

## ***Cercophora aquatica* sp. nov. from a streambed in southern France**

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Chaudhary P., Fournier J. & Miller A. N. (2007) *Cercophora aquatica* sp. nov. from a streambed in southern France. – *Sydowia* 59 (2): 217–225.

*Cercophora aquatica* sp. nov., collected from a streambed in France, is described and illustrated. The morphological features of the fungus are glabrous ascomata which possess an outer areolate wall, asci with a double apical ring but which lack a subapical globule, and ascospores with long gelatinous appendages. It produced a *Phialophora*-like anamorph as well as blastoconidia in culture. It is compared to other *Cercophora* species characterized by glabrous ascomata. *Cercophora* species previously isolated from aquatic habitats are also discussed. *Cercophora crustosa* (Massee) P. Chaudhary, J. Fournier & A. N. Mill comb. nov. and *Cercophora hydrophila* (Kirschst.) P. Chaudhary, J. Fournier & A. N. Mill comb. nov. are proposed as new combinations.

Keywords: Ascomycota, freshwater, Sordariales, systematics, taxonomy.

While exploring aquatic habitats for ascomycetes, an interesting species of *Cercophora* was found on partially submerged wood collected from a streambed in southern France. The fungus was encountered three times in the same stream in July, October and November when the water level was very low. It differs from other *Cercophora* species in possessing glabrous ascomata with an outer areolate wall, asci with a double apical ring, and ascospores with long gelatinous appendages. Given the unique combination of morphological characters and distinct habitat, this fungus is proposed as a new species.

### **Materials and Methods**

Small partially submerged branches were collected from a streambed and examined for ascomata with the aid of a hand lens in

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the field. Ascomata were squash-mounted in water and images of micromorphological structures were captured with a QImaging QColor 3 digital camera mounted on either a Leica MZ7.5 dissecting microscope with a Schott KL1500 fiber optics light source or an Olympus BX51 compound microscope using differential interference or phase contrast microscopy. Images were processed using Adobe Photoshop 7.0 (Adobe Systems Inc., Mountain View, California). A minimum of 30 measurements was taken for all morphological structures using NIH Image 1.63 (National Institute of Health, Bethesda, Maryland). Mean and standard deviation (shown in brackets) were calculated for ascospores and conidia.

Single spore (SS) and multispore (MS) isolates were obtained for culture studies. Air-dried ascomata were placed in a drop of sterile distilled water and ascomatal walls were broken apart with flame-sterilized needles to release the centrum. Centrum material was then spread on 1 % water agar (WA, Difco Bacto) in a 60-mm-diam plastic Petri plate to separate asci and ascospores. The plate was incubated at room temperature (21–25 °C) for 24–48 hours. Germinating MS and SS isolates were transferred to cornmeal agar (CMA, Difco) and incubated at room temperature for 1–2 weeks. Two subcultures of each MS and SS isolate were transferred to fresh CMA plates and incubated for 3 weeks to standardize growth rates. Culture studies were performed on WA, CMA and oatmeal agar (OA, Difco). Approximately 5-mm-diam agar plugs were cut from the margins of each isolate and placed inverted in the center of WA, CMA and OA in 60-mm-diam plastic Petri plates. Plates were placed in Rubbermaid® plastic storage containers and incubated at room temperature under ambient light. Growth rates and colony characteristics were recorded at 7 d intervals for 6 weeks. Anamorphs were observed in water mounts at 42 d. Color terms are taken from Kornerup & Wanscher (1978).

### Taxonomy

***Cercophora aquatica*** P. Chaudhary, J. Fournier & A. N. Mill., **sp. nov.** – Fig. 1–17.

Anamorph. – *Phialophora*-like.

