and size. The former species, however, forms witches' broom on *Q. ilex* L. in

the Mediterranean districts, and this is the chief point of distinction from the latter species. Dimensions of asci of our fungus are 47–60 × 17–23 μm, which fall within the limits for *T. caerulescens*. Morphology of ascus base in our fungus also coincided to that of *T. caerulescens*. In all morphological aspects, our fungus causing the leaf blister of *Q. phillyraeoides* is identical with *T. caerulescens*.

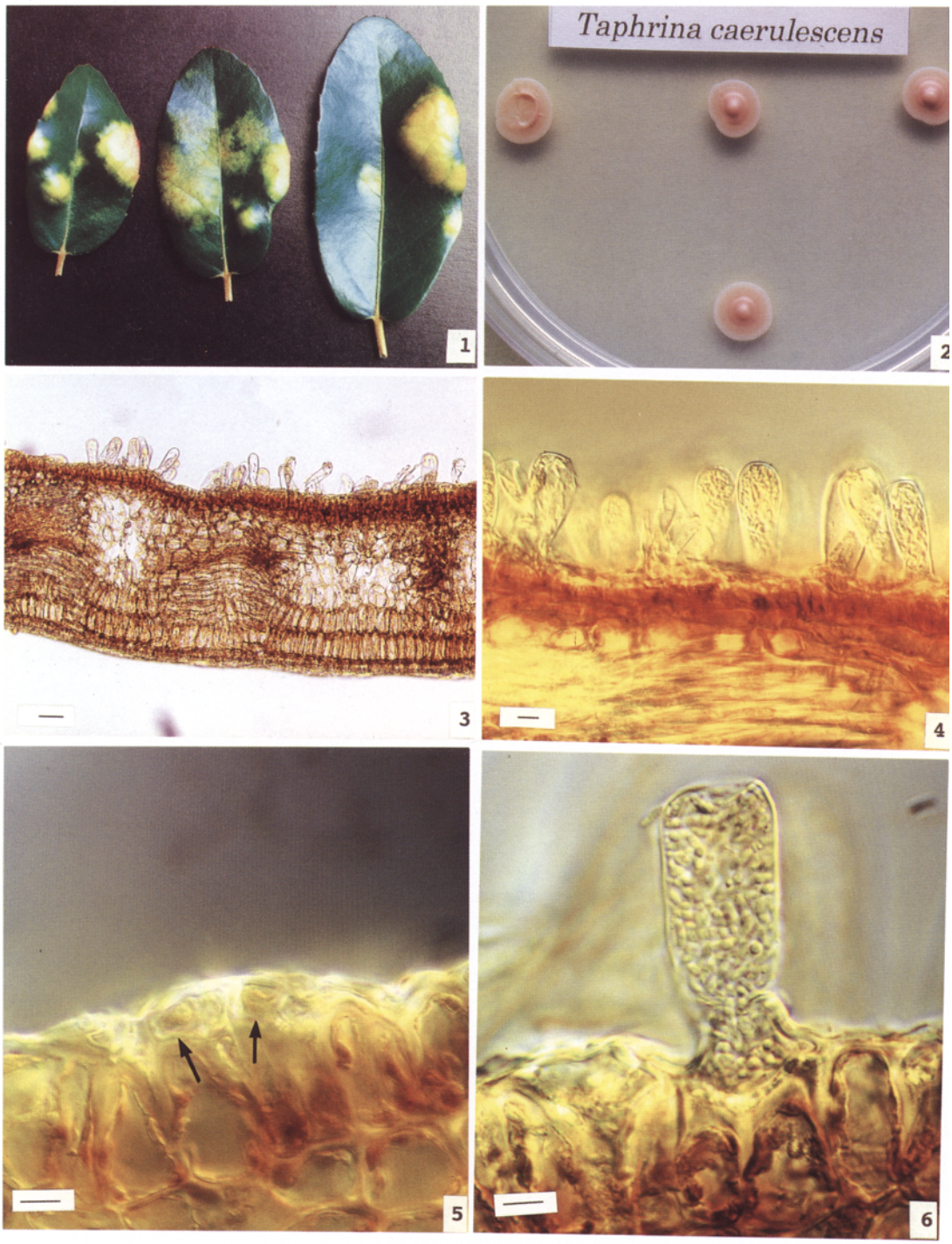


Fig. 1. Symptoms on leaves of *Quercus phillyraeoides* (upper surface view).
Figs. 2–6. *Taphrina caerulescens*.
2. Colonies on PDA by single-blastospore isolation. 3. Asci formed on the lower leaf surface of *Q. phillyraeoides*. 4. Asci filled with numerous blastospores. 5. Ascogenous cells (arrowed) formed on the epidermis. 6. Elongated ascus from ascogenous cell on the epidermis. Scales indicate 50 μm (Fig. 3) and 10 μm (Figs. 4 and 5).

Taphrina caerulescens (Mont. et Desm.) Tul., Ann. Sci. Nat. 5e Sér. Bot. **5**: 127. 1866.

≡*Ascomyces caerulescens* Mont. et Desm., Ann. Sci. Nat. 3e Sér. Bot. **10**: 345. 1848.

≡*Exoascus caerulescens* (Mont. et Desm.) Sadeb., Jahrb. Hamburg. Wiss. Anst. **1**: 119. 1884.

≡*Taphrina caerulescens* (Mont. et Desm.) Schröt. in Engler & Prantl, Naturl. Pflanzenfam. 1, **1**: 161. 1897. (comb. superfl.)

=*Ascomyces quercus* Cooke in Ravenel, Fungi Americ. no. 72.

≡*Taphrina quercus* (Cooke) Sacc., Syll. Fung. **8**: 814. 1889.

=*Ascomyces alutaceus* Thüm., Verhandl. K. K. Zool. Bot. Ges. Wien **29**: 523. 1880.

≡*Taphrina alutacea* (Thüm.) Sacc., Syll. Fung. **8**: 815. 1889.

=*Ascomyces extensus* Peck, Rept. New York St. Mus. **39**: 50. 1886.

≡*Taphrina extensa* (Peck) Sacc., Syll. Fung. **8**: 815. 1889.

=*Ascomyces rubrobrunneus* Peck, Rept. New York St. Mus. **40**: 67. 1887.

≡*Taphrina rubrobrunnea* (Peck) Sacc., Syll. Fung. **10**: 67. 1892.

Specimen examined: On leaves of *Quercus phillyraeoides* at Oouchimihori, Yamaguchi Pref., April 28, 1997, K. Katumoto. The material is deposited in the Herbarium of National Science Museum, Tokyo (TNS-F 238402). Anamorph cultures are deposited in the Institute for Fermentation, Osaka, Japan (IFO) and in the Culture Collection Center, Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan (IAM).

