Dung fungi as a proxy for megaherbivores: opportunities and limitations for archaeological applications

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Abstract

The use of spores of coprophilous fungi from sedimentary sequences as proxy evidence for large herbivore abundance has garnered pronounced attention and scrutiny over the past three decades. In response to the rapid rate at which new information is being discovered on this topic, this paper presents a brief review of the archaeological applications so far, and outlines opportunities and limitations of using *Sporormiella* as a proxy for herbivore abundance. Specific archaeological uses of this proxy include understanding megaherbivore extinctions and human land use patterns such as pastoralism and agriculture. We analyze how dung fungal records are formed and review the mycological literature to outline factors affecting spore reproduction and preservation. These include how strongly each commonly used dung fungal taxon relies on dung as a substrate and environmental factors affecting dung fungal reproduction and coprophilous fungi deposition. Certain laboratory preparation techniques adversely affect spore representation on pollen slides. The methods of analysis and quantification of spore records also impact on our understanding. We describe good practice to increase precision of analytical methods. Due to limitations imposed by some of these factors, it is possible that an absence of dung fungi from a palaeoecological record does not imply an absence of herbivores. However, consideration of these factors and inclusion of as wide a range of coprophilous spore records as possible increases the reliability of such inferences.

Acknowledgements

1. Introduction

The use of *Sporormiella* spores from sedimentary sequences as a proxy for large herbivore abundance has garnered pronounced attention and scrutiny over the past three decades. Since it was first proposed as a proxy for Pleistocene megaherbivore abundance in the 1980s (Davis 1987), increasing research has been devoted to developing sampling, recovery and quantification techniques, as well as understanding the applications and limitations of this method. In response to the rapid rate at which new information is being discovered on this topic, this paper presents a brief review of the archaeological applications so far, and outlines opportunities and limitations of using *Sporormiella* as a proxy for (mega)herbivore abundance.

Sporormiella is one of a number of genera of coprophilous fungi, also known as dung fungi. These are fungi that show a strong preference for (mainly herbivore) dung as their primary substrate. *Sporormiella* is one of many organisms that participate in the breakdown of herbivore dung after it is evacuated. This fungus belongs to a group of most commonly used fungal indicators of herbivore abundance, Ascomycota, which reproduce on the dung two to four weeks after it is deposited (e.g. Harper and Webster 1964). There are some indications that the spores produced by *Sporormiella* and similar genera need to pass through the digestive tract of an herbivore in order to activate germination, but evidence for this is limited (Janczewski 1871; Massee and Salmon 1902; Krug et al. 2004). Regardless of these uncertainties, the abundance of *Sporormiella* spores in sedimentary sequences has been demonstrated repeatedly to reflect herbivore abundance in both modern (e.g. Baker et al. 2016; Gill et al. 2013; Parker and Williams 2012; Raczka et al. 2016; Wood et al. 2013) and ancient settings (e.g. Burney et al. 2003; Davis 1987; Davis and Shafer 2006; Doyen and Etienne 2017; Gill et al. 2009).

2. Applications for Archaeologists

24   Dung fungal records are important datasets for archaeologists because of the frequency of

25   interactions between ancient people and large herbivores. *Sporormiella* continues to be used as a

26   proxy to detect declines in late Pleistocene megaherbivore communities (e.g. Davis 1987; Gill et

27   al. 2009, 2014; Graham et al. 2016; Johnson et al. 2015; Perrotti 2018). The North American

28   extinction of over 30 species of large mammals at the end of Pleistocene is of particular interest

29   to archaeologists because it roughly coincides with the earliest human colonization of the

30   continent. The cause of these extinctions remains the subject of intense debate. Disagreement

31   concerns the relative impacts of human hunting (Alroy 2001; Frank et al. 2015; Martin 1984;

32   Surovell et al. 2016; Surovell and Waguespack 2009), climate change (Guthrie 1984; Grayson

33   and Meltzer 2003), disease (MacPhee 1997) and a potential extraterrestrial impact (Firestone et

34   al. 2007) on Pleistocene megafauna.