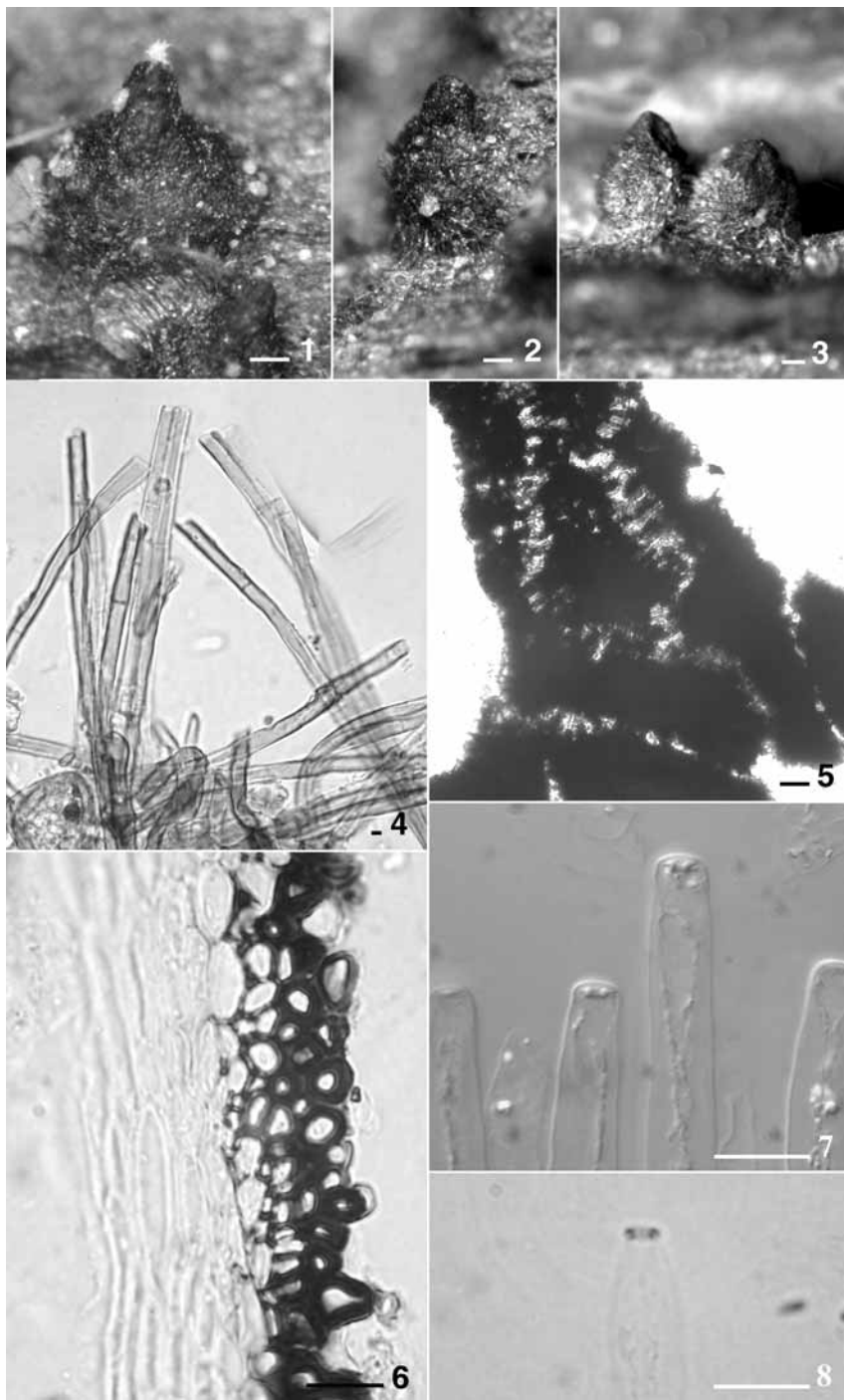
Ascomata subglobosa vel obpyriformia, 350–550 µm diam, nigra, solitaria vel gregaria, glabrata, papillata, erumpentia; subiculum ex hyphis brunneis, septatis compositum; collum papillatum. Papillae conicae, periphysibus indutae. Paraphyses filiformes, septatae. Asci cylindrici, unitunicati, annulo apicali incrassato, duplici, refracti, octospori. Ascosporae juvenes, cylindricae, 36–51 × 4–6 µm, hyalinae, aseptatae, appendicibus bipolaribus, 38–45 µm, gelatinis, deinde bicellulares; cellula superior inflata, ellipsoidea, 15–18.5 × 7–12 µm, hyalina vel brunea; cellula pedicelli 23–34 × 4–6 µm, hyalina. Ascosporae demum usque 5-septatis.