Quercus phillyraeoides is here added to the host plants of *T. caerulescens*. We propose to name this disease of *Q. phillyraeoides* "leaf blister."

Taphrina caerulescens is distributed in Europe, temperate North America, northern Africa (Algeria and Morocco) and Japan (Mix, 1949). It was reported by Mix (1949) on 44 taxa of *Quercus,* of which *Q. acutissima* Carr. was a host plant in Japan. Six other host plants, *Q. crispula* Blume (syn. *Q. mongolica* Fisch. ex Turcz. var. *grosseserrata* Rehd. et Wils.), *Q. dentata* Thunb., *Q. glauca* Thunb., *Q. myrsinaefolia* Blume, *Q. serrata* Thunb., and *Q. variabilis* Blume were listed along with *Q. acutissima* as host plants of *T. caerulescens* in Japan (Nishida, 1911; Sawada, 1952; Terashita, 1957; Ito, 1964). Nishida (1911) pointed out that the dimensions of asci in Japanese collections (80–120×20–24 μm on *Q. dentata*; 40–80×15–25 μm on *Quercus* spp.) were larger than those reported from abroad (Ito, 1964). Mix (1949) recognized that the dimensions of asci in *T. caerulescens* varied depending upon its host plants, and he determined its ascus-size limits to be 30–120× 11–34 μm. This value was estimated from all materials on known host-species including a Japanese collection. Accordingly, ascus sizes measured by Nishida generally fell within the range determined by Mix. Dimensions of asci in our fungus on *Q. phillyraeoides* (47–60×17–23 μm) were similar to those of materials on *Q. cinerea* Michx. in North America, *Q. prinoides* L. in North America, and *Q. sessiflora* Salisb. in Italy, falling at the lower end of Mix's range. Terashita (1957) assigned the causal fungus of leaf blight of *Q. mysinaefolia* Blume to *T. caerulescens*, and reported ascus dimensions of 22–40× 14–21 μm. The ascus size in his fungus was more smaller than that in our fungus on *Q. phillyraeoides*. Further his fungus caused leaf blight and it was said never to cause leaf blister. Ascus size and symptoms on infected leaves by *T. caerulescens* may vary with the host plant species.

