
***Phomatospora luteotingens* sp. nov., a new aquatic species of *Phomatospora* from France and Spain**

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A distinctive species of *Phomatospora* was repeatedly encountered on submerged wood in aquatic (lotic) habitats in various parts of France and northwestern Spain. It is described and illustrated herein based on many collections and its affinities and differences with other aquatic species known in the genus are discussed. *Phomatospora luteotingens* sp. nov. is mainly characterized and distinguished from other related taxa by being consistently associated with a yellow stain of the woody substrate and ascospores lacking a mucilaginous sheath or appendages. The ecological data observed during this study are briefly reported.

Key words – freshwater ascomycota – Sordariomycetes – taxonomy

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Introduction

In the course of taxonomic investigations of fungi on submerged woody substrates in aquatic (lotic) habitats in Ariège at the foot of Pyrénées, a distinctive ascomycete species staining the wood yellow was frequently encountered. Further sampling in other regions showed it is widespread in France and northwestern Spain. Based on the morphology of ascomata, asci and ascospores this taxon clearly belongs to the genus *Phomatospora* Sacc. Barr (1994) commented upon the genus, that she considered terrestrial and restricted to “herbaceous stems, monocot culms and coriaceous leaves” and provided a key to the North American species. New taxa have since been described from freshwater habitats (Scheuer 1988, Fallah & Shearer 1998, Raja & Shearer 2008) and from marine habitats (Hyde 1988, 1992, 1993), but there has been no monographic study on the genus *Phomatospora*. The new taxon described herein

broadens the generic concept of *Phomatospora* in introducing a species producing yellow pigments in the substrate, a feature so far not recorded.

Methods

Submerged wood samples varying from 5 mm to 10 cm diam were cut into 15–30 cm long pieces and placed in plastic bags. At the laboratory each piece was washed under tap water to remove surface debris and sand, and superficially air-dried before direct examination under a stereomicroscope.

All microscopic observations and measurements were made in water, ascospore ornamentation was best observed in lactic cotton blue and mucilaginous sheaths or appendages stained in aqueous nigrosin, 3% KOH or India ink. Paraphyses, asci and their apical apparatus were stained in chlorazol black, toluidine blue or Waterman blue-black ink. The reaction of the ascal apical apparatus

to iodine was tested in Melzer's reagent. Longitudinal median sections were made manually with a razor blade and mounted in chloral-lactophenol.

Cultures of living specimens were made on CMA (Corn Meal Agar) with 5mg/l of streptomycin in 9 cm diam Petri dishes. A mass of ascospores and asci was removed from an ascoma with a fine needle and placed in a drop of sterile water that was stirred with a needle to distribute the elements on the slide. A part of the drop containing some ascospores was taken with a sterile micropipette and placed on CMA, then incubated at 25°C. Later a single germinating ascospore was transferred into a new Petri dish and incubated at 25°C.

Results

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

Etymology – named for the yellow stain of the wood consistently associated with this taxon.

Ascomata dispersa vel conferta, plerumque tote immersa in ligno luteo tincto, subglobosa, lateriter compressa, 380–590 µm longa × 170–340 µm lata × 170–250 µm alta, maturescentia partim erumpentes; rostrum nigrum, crassum, conicum, 80–120 µm altum, periphysatum. Peridium 15–35 µm crassum, ex parvis cellulis tenuiter tunicatis pallidis brunnaeis compositum, ad 80 µm inspissatum et nigrum opacum circum apicem. Asci 140–165 µm tota longitudina, pars sporifera 120–140 µm longa × 7–8 µm lata, stipes 20–25 µm longus, unitunicati, cylindrici, cum octo uniseriatis ascosporis, anulo apicali refractivo, inamyloideo, 1.8 µm alto 2.5 µm lato. Paraphysae septatae, 6–8 µm latae ad basim, mox deliquescentes. Ascosporae 14.5–17.8 × 5–6 µm, anguste ellipticae ad oblongae, aseptatae, hyalinae, longitudinaliter striatae, sine bipolaribus appendicibus.