Holotypus. – FRANCE, Ariège, Rimont, ruisseau de Peyrau, 400 m elev., 29 Nov 2006, on recently exposed, submerged wood of *Quercus* sp. collected from a streambed, leg. J. Fournier (JF06314, ILLS 58443)

Ascomata subglobose to obpyriform, 350–550 µm diam, 400–600 µm high, brownish black to black, solitary or gregarious, glabrous, strongly papillate, erumpent; subiculum composed of brownish, septate hyphae 2.5–3 µm broad; neck papillate with a rounded apex, ostiolate, brownish black to black, sulcate or roughened. Ascomal wall areolate in surface view; in longitudinal section 2-layered, outer layer 15–21 µm thick, composed of 3–6 layers of dark brown, thick-walled, scleroplectenchymatous polygonal cells, 4–8.5 µm diam, cell walls 1.5–2.5 µm thick; inner layer 14–17 µm thick, composed of 4–6 layers of hyaline, thin-walled pseudoparenchymatous flattened cells up to  $17 \times 3.5$  µm. Ascomatal apex with periphyses. Centrum hyaline. Paraphyses filiform, 4–9 µm wide, hyaline, numerous, septate, unbranched, persistent. Asci cylindrical,  $180\text{--}250 \times 13\text{--}16$  µm, long-stipitate, stipe  $70\text{--}100 \times 2.5$  µm, numerous, unitunicate, thin-walled, apex rounded; apical ring thickened, double, refractive, 3–3.5 µm broad, 1 µm high; subapical globule absent, with eight biseriate to triseriate ascospores. Ascospores cylindrical,  $36\text{--}51 \times 4\text{--}6$  µm [ $43.9 \pm 3.4 \times 4.4 \pm 0.5$ ], sigmoid to geniculate, hyaline, aseptate, with several small oil droplets; bipolar appendages long, 38–45 µm, gelatinous, lash-like; becoming differentiated into an apical swollen head and a basal pedicel while inside the ascus; head ellipsoid, (13–)  $15\text{--}18.5$  (–21)  $\times 7\text{--}12$  µm, rounded at the apex, truncate at the base, hyaline to dark brown; pedicel  $23\text{--}34 \times 4\text{--}6$  µm, hyaline; ascospore up to 5-septate after liberation from the ascus, each cell with a single, large refractive oil droplet, occasionally with inflated cells due to constrictions at the septa; occasionally producing phialides directly from the ascospore.

Colonies on WA and CMA slow-growing, approximately 15 mm diam on WA and 30 mm diam on CMA in 28 d, fast-growing on OA, covering the plate in 21 d, mat silky on WA and CMA, hyaline, margin plumose, mat felty at the center on OA, yellowish-grey (4B2) to grayish orange (5B3–5B4), surrounded by silver gray (4E2) to bronze (5E5) slightly appressed subfelty mycelia, margin even, appressed, hyaline, not distinct, developing ridges with deep furrows by the fourth week of incubation; reverse same as the mat in all media.

Hyphae hyaline to pale brown, thin-walled, 1–3 µm wide. Conidiogenous cells phialides, produced from pale brown hyphae as a single terminal phialide, often branching into two or more phialides, sometimes percurrently proliferating, delimited by a basal septum, monophialidic, pale brown, cylindrical,  $16\text{--}23 \times 2.5\text{--}3$  µm at widest part, slightly constricted at the collarete, 1–1.5 µm just below the collarete; collarete flaring, brown, darker than the phialide, 3–4.5 µm wide. Conidia pyriform, truncate at base, hyaline to pale brown,  $2.5\text{--}3.5 \times 2\text{--}3$  µm [ $3.1 \pm 0.2 \times 2.5 \pm 0.2$ ]. Blas-



toconidia produced laterally on stalks 3–11  $\mu\text{m}$  long or directly from hyphae, obovate to oblong, larger than the phialoconidia,  $4\text{--}7 \times 2\text{--}3.5 \mu\text{m}$ , hyaline. Anamorphs produced only on CMA.