Culture

Colonies formed on PDA (Fig. 2) were pinkish and grew well at 20–25°C. These isolates exhibited positive urease activity, and negative DNase activity and DBB reaction. Three isolates of two other *Taphrina* species (*T. deformans* (Berk.) Tul. C93051 and *T. wiesneri* (Rath.) Mix C9201 and C10101) showed the same results. These were typical cultural characteristics of *Taphrina* (Table 1).

Table 1. Phenotypic characterization of anamorph isolates from *Taphrina caerulescens*, other *Taphrina* species and ascomycetous and basidiomycetous yeasts.

Isolate	Source	DNase activity	Urease activity	DBB reaction
T. caerulescens K9701 (IFO 32988)[a]	*Quercus phillyraeoides*	−	+	−
T. caerulescens K9702 (IFO 32989)	*Q. phillyraeoides*	−	+	−
T. caerulescens K9703 (IFO 32990)	*Q. phillyraeoides*	−	+	−
T. caerulescens K9704 (IFO 32991)	*Q. phillyraeoides*	−	+	−
T. deformans C93051 (IFO 32994)	*Prunus persica* var. *vulgaris*	−	+	−
T. wiesneri C9201 (IFO 32992)	*Prunus yedoensis*	−	+	−
T. wiesneri C10101 (IFO 32993)	*Prunus yedoensis*	−	+	−
Saccharomyces cerevisiae JCM 7255		−	−	−
Rhodotorula glutinis JCM 8208		+	+	+

a) Cultures are deposited in Institute for Fermentation, Osaka, Japan (IFO).

