# **The Journal of Fungi**

# A novel aquatic species of Phomatospora, Phomatospora luteotingens sp. nov., has been discovered in France and Spain.

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### INTRODUCTION

A unique ascomycete species that discolored the wood yellow was commonly found during taxonomic studies of fungi on submerged woody substrates in aquatic (lotic) settings in Ariège, at the foot of the Pyrénées. Additional sampling in additional areas revealed that it is common throughout northwest Spain and France. Ascomata, asci, and ascospore morphology all strongly indicate that this taxon is a member of the genus Phomatospora Saccharini. Barr (1994) offered a key to the North American species and made comments about the genus, which she limited to "herbaceous stems, monocot culms, and coriaceous leaves" and classified terrestrial. Since then, new taxa from marine habitats (Hyde 1988, 1992, 1993) and freshwater habitats (Scheuer 1988, Fallah & Shearer 1998, Raja & Shearer 2008) have been reported.

However, no monographic investigation has been conducted on the genus Phoma-tospora. The novel taxon presented here adds a species that produces yellow pigments in the substrate, a characteristic not previously documented, to the general taxonomic group Phomatospora.

#### Techniques

Samples of submerged wood with diameters ranging from 5 mm to 10 cm were chopped into segments that were 15–30 cm long and put inside plastic bags. Each piece was cleaned in the lab using tap water to get rid of any sand and surface debris. It was then given a brief air drying period before being examined closely with a stereomicroscope.

Ascospore ornamentation was best detected in lactic cotton blue and mucilaginous sheaths or appendages stained in aqueous nigrosin, 3% KOH, or India ink. All microscopic observations and measurements were conducted in water.

The asci, paraphyses, and their apical apparatus were stained with Waterman blue-black, Toluidine blue, or Chlorazol black ink. Melzer's reagent was used to examine the ascal apical apparatus 40's response to iodine.

Using a razor blade, longitudinal median sections were manually cut and then mounted in chloral-lactophenol.

In 9 cm diameter Petri dishes, cultures of living specimens were created using Corn Meal Agar (CMA) and 5 mg/l of streptomycin. Using a fine needle, a mass of ascospores and asci was extracted from an ascoma and put in a drop of sterile water, which was then swirled with a needle to spread the components on the slide. Using a sterile micropipette, a portion of the drop containing a few ascospores was removed and placed on CMA before being incubated at 25°C. A single ascospore that was germinating was then moved to a fresh Petri dish and incubated at 25°C.

#### Outcomes

Figures 1–6 of Phomatospora luteotingens J. Fourn. & C. Lechat, sp. nov. MycoBank 518031

**Etymology:** so called because of the yellow tint of the wood that is regularly connected to this species.

The ascomata disperses by conferta, plenamente immersa in ligno luteo tincto, subglobosa, lateriter compressa, 380-590  $\mu$ m longa × 170-340  $\mu$ m lata × 170-250  $\mu$ m alta, maturescentia partim erumpentes; rostrum nigrum, crassum, conicum, 80-120  $\mu$ m altum, periphysatum. Ad 80  $\mu$ m inspissatum et nigrum opacum circum apicem, peridium 15–35  $\mu$ m crassum, ex parvis cellulis tenuiter tunicatis pallidis brunnaeis compositum. The ascidians are 140–165  $\mu$ m longitudinally, the sporifera pars are 120–140  $\mu$ m long and 7-8  $\mu$ m wide, and the stipes are 20–25  $\mu$ m long. They are unitunicati, cylindrici, and cum octo uniseriatis ascosporis. Refractive apical annulus inamyloideo, 1.8  $\mu$ m high to 2.5  $\mu$ m long.

Septate paraphysae, latae 6–8  $\mu$ m ad basim, mox deliquescentes. Aspectrum ellipticae ad oblongae, aseptatae, hyalinae, longitudinaliter striatae, sine bipolaribus appendicibus, ascosporae 14.5-17.8 × 5- 6  $\mu$ m.