**Etymology.** – Refers to the habitat where the fungus was collected.

**Habitat** – Found on wood of *Alnus glutinosa* and *Quercus* sp., partially submerged in a shallow freshwater streambed.

**Distribution.** – Only known from type locality.

**Specimen used in culture studies.** – ILLS 58443-1, 2 (MS), 3, 4 (SS).

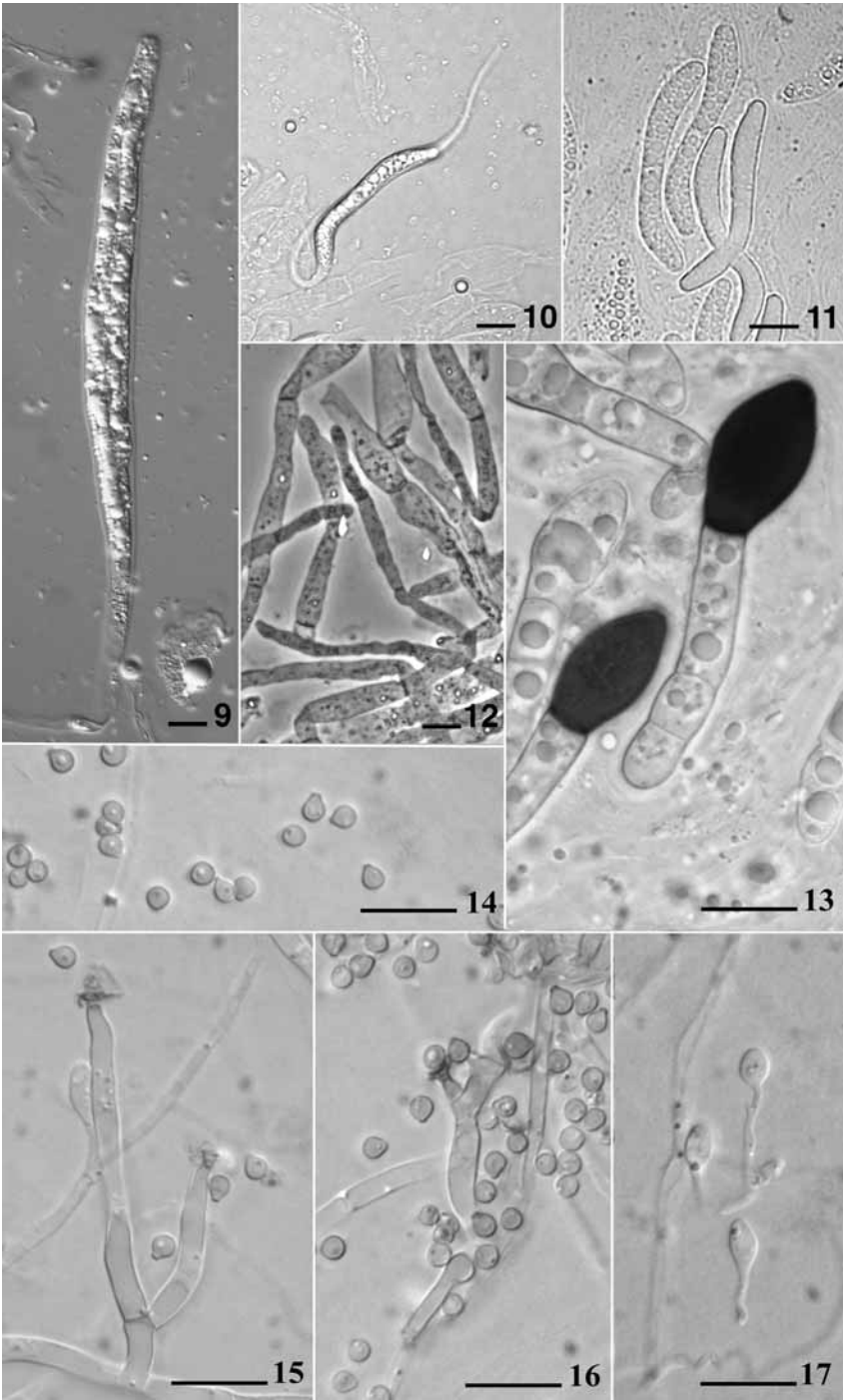
**Other material examined.** – FRANCE, Ariège, Rimont, ruisseau de Peyrau, 400 m elev., 26 July 2006, on recently exposed, submerged wood of *Alnus glutinosa* collected from a streambed, leg. J. Fournier (JF06165, ILLS 58442).