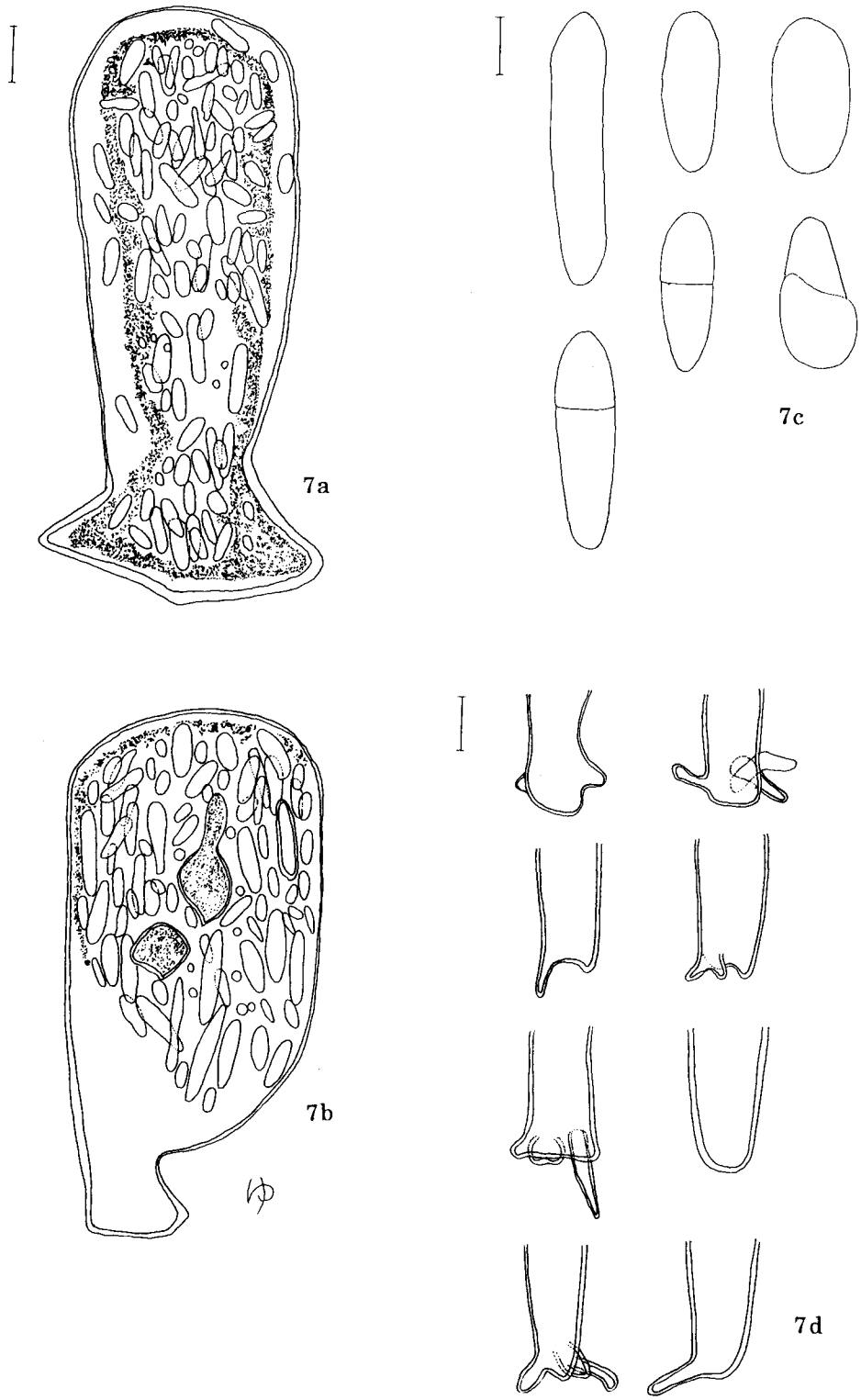
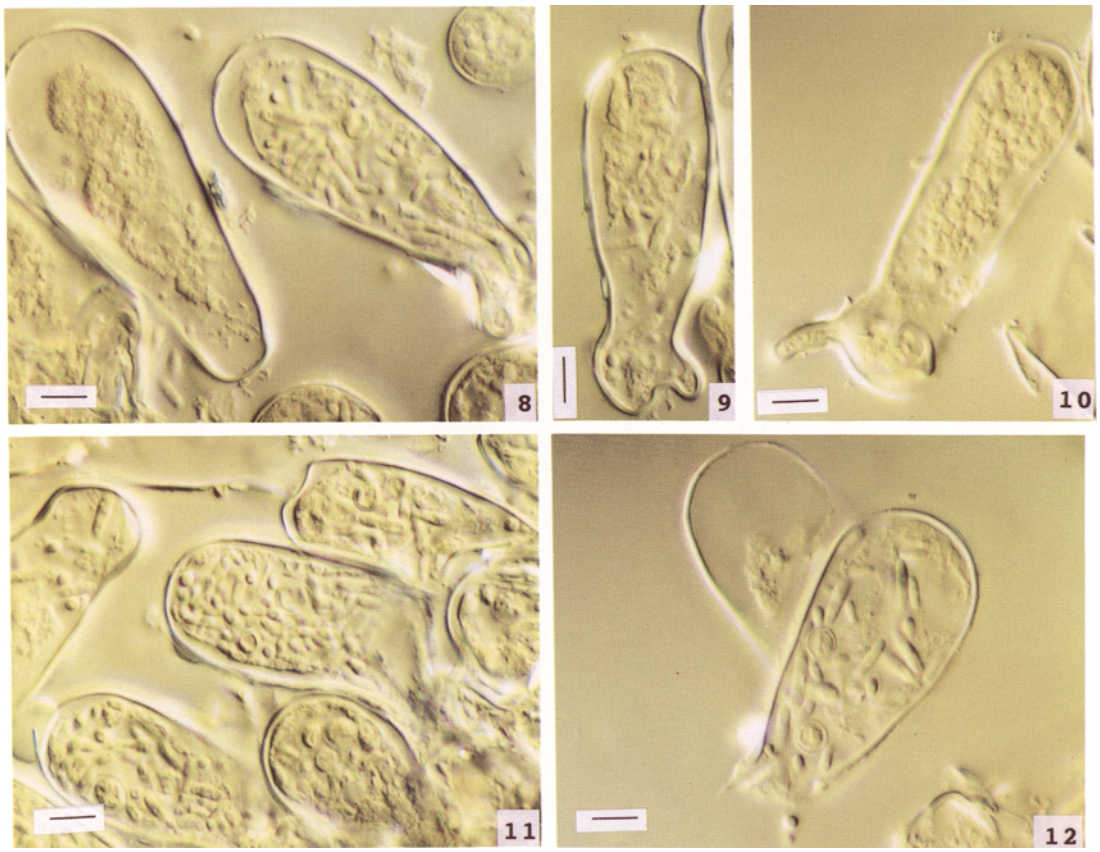