Ascomata are sporadic to gregarious, infrequently come into contact, submerged in the wood until it becomes half

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erumpent with age; the wood is stained yellow at depth, bleached or yellow at the surface, depressed-spherical, laterally flattened and elongated in the wood grain; they are 380-590  $\mu$ m long × 170-340  $\mu$ m broad × 170-250  $\mu$ m high, with a central or eccentric black neck that is 80-120  $\mu$ m high, hardly protruding above the host surface, and periphysate. Thinner at the base than the sides, the peridium is made of tiny pseudoparenchymatous cells that create a textura angularis, measuring 15–35  $\mu$ m in thickness.

**Textura prismatica:** a broad layer of tiny, virtually opaque cells with thick walls encircling the ostiolar neck; the cells are faintly colored to dark brown and can reach up to 80  $\mu$ m in thickness at the apex. Paraphyses Very thin-walled, brittle, and early deliquescent, 6-8  $\mu$ m broad at base, tapering above, septate, constricted at septa, shorter than asci.

The asci measure 140-165  $\mu$ m in total length, the sporebearing parts measure 120-140  $\mu$ m in length × 7-8  $\mu$ m broad, and the stipes measure 20-25  $\mu$ m in length. They are cylindrical, unitunicate, and easily deliquescent in water. They have eight uniseriate ascospores, a refractive thimble-shaped apical ring measuring 1.8  $\mu$ m in height and 2.5  $\mu$ m in width, and are J-shaped, purple-stained in Tulipae.

14.5–17.8 × 5–6  $\mu$ m (x = 15.9 × 5.7  $\mu$ m, n = 30) ascospores, one-celled, hyaline, typically biguttulate, longitudinally striate, and without a sheath. They are narrowly ellipsoid to oblong with broadly rounded ends.

like polar limbs. Notably, the ascospore cirrhi that are discharged after drying are pinkish-white in color.

**Anamorph:** not found on a naturally occurring substrate.

**Holotype:** France, Ariège, Rimont; 1.5 km from the village on road D 18, Le Baup Brook; approximately 500 m on a submerged Alnus glutinosa twig; linked to Pseudohalonectria lutea Shearer and an unidentified discomycete; June 12, 2009, JF 09145 (LIP).

**Culture:** After 15 days on CMA, the colony grew slowly and reached a diameter of 0.8 to 1.5 cm. It was white to pale yellowish in color, not diffusing any color in the medium, and its color changed from yellow to ochraceous. Aerial mycelium consisting of  $2.5-3 \mu m$  diam. smooth, hyaline, septate hyphae. Despite multiple tries using a wide variety of medium and extended incubation times, the cultures stayed sterile and no anamorph could be found.

Distribution known to exist in France and Spain (Europe). Decorticated twigs or branches immersed in freshwater are referred to as substrates.

**Material examined:** France, Ariège: Caussou, Caussou Brook, 750 m, on submerged branch of Fraxinus excelsior, 7 May 2009, JF 09087 (LIP, paratype); Lescure, Volp Brook, Le Pas du Baup, 500 m, on submerged branch of Fraxinus excelsior, 26 October 2006, JF 06276; Prat Communal, Loumet, 950 m, on submerged branch of Salix sp., 8 September 2006, JF 06219; Rimont, Peyrau Brook, Palettes, 400 m, on submerged twig of Alnus glutinosa, 24 June 2006, JF 06129; same location and host, 7 Nov. 2006, JF 06288. Coast of Gold: Avot, La Tille, August 5, 2008, A. Gardiennet, AG08AT18. Haute Marne: A. Gardeniennet, AG08JT51, Pressigny, Val Morel Brook, July 18, 2008.