## Discussion

The genus *Cercophora* Fuckel was first described by Fuckel in 1870 who soon synonymized it under *Sordaria* Ces. & De Not. It remained obscured until Lundqvist revived it in 1972 (Lundqvist 1972). It is characterized by large, membranous to carbonaceous, ostiolate, ornamented or glabrous ascomata, asci usually with an apical ring and a subapical globule, and hyaline ascospores which develop a swollen head that often turns brown at maturity. Molecular studies have shown that *Cercophora* is a highly polyphyletic genus and species cannot be grouped according to ascospore morphology (Miller and Huhndorf 2005). However, new species which fit the traditional morphological circumscription of the genus should be placed within *Cercophora* until additional work can be completed. *Cercophora* species are mainly differentiated by ascomatal characters such as color, shape and type of ornamentation, ascal characters such as the size of ring (single or double) and presence or absence of a subapical globule, ascospore features such as size and shape and presence or absence of appendages, and habitat. Most species are characterized based on elaborate ornamental structures, including hairs, setae, tomentum, colored granules, tubercles, or neck cells present on the ascomata (Lundqvist 1972). However, a few species possess glabrous ascomata making it difficult to characterize them as they do not possess any of these distinctive ascomatal fea-

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**Figs. 1–8.** *Cercophora aquatica*: 1–3. Ascomata on substrate. 4. Subiculum hairs. 5. Surface view of outer areolate ascomatal wall. 6. Longitudinal section through ascomatal wall. 7–8. Ascus apices with thickened, double apical ring. Scale bars: 1, 2, 3 = 100  $\mu\text{m}$ ; 4 = 1  $\mu\text{m}$ ; 5, 6, 7, 8 = 10  $\mu\text{m}$ .



tures. Such taxa are mainly delimited by a unique combination of morphological characters and habitat.

*Cercophora aquatica* is characterized by glabrous ascomata with an outer areolate wall, asci with a thickened, double apical ring, but without a subapical globule, ascospores with long gelatinous appendages, and an aquatic habitat. It is morphologically similar to an unpublished species referred to as *Cercophora* sp. 2 by Lundqvist (1972, pg 115), which was also collected on wood in fresh water. *Cercophora aquatica* can be distinguished in the absence of a subapical globule and in having a hyaline centrum, whereas *Cercophora* sp. 2 possesses a subapical globule and a yellow centrum. The taxonomic position of *Cercophora* sp. 2 still needs to be resolved. Lundqvist (1972) recognized that it could be a new species, but refrained from formally describing it mainly due to the lack of unique morphological characters. During examination of *Cercophora* sp. 2, a very prominent subapical globule was seen in the asci and gelatinous ascospore appendages appeared to be present. These observations taken together with the information available on *Cercophora* sp. 2 suggest that it is a new species, but, like Lundqvist, we refrain from formally describing it until additional material can be collected.

The morphology of *Cercophora crustosa* (Massee) P. Chaudhary, J. Fournier & A. N. Mill. **comb. nov.** (Bas.: *Sordaria crustosa* Massee Bull. Roy. Bot. Gard. Kew, 1910, 1: 2–3) is strikingly similar to that of *C. aquatica* in that the ascomata are glabrous with a conical neck, asci lack a subapical globule, and ascospores eventually develop a swollen pigmented head and possess long, lash-like, gelatinous appendages. However, it differs in the size of the ascospores ( $60\text{--}80 \times 6\text{--}10$  vs.  $36\text{--}51 \times 4\text{--}6$   $\mu\text{m}$ ) and habitat (herbivorous dung vs. wood in fresh water).