35   Because faunal remains are sparse and can be difficult to date, it is hard to reliably

36   establish extinction dates. The use of the *Sporormiella* proxy allows researchers to fill in

37   geographic gaps where there may be no dateable fauna. Since *Sporormiella* spores occur in the

38   same deposits as pollen and plant macrofossils, patterns in their abundance can be linked directly

39   with trends and changes in the vegetation record, as well as with absolute dates obtained for

40   these deposits. The fungus has been found in sedimentary records across North America, and is

41   aiding in the understanding of the timing and process of megafaunal extinction, and its effects on

42   vegetation communities (Gill et al. 2009, 2014; Perrotti 2018).

43   If humans are unequivocally tied to megaherbivore extinction, it is possible that the

44   timing of local extinctions could be used as a signal of human colonization in different regions,

45   particularly when complemented with archaeological evidence. Studies incorporating

46   *Sporormiella* in western North America are rare, but declines in *Sporormiella* coincide with

47 generally accepted dates of human colonization in the northeastern United States (Davis and

48 Shafer 2006; Gill et al. 2009; 2014). However, the *Sporormiella* record and archaeological

49 evidence from Page-Ladson, Florida (Halligan et al. 2016; Perrotti 2018) indicate that humans

50 and megaherbivores coexisted in the region for ~2,000 years. Fiedel (2018) suggests that

51 *Sporormiella* may not be a reliable indicator of megaherbivore extinction in eastern North

52 America because current *Sporormiella* records (i.e. Gill et al. 2009, 2014) point to a decline in

53 megaherbivores around 14,800 BP, while some mammoth remains in the region are dated to as

54 late as ~12,000 BP. Furthermore, it is unclear whether absence of spores is equally informative

55 as spore presence (e.g. see below for factors other than herbivore abundance influencing fungal

56 growth, and thus, potential spore presence; see also Raper and Bush 2009; Jones et al. 2017).

57 However, researchers using *Sporormiella* as a proxy for large herbivore abundance acknowledge

58 that declines in *Sporormiella* do not necessarily indicate a complete extinction of all

59 megaherbivores; but rather, a functional decline in grazing pressure that represents shrinking

60 herbivore populations relegated to patchy environments prior to extinction (Gill et al. 2009,

61 2012). Nonetheless, because of the discrepancies between regions and the inconclusive evidence

62 for a human driven extinction of North American megafauna, at present *Sporormiella* or other

63 coprophilous fungi cannot be used as a proxy for human activity and migration in this region

64 (Fiedel 2018).

65   Human colonization seems to coincide with a decline in coprophilous fungi in other parts

66 of the globe, including Australia (Rule et al. 2012; van der Kaars et al. 2017), New Zealand

67 (Wood et al. 2011) and Madagascar (Burney et al. 2003). In some cases, the initial fungal spore

68 decline is followed by an increase after the introduction of domesticated animals (Burney et al.

69 2003; Davis and Shafer 2006; Graham et al. 2016) or other non-native herbivores (Wood et al.

70 2011).

71   Second, many archaeologists use *Sporormiella* and other coprophilous fungi as markers

72 of pastoral and other human land use activities across Europe, Africa and Asia (Ahlborn et al.

73 2015; Felauer et al. 2012; Ghosh et al. 2017; Lehmkuhl et al. 2011; Miehe et al. 2009;

74 Shumilovskikh et al. 2016a, 2016b, 2017; Szymanski 2017; van Geel et al. 2003). Coprophilous

75 ascomycetes other than *Sporormiella* have also been verified to reflect pastoral activities in

76 mountainous, pasture-woodland landscapes (Cugny et al. 2010), in upland grasslands and bogs

77 (Feeser and O'Connell 2010) and in boreal forest (Kamerling et al. 2017). Evidence of direct

78 domestication of herbivores in the pre-Columbian Americas is rare. However, dung fungi could

79 potentially be used to provide more information about communal hunting in the Great Basin

80 region of North America, where pronghorn and mountain sheep were rounded up and potentially

81 kept in pens constructed of stone and brush (Hockett 2005; Hockett and Murphey 2009).