**Lozère:** Y. Mourgues, Banassac, Pratnau, Lot river, on submerged decorated wood, January 10, 2010. Nièvre: A. Gardiennet, Planchez, July 28, 2008, AG08JT52. La Gacilly at Moulin de Huno, Morbihan: July 21, 2008, 14 m, on submerged wood; JPP 28127, J. P. Priou; 47° 46′ 55″ N, 02° 07′ 46″ W. Puyde. **Dôme:** P. Ribollet, Royat, l'Arboretum, 600 meters above drowned timber, June 10, 2009. Hautes Pyrénées: Le Mazeau, on submerged branch of Fraxinus excelsior, 13 May 2009, JF 09117;

**Vendée:** Asque, La Gourgue, Arros stream, 485m, on submerged twig of Alnus glutinosa, 29 May 2009, JF 09131 (LIP, paratype). Road to La Endriga, Arbeyales, Spain, 43° 05' 54.07" N; 6° 10' 14.78" W, on submerged Alnus glutinosa wood, June 19, 2009, ER. Dominguez, ERD-4827;

Páramo, via submerged wood in a beech forest, to Puerto de Ventana, 1200m, 43° 05' 50.31" N 6° 02' 00.53" W Dominguez ER.

#### Talk

The genus Phomatospora is distinguished by ostiolate immersed ascomata with peridium made up of tiny pseudoparenchymatous cells and typically noticeably thickened in the upper part; cylindrical unitunicate asci with uniseriate ascospores; a refractive J-shaped apical ring; and ellipsoid, generally one-celled hyaline ascospores with longitudinally striate wall and frequently a mucilaginous sheath and/or differently shaped bipolar appendages (Barr 1994, Cai et al. 2006).

The only difference between the current fungus and this generic description is that the ascospores lack a sheath or other appendages.

It was demonstrated that the type species, P. berkeleyi and P. arenararia, could generate a Sporothrix anamorph in culture. It resulted in the species being assigned to the Xylariales order, where it is previously known that such anamorphs exist (Rappaz 1992). The most recent phylogenetic analyses (Lumbsch & Huhndorf 2007) did not support this conclusion, and Phomatospora is currently classified in the Sordariomycetes genus incertae sedis.

According to Shearer & Raja (http://fungi.life.uiuc.edu/world\_ records), there are currently five species of Phomatospora known to exist in freshwater environments. These species are P. aquatica Minoura & Muroi (Minoura & Muroi 1978), P. berkeleyi Sacc. (Fallah & Shearer 1998), P. muskellungensis Fallah & Shearer (Fallah & Shearer 1998), P. striatigera

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Scheuer (Scheuer 1988), and P. triseptata Raja & Shearer (Raja & Shearer 2008). Ascospore sizes in the first two species are less than those of P. luteotingens, measuring  $11-13 \times 3.2-4$  µm and  $8-10 \times 3-5$  µm, respectively, whilst ascospore sizes in the last two species are greater, measuring  $20-26 \times 4-5$  µm.

as well as  $23-27 \times 4-6 \mu m$ . P. triseptata ascospores are broader (18–20 × 7-8 µm) and have three septa, but they are not much longer than P. luteotingens'. Furthermore, none of these five species have been found to stain wood yellow like P. luteotingens, and their ascospores have bipolar appendages. The presence of a similar yellow color in mycelium-filled ancient ascomata has not been documented in Phomatospora species either in terrestrial or marine environments. Table 1 provides a summary of the differential characters that were previously compared.

It should be mentioned that the holotype collection included Pseudohalonectria lutea Shearer (Sordariomycetes), which is frequently found on submerged yellow-stained wood. However, both species create colonies that are clearly separated from one another and cannot be mistaken based on the fact that P. lutea's stain is olivaceous yellow, whereas P. luteotingens' is bright yellow.

Furthermore, whether P. lutea or other taxa are present or not, the yellow stain is always linked to P. luteotingens.

Phomatospora luteotingens is a purely aquatic species that is frequently found in different lotic environments near the base of the Pyrénées, even during the winter. It has also been collected in other regions of France and Spain. It seems to be one of the most common species seen in the area on submerged wood in various brooks surveyed. It has only been seen on deciduous, always decorated, submerged wood thus far, with alder and ash appearing to be the primary hosts.

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