Several other *Cercophora* species also possess glabrous ascomata similar to those found in *C. aquatica*, but can be distinguished based on ascomatal morphology and habitat. *Cercophora areolata* Lundq. is characterized in having strongly areolate ascomata composed of broad polygonal cells and yellow centrum pigments (Lundqvist 1972). *Cercophora heterospora* Mukerji, R.N. Kumar & N. Singh differs by its cleistothecial ascomata, 4-spored asci, and ascospores which lack gelatinous appendages (Mukerji *et al.*, 1995). *Cercophora minuta* Mukerji, R.N. Kumar & N. Singh is characterized by smaller ( $350\text{--}475 \times 380\text{--}400$  vs.  $350\text{--}550 \times 400\text{--}600$   $\mu\text{m}$ ) ascomata with an

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**Figs. 9–17.** *Cercophora aquatica*: **9.** Ascus **10.** Ascospore with long gelatinous appendages. **11.** Ascospores. **12.** Paraphyses. **13.** Mature, pigmented ascospores. **14.** Phialoconidia. **15.** Percurrently proliferating phialide. **16.** Phialides and phialoconidia. **17.** Blastoconidia. Scale bars = 10  $\mu\text{m}$ .

indistinct neck, asci with a subapical globule, and larger ascospores ( $60\text{--}75 \times 5\text{--}8.5$  vs.  $36\text{--}51 \times 4\text{--}6$   $\mu\text{m}$ ). *Cercophora sordarioides* (Speg.) Lundq. is distinguished by its semi-transparent, yellow-olivaceous ascomata, asci with a simple ring and subapical globule, and coprophilous habitat (Lundqvist 1972). *Cercophora striata* (Ell. & Ev.) Lundq. differs in having ascomata with a striate neck and white to grayish granules that form below the neck upon drying (Miller and Huhndorf 2001). Finally, *Cercophora tuberculata* Mukerji, R.N. Kumar & N. Singh differs in having tubercles at the base of the neck and asci with a subapical globule (Mukerji *et al.* 1995).

While most *Cercophora* species are coprophilous, lignicolous, or terricolous, a few species have been found in aquatic habitats. *Cercophora terricola* Ueda, which was isolated from river sediment, is characterized by ascomata covered by brown, thick-walled hairs (Ueda 1994). *Cercophora hydrophila*\* (Kirschst.) P. Chaudhary, J. Fournier & A. N. Mill. **comb. nov.** (Bas.: *Bombardia hydrophila* Kirschst. Kryptogamenflora der Mark Brandenburg, Sphaeriales, 1911, 7(2):184) and *Cercophora natalitia* (Speg.) Lundq. have been reported from a similar habitat, but can be distinguished in having ascomata with short, light brown cells covering the ascomatal necks (Gnanananthan 1972, Lundqvist, 1972). *Cercophora hydrophila* can further be separated by its longer ascospores ( $60\text{--}75$  vs.  $36\text{--}51$   $\mu\text{m}$ ). Several lignicolous species, viz., *Cercophora appalachianensis* O. Hilber and R. Hilber, *Cercophora caudata* (Curr.) Lundq., *Cercophora costariensis* (Carroll & Munk) O. Hilber & R. Hilber, *Cercophora newfieldiana* (Ellis & Everh.) R. Hilber, *Cercophora scortea* Cain, and *Cercophora sulphurella* (Sacc.) R. Hilber known to have a terrestrial habitat have also been reported from aquatic habitats (<http://www.life.uiuc.edu/fungi/wr-search-frame.html>). These species, which are believed to be immigrants that have washed into aquatic habitats, differ from *C. aquatica* in having some type of ascomatal vestiture.

### Acknowledgments

The production of this manuscript was supported by a National Science Foundation Biodiversity Surveys & Inventories Grant (DEB-0515558) to ANM. The authors would like to thank Drs. Thomas Læssøe and Hennig Knudsen for the loan of material from C. We also wish to thank J. L. Crane for his comments on an earlier draft of this manuscript.

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\* Although Lundqvist (1972) used the name "*Cercophora hydrophila* (Kirschst.)" on page 115, he did not validly transfer this species on this page or in his summary of new combinations.



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(Manuscript accepted 3 Aug 2007; Corresponding Editor: R. Pöder)



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