

Xylobolus frustulatus (Stereaceae): Developmental Observations, Morphology, and Ecology

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ABSTRACT—*Xylobolus frustulatus*, a wood-rotting fungus known as the ceramic or parchment fungus in the Stereaceae, was observed continually over a twelve-month period in the Fort Worth Nature Center and Refuge near the city of Fort Worth, Texas. The distribution, habitat, and morphology of this fungus is described. The moist young stages in early development of this fungus produce clear to amber-colored liquid droplets (guttation) and look morphologically different from the fruiting bodies when fully mature and dried. This pattern of development has not been previously described in detail. Guttation in fungi is discussed and some examples given. Photographic images record the morphological changes over time. The mature fungus was embedded in paraffin and 4- μ m sections were stained with Gomori-methenamine silver, hematoxylin and eosin, and also the periodic acid Schiff method to show the sterile acanthohyphidia on the spore bearing surface. Decayed logs with fruiting bodies of the fungus were transported to the BRIT laboratory to make daily observations and photograph a fungal beetle in the genus *Cis*. A series of photographs documents the beetle morphology and emergence from the cavities where it feeds and lives inside the fungus. This beetle is tiny, about 1 mm in length, dark brown to black, and barely visible to the naked eye. This group of *Cis* beetles is only known to occur on fungi and is reported here as a species new to science that will be described elsewhere.

Key Words—basidiomycete, beetle, ceramic, *Cis*, crust, parchment, droplets, fungus, guttation, wood-rotter

Introduction

Xylobolus frustulatus is a well described crust fungus, widely known as the ceramic or parchment fungus because of its striking appearance when mature (Persoon, 1801; Boidin, 1958; Kuo, 2008). The crust breaks into irregular ivory colored plates “frustules” separated by a dark sepia “grout.” Its preferred host is mature, dead, decaying, decorticate, oak trees of a variety of taxa. Most of the records are in the eastern USA as well as in Europe and Asia, perhaps because of its host association, although there is one record from Oregon. This fungal record occurred in 1922, growing on an Pacific wax myrtle (see www. MyCoPortal.org). It has been noted in Louisiana and Texas. W. H. Long collected specimens in Denton, TX, in North Central Texas in the early

1900’s (see www. MyCoPortal.org). At that time, many classified it in the genus *Stereum*, another crust basidiomycete fungus (Boidin, 1958). It currently is classified in the family Stereaceae with other corticioid, perennial, effused, wood-rotting fungi such as *Stereum*.

Review of Guttation Droplets

Liquid droplets (guttation) form on the young, developing fruiting bodies of *Xylobolus frustulatus*. The mechanism of guttation in fungi is poorly understood, although it is widespread in some wood-rotting genera, especially in the Polyporaceae. In *Fomitopsis pinicola* guttation of reddish liquid droplets are frequently seen and usually observed in the early rapid growth phase, but may also be temperature and humidity dependent (Parmasto and Voitk, 2010). *Abortiporus biennis* is another polypore

Figures 1–4. Habit photographs of *Xylobolus frustulatus* from Greer Island, Fort Worth Nature Center and Refuge on decorticated post oak logs.



Figure 1. Ground site habitat with fungus on 6-ft log mostly on top of leaves, January 21, 2017.



Figure 2. Log partially buried in leaves. Note the lobed cross-shaped oak leaves resembling a Maltese cross, December 21, 2016.



Figure 3. Cut end of log showing fungus on outer portion and inner rotted center with no fungi growing in the hollow dark interior, December 21, 2016.



Figure 4. Log with weathered frustules, May 22, 2016.

that produces characteristic reddish liquid droplets (Bunyard, 2017). Others have photographed similar droplets on immature specimens of *Xylobolus* (Smith, 2016).