82 **3. Opportunities and Limitations**

83 3.1 Dung Fungus Reproduction

84   Interpreting the abundance of spores of coprophilous fungi in a sedimentary record

85 requires an understanding of the different factors influencing fungal reproduction. *Sporormiella*

86 is strongly coprophilous and is observed almost entirely in association with herbivore dung

87 (Doveri 2007: 613). Gelorini et al. (2011) emphasized that only genera that are obligate to

88 herbivore dung, such as *Sporormiella* and *Podospora*, could serve as a reliable signal of

89 herbivore presence. However, the precise lifecycle and substrate preferences remain ambiguous

90 for even the most commonly noted spores of coprophilous fungi (Table 1). *Apiosordaria* and

91 *Coniochaeta* are two taxa that are often taken to indicate herbivore presence but have recently

92   been found to be poor indicators of herbivore abundance (Doyen and Etienne 2017). This is not

93   surprising, since the mycological literature indicates they primarily grow in soil (Bell 1983: 33;

94   Doveri 2007: 760; Krug et al. 1983; Guarro et al. 2012: 47-51, 118). Other commonly observed

95   semi-coprophilous ascomycetes such as *Cercophora* and *Sordaria* are also found on other

96   organic substrates, such as plant debris, decaying wood, or soil, with some frequency (Bell 1983:

97   36, 40; Doveri 2007: 826, 847; Guarro et al. 2012: 111, 383; Hanlin 1990; Kruys and Wedin

98   2009). Newcombe et al. (2016) found evidence that *Sordaria*, *Preussia*, and even *Sporormiella*

99   may be epiphytic and concluded that the presence of these spores is not undisputable evidence of

100   herbivore abundance. Because herbivore dung consists largely of partly digested plant remains,

101   the ability of some dung fungi to opportunistically grow on plants is not surprising. However,

102   coprophilous fungal taxa *Sporormiella* and *Podospora*, as well as a number of less common taxa

103   such as *Ascodesmis, Arnium, Bombardioidea, Delitschia* and *Trichodelitshia*, have a strong

104   preference for dung as a substrate and therefore typically reflect the presence of large herbivores.

105   Despite its strong preference for dung as a substrate, caution still must be applied when

106   observing *Sporormiella* in sediment samples, as *Sporormiella* spores can be indistinguishable

107   from the spores of *Preussia* (Barr 2000; Cain 1961; Kruys and Wedin 2009). Though these two

108   fungi are closely related, only *Sporormiella* depends on dung as a growth substrate (Von Arx and

109   Van der Aa 1987).

110   Even if spores of coprophilous fungi may not necessarily have to pass through the gut of

111   an herbivore to complete reproduction, many fungal spores are consumed and move through the

112   digestive system. After consumption by an herbivore, spores of coprophilous fungi are expelled

113   with the dung after which they germinate and propel spores away from the dung. The spores

114   typically adhere to nearby vegetation and are inadvertently consumed along with the vegetation.

115   After passing through the digestive tract, the spores are expelled along with the dung to complete

116   the lifecycle again (Figure 1).