Ascomata scattered to gregarious, rarely in contact, immersed in submerged wood to half erumpent with age, the wood stained yellow at depth, bleached or yellow at the surface, depressed-spherical, laterally flattened and elongated in the grain of wood, 380–590 µm long × 170–340 µm broad × 170–250 µm

high, with a central or eccentric broadly conical black neck 80–120 µm high, hardly protruding above host surface, periphysate. Peridium 15–35 µm thick at base and sides, thinner at base than at side, composed of small pseudoparenchymatous cells forming a *textura angularis* to *textura prismatica*, weakly pigmented to dark brown, up to 80 µm thick at apex, with a wide layer of small very thick-walled, nearly opaque cells surrounding the ostiolar neck. Paraphyses 6–8 µm broad at base, tapering above, septate, constricted at septa, shorter than asci, very thin-walled, fragile and early deliquescent. Asci 140–165 µm total length, the spore-bearing parts 120–140 µm long × 7–8 µm broad, the stipes 20–25 µm long, unitunicate, cylindrical, readily deliquescent in water, with eight uniseriate ascospores, with a refractive thimble-shaped apical ring, 1.8 µm high × 2.5 µm broad, J-, stained purple in Toluidine blue. Ascospores 14.5–17.8 × 5–6 µm (\bar{x} = 15.9 × 5.7 µm, n = 30), narrowly ellipsoid to oblong with broadly rounded ends, one-celled, hyaline, usually biguttulate, longitudinally striate, lacking a sheath or polar appendages. It is noteworthy that cirrhi of ascospores released upon drying are white with a pinkish tinge.

Anamorph – not observed on natural substrate.

Holotype – France, Ariège, Rimont, 1.5 km from the village on road D 18, Le Baup Brook, ca. 500m, on submerged twig of *Alnus glutinosa*, associated with *Pseudohalonestria lutea* Shearer and an unidentified discomycete, 12 Jun. 2009, JF 09145 (LIP).

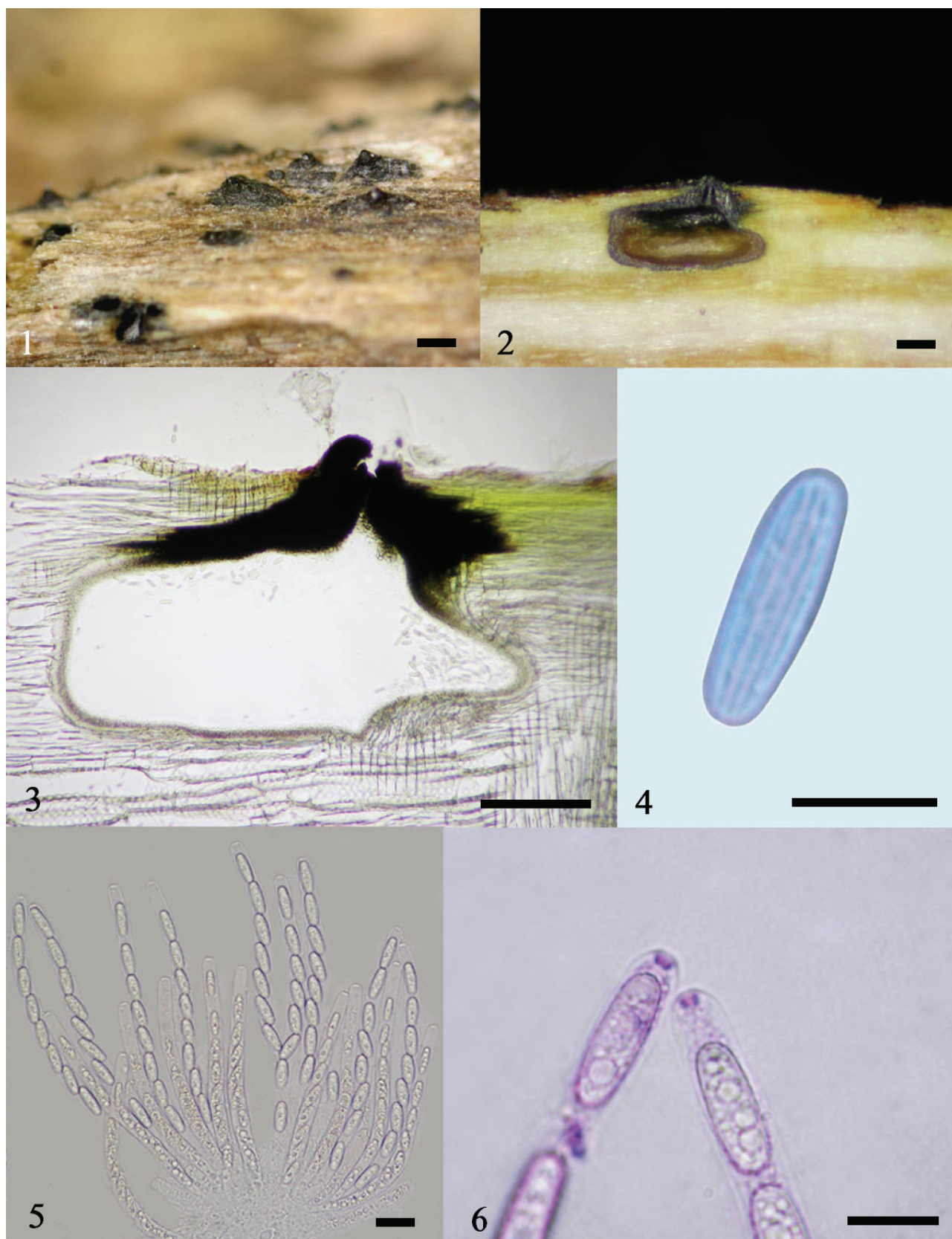
Culture – colony slow-growing, 0.8–1.5 cm diam after 15 days on CMA, white to pale yellowish, not diffusing any coloration in the medium, reverse of the colony yellow to ochraceous. Aerial mycelium composed of smooth, hyaline, septate hyphae 2.5–3 µm diam.