Spectacular fruiting bodies of *Inonotus glomeratus* on decayed logs often produce copious amounts of shiny black exudate that is thick and sticky and will stain fingers deep auburn brown (Thornhill, 2014). There are few studies on the nature of the fluid in these droplets or their biological function. It is unlikely that they are involved in spore dispersal since they are usually present prior to spore maturation. Guttation may function in different ways in different fungal species.

The Location

The Fort Worth Nature Center and Refuge (FWNC&R) is a 3,621-acre area located in the Fort Worth Prairie and comprised of forests, prairies, and wetlands only 10 miles from Fort Worth, Texas. It has more than 20 miles of hiking trails and is one of the largest city-owned parks in the United States of America. In 1964, land was set aside to create a nature preserve and wildlife sanctuary that also included Lake Worth and the West Fork of the Trinity River. In 1980, this area was designated as a National Natural Landmark by the U.S. Department of Interior. Of the 55-tree species recorded for the area, most are deciduous and dominated by *Quercus stellata* (Post Oak) and *Ulmus crassifolia* (Cedar Elm). This eco-region is known as the Eastern Cross Timbers underlain by limestone and sandstone (FWNC&R, 2017).

The Habitat

Field trips were made several times per week from May 1, 2016 to May 1, 2017 and to several areas in FWNC&R. These habitats had scattered decaying post oak logs among leaf litter on ground sites (Figures 1, 2). This forested area was more open since leaf fall had occurred during the winter months. Two sites were observed during this time-period: Greer Island, GPS 32.828782–97.463042, sandy soil and Ten Mile Bridge Road in a sandy forest. The well-decayed logs were decorticated and extremely hard, predominantly covered with the crust-like *Xylobolus frustulatus* (Figures 3, 4). All specimens



Figure 5. *Stereum rugosum* growing (below) with *Xylobolus* (above) on the same decayed post oak log, February 19, 2017.

Figures 6–8. Stages of *Xylobolus frustulatus* fruiting bodies.



Figure 6. Immature early stage of cushion-shaped frustules with clear guttation droplets covering most of surface area with fewer amber-colored droplets at left margin, May 1, 2016.

were on dead *Quercus stellata* as this is the predominate oak in this region (Figures 3, 4). This fungus grows on the top, sides, and cut ends of decayed logs. The only other fungus observed

on these rotten logs was *Stereum rugosum* (Figure 5). *Xylobolus* is visible and conspicuous throughout the year. The field observations of the early developmental stages of *Xylobolus* were

made continuously from December 11, 2016 to May 1, 2017. The mature frustules were observed once or twice a week for over a year without any obvious additional changes.



Figure 7. Mature frustules showing polygonal, flattened, ivory-colored frustules of varying sizes with margins separated by black spaces, December 21, 2016.