GENUS	SUBSTRATE
Ascodesmis	Primarily herbivore and carnivore dung; occasionally soil or decaying vegetation (Doveri 2007: 492; Guarro et al. 2012: 79)
Apiosordaria	Primarily soil; occasionally dung (Krug et al. 1983; Guarro et al. 2012: 47-51)
Arnium	Primarily dung; occasionally soil (Bell 1983: 46; Doveri 2007: 872; Guarro et al. 2012: 59)
Bombardioidea	Exclusively dung (Bell 1983: 49; Doveri 2007: 870)
Cercophora	Primarily decaying wood and vegetation; occasionally charcoal, soil and dung (Bell 1983: 40; Doveri 2007: 847; Hanlin 1990: 46-47; Guarro et al. 2012: 111)
Chaetomium	Primarily decaying vegetation; also dung, soil, and a range of other organic substrates (Bell 1983: 33; Doveri 2007: 760; Guarro et al. 2012: 118)
Coniochaeta	Primarily soil; also dung and decaying wood (Bell 1983: 39; Hanlin 1990; Doveri 2007: 810; Guarro et al. 2012: 132-142)
Delitschia	Almost exclusively herbivore dung; occasionally soil and decaying wood (Bell 1983: 51; Guarro et al. 2012: 159)
Podospora	Almost exclusively herbivore dung; occasionally soil (Bell 1983: 14; Doveri 2007: 905; Guarro et al. 2012: 340; Schlütz and Shumilovskikh (2017)
Sordaria	Almost exclusively herbivore and omnivore dung; occasionally soil or vegetation (Bell 1983: 36; Doveri 2007: 826; Guarro et al. 2012: 383)
Sporormiella	Mostly (75%) herbivore dung; occasionally decaying wood or soil (Doveri 2007: 613); NB closely similar to the soil-inhabiting genus *Preussia*
Trichodelitschia	Exclusively dung (Bell 1983: 51)

Table 1. Substrates of commonly encountered spores of coprophilous fungi in sedimentary sequences

117   Extensive research exists on the fungal community composition of different types of

118   herbivore dung (e.g. Ebersohn and Eicker 1992; Mungai et al. 2011, 2012; Nyberg and Persson

119   2002; Piontelli et al. 1981; Richardson 2001; Van Asperen 2017; Wicklow et al. 1980). Often, a

120   few species are abundant, alongside a large number of rare species (Ebersohn and Eicker 1992;

121   Krug et al. 2004; Richardson 2001; Nyberg and Persson 2002). Most coprophilous fungi occur

122   on a wide range of dung types (Angel and Wicklow 1983; Richardson 1972, 2001), but many

123   genera show a preference for certain types of dung. While these genera also occur on other dung

124   types, they occur more often and more abundantly on their preferred dung type (Bell 2005;

125   Lundqvist 1972; Richardson 1972, 2001; Van Asperen 2017).

126   Spores of coprophilous fungi are typically a very local indicator of herbivore dung (Graf

127   and Chmura 2006) due to their short dispersal distances (Ingold 1961; Ingold and Hadland 1959;

128   Trail 2007; Yafetto et al. 2008). They can become airborne, but are likely deposited within 100

129   meters of the dung source (Gill et al. 2013). However, it is possible to get a more regional

130   assemblage of dung fungi if water is present because spores can enter a river, pond, or lake via

131   slopewash. The spores tend to settle out fairly rapidly (Raczka et al. 2016), so spore

132   concentration declines toward the center of lakes and ponds (Raper and Bush 2009). This

133   discrepancy could be addressed by analyzing multiple cores from various locations within the

134   same site. Overall, spores within smaller bodies of water are more likely to reflect herbivore

135   abundance (Johnson et al. 2015).

136   3.2 Environmental factors

137   Dung fungi have species or genera-specific responses to microenvironmental factors that

138   affect the success of reproduction (Dix and Webster 1995). These species-specific responses to

139   environmental changes could potentially encourage or limit growth of a particular fungal type,

140   which leads to a fluctuation in spore abundance that is not actually representative of megafaunal

141   activity. In part, this issue can be ameliorated by completing a comprehensive palynological

142   study, including multiple coprophilous fungal taxa.