In spite of several attempts on a large range of media and long incubation, the cultures remained sterile and no anamorph was obtained.

Known distribution – France and Spain (Europe).

Substrate – decorticated twigs or branches submerged in freshwater.

Material examined – France, Ariège: Caussou, Caussou Brook, 750 m, on



Figs 1-6 – *Phomatospora luteotingens* JF 09145 (holotype). **1** Wood surface with prominent ostiolar necks in side view. **2** Vertical section of an ascoma *in situ*. **3** Thin vertical section of an ascoma in chloral-lactophenol. **4** Ascospore stained in lactic cotton blue. **5** Asci in chlorazol black. **6** Ascus apical apparatus stained in toluidin blue— Bars **1-3**:100 μm ; **4, 6**:10 μm ; **5**: 20 μm .

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 Oct. 2006, JF 06276; Prat Communal, Loumet, 950m, on submerged branch of *Salix sp.*, 8 Sept. 2006, JF 06219; Rimont, Peyrau Brook, Paletes, 400m, on submerged twig of *Alnus glutinosa*, 24 Jun. 2006, JF 06129; same location and host, 7 Nov. 2006, JF 06288. Côte d'Or: Avot, La Tille, 5 Aug. 2008, A. Gardiennet, AG08AT18. Haute Marne: Pressigny, Val Morel Brook, 18 Jul. 2008, A. Gardiennet, AG08JT51. Lozère: Banassac, Pratnau, Lot river, on submerged decorticated wood, 10 Jan. 2010, Y. Mourgues. Nièvre: Planchez, 28 Jul. 2008, A. Gardiennet, AG08JT52. Morbihan: La Gacilly, Moulin de Huno, 47° 46' 55'' N, 02° 07' 46'' W, 14 m, 21 Jun. 2008, on submerged wood, JPP 28127, J. P. Priou. Puy de Dôme: Royat, l'Arboretum, 600m, on submerged wood, 10 Jun. 2009, P. Ribollet. Hautes Pyrénées: Asque, La Gourgue, Arros stream, 485m, on submerged twig of *Alnus glutinosa*, 29 May 2009, JF 09131 (LIP, paratype); Vendée: Le Mazeau, on submerged branch of *Fraxinus excelsior*, 13 May 2009, JF 09117. Spain, Asturias: road to La Endriga, Arbeyales, 43° 05' 54.07" N; 6° 10' 14.78" W, on submerged wood of *Alnus glutinosa*, 19 Jun. 2009, ER. Dominguez, ERD-4827; Páramo, road to Puerto de Ventana, 1200m, 43° 05' 50.31" N 6° 02' 00.53" W, on submerged wood in a beech forest, ER. Dominguez.