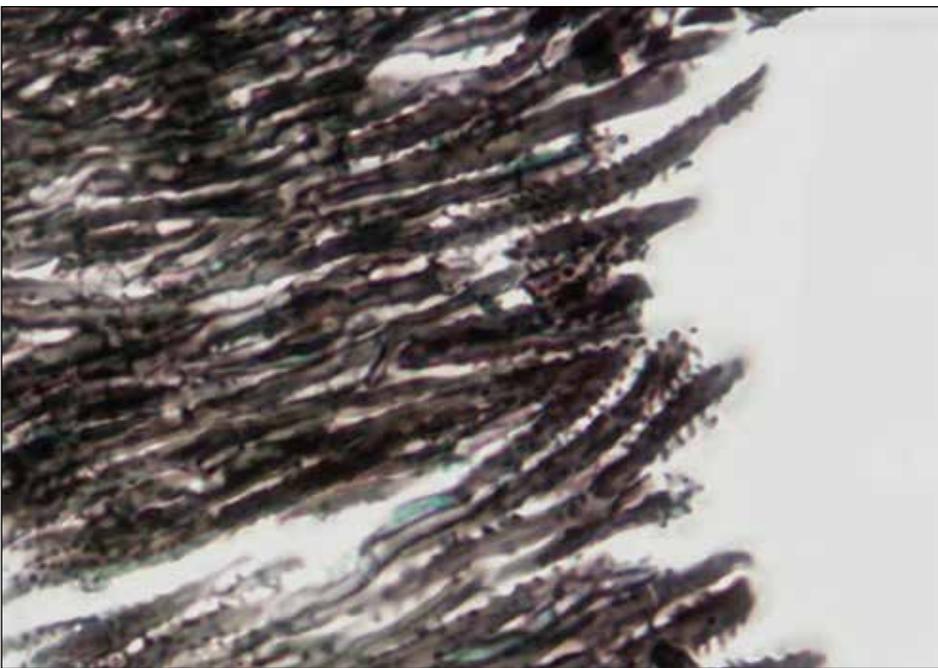


Figure 8. Gomori Methenamine Silver stained preparation of *Xylobolus* showing acanthohyphidia “bottle brushes.” Fungal cell walls stain black against a green counterstain background.

Materials and Methods

Three logs, each about 1 meter long, were brought to the BRIT research area and examined daily with a hand lens. Dark colored beetles were found growing inside tiny fungal cavities about the same size as the beetle. Beetle image stacks were captured using a Canon 6D SLR with 65mm MP-E 1-5x macro lens, controlled with Helicon Remote

(Version 6). Stacking was done using Helicon Focus. Macrophotography was done using a Nikon Cool-Pix S9900 camera. Fungal fragments were embedded in paraffin and 4- μ m sections stained with Gomori-methenamine silver, hematoxylin and eosin, and by the periodic acid Schiff method. Sections were examined and photographed at 1000X with an Olympus microscope.



Figure 9. Adult male beetle walking on edge of ruler slightly larger than 1 mm.

Spores were stained with Melzer’s reagent and 100 spores measured.

Specimens Examined

Beetle specimens were deposited in the Clemson University Arthropod Collection, Clemson University, Clemson, South Carolina (CUAC). Fungal specimens were deposited in the Philecology Herbarium at the Botanical Research Institute of Texas as *Xylobolus frustulatus* (Pers.) Boidin, U.S.A., Texas: Tarrant County, Fort Worth Nature Center and Refuge, Greer Island, May 21, 2016, *R. J. O’Kennon 31027* (BRIT barcode BRIT59723).

Morphological Observations

Field and laboratory observations were made through early growth phases to mature fruiting bodies of *Xylobolus frustulatus*. These observations extend this species range further to the west, and formally notes the phenomenon of “guttation,” the exudation of liquid droplets, in the early rapidly growing fruiting body.

Xylobolus in its early development consists of relatively soft, pale, ochraceous, rounded nodules and cushions. These are joined to one another through shallow grooves that define each future “frustule.” The nodules vary from 0.3 to 1.0 cm in size. It is at this stage that droplets of liquid are noted oozing on the surface. Most contain clear liquid, although over time (days to a couple of weeks) some become pale to dark amber, but remain crystal clear (Figure 6). Even though some of these liquid droplets may be amber colored they do not stain the wood or fungal tissue. There are few studies on the nature of the guttation fluid or droplets or their



Figure 10. Mature *Xylobolus* frustules inhabited by *Cis* beetles. Note spherical hole where the beetle lives (upper left) and how feeding activities alter fruiting body shape. Two beetles outside of frustules showing fractured and empty frustule remains after heavy feeding by beetles, December 24, 2016.

biological function. It is unlikely that they are involved in spore dispersal as they are usually present prior to spore maturation.

As the specimen matures the frustules separate into discrete plates, sometimes with upturned margins, becoming ivory-colored and then changing to a grey-brown with age. The context changes from a firm spongy texture to hard, brittle, fructose irregular polygons (Figure 7). Changes in these specimens occurred for more than three weeks.