Fig. 7. *Taphrina caerulescens*.
7a, b. Asci filled with blastospores. 7c. Blastospores. 7d. Ascus-bases. Scales indicate 5 μm (a and b), 1 μm (c), and 10 μm (d).



Figs. 8–12. *Taphrina caerulescens*.
8. Asci: the bases blunt or rounded. 9. Ascus: the bases rounded with rhizoidal appendage. 10. Ascus: the base truncate with rhizoidal appendage. 11. Asci filled with blastospores. 12. Asci filled with two round ascospores and numerous blastospores. Scales indicate 10 μm.

Discussion

Mix (1949) discussed two types of germination of ascogenous cells, citing the works of several authors. In his observations, ascogenous cells elongate to asci except for *Taphrina acericola* Massal, *T. acerina* Eliasson, *T. amentorum* (Sadeb.) Rostr., *T. epiphylla* Sadeb., *T. occidentalis* Ray, *T. sadebeckii* Johanson, *T. thomasii* Mix, and *T. tosquinetii* (West.) Tul. In our observation, the thick-walled ascogenous cells of *T. caerulescens* appeared to elongate to asci.

Taphrina shares some characteristics with the Ascomycetes and Basidiomycetes; the negative diazonium blue B (DBB) reaction and negative extracellular DNase activity resemble characteristics of ascomycetous yeasts, whereas the positive urease activity and major ubiquinone system Q-10 resemble those of basidiomycetous yeasts (Nakase and Komagata, 1971; Sugiyama et al., 1985; Goto et al., 1987). Furthermore, the mode of budding of spores in *Taphrina* was enteroblastic, which is typical of the basidiomycetous yeasts (von Arx et al., 1982). It is not enough to determine the accommodation of these anamorph cultures by only three phenotypic characterizations. However, our results agree with the phenotypic characterization of *Taphrina* (Nishida and Sugiyama, 1993). Mix (1954) mentioned that each species of *Taphrina* exhibited an individual pattern of carbon utilization and that host-forms within *T. caerulescens* behave like separate species. Kramer (1987) provided a key to 26 species of *Taphrina* in 55 host-forms of the yeast phase based on the utilization of 32 carbon compounds adopted from data by Mix. In his key, isolates of *T. caerulescens* from 14 host species of *Quercus* were distinguished from each other based on the utilization of carbon compounds. Moore (1990) erected the genus *Lalaria* R. T. Moore to accommodate the yeast-phase anamorphs of *Taphrina*, and described 23 anamorphic species. He described two different species, *Lalaria caerulescens* R. T. Moore and *L. coccinea* R. T. Moore, both being anamorphic phase of *T. caerulescens* derived from two different isolates: the former from *Quercus alba* L. and the latter from *Q. coccinea* Munch. These two species were distinguished by the pattern of carbon utilization.

Carbon utilization of *T. caerulescens* from different hosts in Japan needs to be verified.

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