Discussion

Phomatospora is a genus characterized by ostiolate immersed ascomata with peridium composed of small pseudoparenchymatous cells and usually markedly thickened in upper part, cylindrical unitunicate asci with uniseriate ascospores, a refractive J- apical ring and ellipsoid, usually one-celled hyaline ascospores with longitudinally striate wall and often a mucilaginous sheath and/or variously shaped bipolar appendages (Barr 1994, Cai et al. 2006). The present fungus fits very well this generic definition except for the lack of sheath or appendages on the ascospores.

The type species, *P. berkeleyi*, and *P. arenaria* were shown to produce a *Sporothrix* anamorph in culture, which led to the

placement of the genus in Xylariales where such anamorphs are already known to occur (Rappaz 1992). This conclusion was not supported by the most recent phylogenetic studies (Lumbsch & Huhndorf 2007) and *Phomatospora* is currently placed in Sordariomycetes genera incertae sedis.

Five species of *Phomatospora* are so far known from freshwater habitats (Shearer & Raja http://fungi.life.uiuc.edu/world_records), namely *P. aquatica* Minoura & Muroi (Minoura & Muroi 1978), *P. berkeleyi* Sacc. (Fallah & Shearer 1998), *P. muskellungensis* Fallah & Shearer (Fallah & Shearer 1998), *P. striatigera* Scheuer (Scheuer 1988) and *P. triseptata* Raja & Shearer (Raja & Shearer 2008). The former two species have smaller ascospores than those of *P. luteotingens*, respectively 11-13 × 3.2-4 µm and 8-10 × 3-5 µm, while the latter two species have larger ascospores, respectively 20-26 × 4-5 µm and 23-27 × 4-6 µm. Ascospores of *P. triseptata* are hardly longer than those of *P. luteotingens*, but are broader (18-20 × 7-8 µm) and feature three septa. In addition, the ascospores of these five species have bipolar appendages and none is reported to stain the wood yellow as with *P. luteotingens*. Such a yellow colour, which is likewise present in old ascomata filled with mycelium has neither been reported for *Phomatospora* species occurring in marine or terrestrial habitats. The differential characters compared above are summarized in Table 1.

It should be noted that *Pseudohalonectria lutea* Shearer (Sordariomycetes), which is commonly encountered on submerged yellow stained wood, was present in the holotype collection. Both species however, form colonies that are strictly delimited from one another and cannot be confused based on the bright yellow stain associated with *P. luteotingens*, while that of *P. lutea* is olivaceous yellow. Moreover, the yellow stain is consistently associated with *P. luteotingens*, regardless of the presence of *P. lutea* or other taxa.

Phomatospora luteotingens is a strictly aquatic species commonly encountered, even in winter, in various lotic habitats at the foot of the Pyrénées and has been likewise collected in other parts of France and Spain. It appears in many brooks to be one of the dominant species encountered on submerged wood in the regions

Table 1. Synopsis of discriminant characters of freshwater species of *Phomatospora*.

Species	Ascospores				Yellow stain of substrate
	Size (µm)	Septa	Sheath	Appendages	
<i>P. aquatica</i>	11-13 × 3.2-4	Aseptate	Absent	Present, rounded	Absent
<i>P. berkeleyi</i>	8-10 × 3-5	Aseptate	Absent	Present, rounded	Absent
<i>P. luteotिंगens</i>	14.5-17.8 × 5-6	Aseptate	Absent	Absent	Present
<i>P. muskellungensis</i>	20-26 × 4-5	0-1-septate	Absent	Present, filiform	Absent
<i>P. striatigera</i>	23-27 × 4-6	Aseptate	Absent	Present, rounded	Absent
<i>P. triseptata</i>	18-20 × 7-8	3-septate	Present, thin	Present, rounded	Absent

surveyed. It has so far been collected on deciduous, always decorticated submerged wood, with an apparently marked host recurrence on alder and ash.

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