143   Presence, abundance and succession of specific fungal genera and species on dung

144   incubated in the laboratory is known to differ from that on dung in field conditions (Angel and

145   Wicklow 1983; Harper and Webster 1964; Richardson 2001). Laboratory conditions are highly

146   artificial, with relatively constant temperatures and humidity. In contrast, under field conditions,

147   temperatures are generally lower and display daily fluctuations, and waterlogging and

148   precipitation vary in frequency and intensity. Although the growth of dung fungi in the

149   laboratory cannot be used as a direct analog to fungal growth in nature, research suggests that

150   coprophilous ascomycetes are not as successful when temperature or relative humidity becomes

151   too high or too low (Asina et al. 1977; Kuthubutheen and Webster 1986a). The effects of low

152   relative humidity can be compounded by competition between ascomycetes (Kuthubutheen and

153   Webster 1986b). Soil hydrology is also likely to affect spore reproduction (Wood and

154   Wilmshurst 2013).

155   Although at varying levels of success, spores can often germinate, and the resulting

156   mycelium and fruit bodies grow, across a wide range of temperatures. For example, Asina et al.

157   (1977) found that three *Sporormiella* species could germinate at temperatures within a range of

158   10-30°C., although the range of temperatures at which germination was maximal was smaller (a

159   range of 5-15 degrees). Dung fungal development is generally slower at lower temperatures, and

160   although the abundant genera tend to be present across a range of temperatures, they produce

161   fewer fruitbodies at lower temperatures (Krug et al. 2004; Wicklow and Moore 1974). However,

162   dung could provide warmer conditions than are prevalent in the surrounding environment as long

163   as decomposing organisms are still capable of growth (Lundqvist 1972; Webster 1970).

164   Most dung fungi germinate, grow and produce fruitbodies more slowly when water

165   availability is low, and fruit for a shorter period of time, although some species produce

166   fruitbodies more quickly (Dickinson and Underhay 1977; Harrower and Nagy 1979;

167   Kuthubutheen and Webster 1986a, 1986b). Dickinson and Underhay (1977) suggest that the

168   rapid decline in water content common in the warm and/or dry season soon inhibits fungal

169   growth, whereas in the cold and/or wet season, growth may be limited or slowed by high water

170   content. Kuthubutheen and Webster (1986b) found that *Sporormiella* was the most tolerant of

171   low water availability of the genera they tested, which included *Podospora*. The interaction

172   between the effects of temperature and moisture availability on dung fungal growth leads to

173   higher dung fungal diversity during the wetter, cooler season in temperate latitudes than in the

174   warmer, drier season (Krug et al. 2004; Wicklow 1992; Richardson 2001; Van Asperen 2017).

175   Although winter temperatures are lower, this temperature drop presents a stress factor which may

176   have the effect of reducing the reproductive fitness of dominant species, thereby releasing less-

177   specialized, more stress-tolerant species. In contrast, in summer the primary factor is the lower

178   substrate humidity negatively affecting germination rate (Harrower and Nagy 1979;

179   Kuthubutheen and Webster 1986a, 1986b).

180   Dung type and animal behavior may also affect the durability of the dung resource. Some

181   animals defecate in latrines to which they return regularly, leading to large accumulations of

182   dung material. Larger dung pats are less susceptible to desiccation, while clusters of pellets

183   create a wider range of microhabitats but are more prone to desiccation (Beynon 2012). Salt from

184   urine may also inhibit sporulation (Schlütz and Shumilovskikh 2017). Furthermore, dietary

185   diversity and the quality of the vegetation consumed also leads to dung with different

186   characteristics. For example, moisture and nitrogen content of dung of a range of herbivores in

187   South Africa was found to be correlated with the amount of precipitation in the 2-6 weeks before

188   sample collection (Edwards 1991). Dung from cattle feeding on the new growth of grass in

189   spring in temperate climates generally has a higher moisture content than later in the growth

190   season (Greenham 1972; Van Asperen, pers. obs.).

191   Another factor that may reduce the number of fruitbodies produced and the duration of

192   fruiting is competition between ascomycetes (Lussenhop & Wicklow 1985; Kuthubutheen and

193   Webster 1986b). *Sporormiella* species often appear relatively late in the incubation period

194   (Angel and Wicklow 1983), so perhaps they are more easily outcompeted by genera that appear

195   earlier when environmental factors favor those genera.