Microscopically, these specimens had characteristic features including ellipsoid amyloid spores (3.5-5.5 μm) and a prominent layer of acanthohyphidia on the spore-bearing surface (Figure 8). These “bottle brushes” are a reliable characteristic feature, but are not specific for *Xylobolus*, and have been described in other taxa (Boidin et al., 1979). No clamp connections were observed. The biological function, either anatomic or physiologic, of these sterile acanthohyphidia is not known. They may contribute to the rigidity of the surface. Others have suggested such functions as osmotic or thermoregulation, nutrient

storage or transfer to developing spores, or secretory or excretory roles. Whether they function to attract beetles or to protect against predation is entirely unknown. It has been suggested that cystidia may play a defensive role in some taxa (Nakamori and Suzuki, 2007).

Taxonomy of *Cis* beetles

The *Cis* beetles belong to the order Coleoptera, Family Ciidae, and genus *Cis* that include more than 90 species as obligate inhabitants of mostly wood-rotting fungi. They complete their entire life cycle in the fungus usually within a two-month period. *Cis* beetles are dark brown to black in a size range of 0.5–5 mm long, depending on the species.

Fungal-Beetle Ecology

Fungivory by a wide variety of organisms is a well-documented phenomenon, including numerous invertebrates (Schigel, 2012). Many fungivorous insects made the basidiocarp of *Xylobolus* home but the most prominent was a *Cis* beetle. Beetles in the family Ciidae are known obligate fungivores, most utilizing the

basidiocarps of lignicolous fungi, so it was not surprising to discover a tiny beetle in the genus *Cis* living in close association with this fungus. There is at least one report from Japan in which a closely related beetle, *Ennearthron chujoi* was noted with *Xylobolus* (Orledge and Reynolds, 2005).

These beetles burrow into frustules, consuming and hollowing out the interior. This results in a spherical hole in the frustule about the body size of the beetle (males slightly larger at 1.1 mm and females 0.9 mm (Figure 9). The adult female apparently feeds on the fungus vertically and fits into the cavity with only its head barely visible (Figure 10). Close inspection of the fungal surface revealed many cavities and adult beetles crawling among the frustules (Figure 10). These beetles lay their eggs (not seen) that develop into tiny larvae seen inside the cavities. Their entire life cycle apparently is conducted within the confines of the fungus. This *Cis* beetle species is sexually dimorphic: males slightly larger than females with protruding horns on their heads and females without horns (Figure 11). However, these beetles have wings, and it is likely that spore dispersal flights occur here as in other *Cis* beetles, spreading fungal spores to other hosts. Beetles were collected and sent to Michael L. Ferro, Collection Manager, Clemson University Arthropod Collection for identification. It was determined that this *Cis* species was a species new to science after comparison with other morphologically similar beetles (Figure 12A and B).

In addition to these beetles, which were clearly the most common inhabitants, other organisms were observed, including a tiny snail *Zonitoides arboreus* about 1 mm. Such fungivores have been described in association with other polypores. Many of these beetles tend to be somewhat specific for a particular fungus or host wood. Despite an extensive overview of these relationships, *Xylobolus* species were not noted by Lawrence (1973).

Conclusions

Regular field observations (several times per week) over a longer period of time (years) are important to connect early developmental stages to mature



Figure 11. Adult female beetle walking on background.

fruiting body stages in many fungi. This kind of dedicated field work often results in surprise discoveries of ecological interactions (the life cycle of a new species of tree beetle) not possible on a hit and miss basis dictated by rainy weather and convenience of the collector's schedule. *Xylobolus frustulatus* is part of a complex local ecosystem, including a variety of insects, responsible for the recycling of dead wood. The interrelationships of this life cycle await further research to elucidate the fungal-invertebrate complex interactions with dead wood and recycling of decomposing organic matter on ground sites.

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Figure 12-A.

Figure 12. Undescrbed species of *Cis* male beetle: A) lateral view; B) dorsal view.



Figure 12-B.

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