196   In addition to direct effects of environmental factors, in temperate latitudes the activity of

197   other dung-inhabiting species, in particular dung beetles (both adults and larvae) and fly larvae,

198   is much higher in wet and warm conditions than in dry or cool conditions (Davis 1994). In North

199   Carolina, beetle activity greatly diminished at temperatures below 10°C, as well as in dry, hot

200   conditions or very wet spells (Bertone et al. 2005). Further north, in the more continental

201   climates of Alberta and Michigan, beetle activity extended from early March to late November,

202   with the main period of activity ranging from May to July (Floate & Gill 1998; Kadiri et al.,

203   2014; Wassmer 2014).

204   Besides potential direct consumption of dung fungi, the grazing activity of these insects

205   has several adverse effects on dung fungal growth: it reduces the amount of dung available for

206   growth, it disrupts fungal hyphae, and it fragments the dung (Lussenhop et al. 1980; Wicklow &

207   Yocom 1982). Fragmentation makes the dung more susceptible to moisture loss, and also

208   removes the competitive advantage of fungal hyphal growth compared to bacterial growth

209   (Lussenhop et al. 1980). Lussenhop et al. (1980) found that the presence of dung beetles reduced

210   dung fungal hyphal density, especially at lower moisture content, but this did not lead to lower

211   rates of fruiting and increased dung fungal diversity, possibly by dispersing the fungi more

212   widely. Wicklow & Yocom (1982) observed that the presence of fly larvae reduced the species

213   diversity of dung fungi. However, they note that *Sporormiella* abundance was not significantly

214   affected, whereas there was a small negative effect on *Podospora* and *Sordaria*. In another study,

215   species diversity was not affected, but there was a highly significant 68% reduction in spore

216   production in the presence of fly larvae (Lussenhop & Wicklow 1985). A study in a savanna

217   environment in Nigeria found that during the dry season, cattle dung was broken down by insects

218   (primarily termites), whereas in the wet season, when termites were absent, dung breakdown was

219   almost entirely due to fungal activity (Omaliko 1981).

220   3.3 Incorporation of fungal spores into sedimentary records

221   Local hydrology has the potential to produce fluctuations in a spore record that are not

222   representative of herbivore abundance at the site. Wood and Wilmshurst (2012) demonstrated

223   that spore fluctuations can correlate with changes in local hydrology. However, these

224   correlations were not consistent. Two bogs demonstrated an increase in *Sporormiella* when water

225   levels were at their peak, while one bog exhibited an increased in *Sporormiella* while water

226   levels were lower than usual. Because the apparent correlations are not consistent between the

227   sites, it is possible that the changes in local hydrology could be affecting herbivore behavior.

228   Depending on local conditions, animals could be preferentially utilizing the site based on water

229   availability, or in contrast may be avoiding it when water levels are too high due to decreased

230   ground stability. Similarly, Ponel et al. (2011) interpreted the coprophilous fungi record at a site

231 in the French Alps to reflect taphonomic processes. Due to hydrological factors, spores of

232 coprophilous fungi were almost completely absent in the lower, lacustrine part of the record,

233 whilst the presence of dung was attested by an abundance of dung beetle remains. In contrast,

234 spores of coprophilous fungi became more abundant and dung beetles declined as the lake

235 infilled and transitioned into a peat bog, as herbivores were able to graze within the bog.

236   Rainfall and the degree of storminess at a site can also affect a dung fungal record.

237 Spores can be flushed into waterways, and if the fecal material is washed away soon after it is

238 deposited, the fungus may not have sufficient time to reproduce and deposit higher numbers of

239 fungal spores into a record. Finally, high energy depositional environments could potentially

240 transport spores further than lower energy systems. In these types of environments, the fungal

241 spore record could be more regional than expected, given the local nature of spore deposition.

242 However, to date there has been little research into the role of these factors in the deposition of

243 spores of coprophilous fungi in sedimentary records.

244 3.4 Laboratory Recovery

245   Spores of coprophilous fungi and other non-pollen palynomorphs are generally extracted

246 from palaeoecological samples along with pollen, usually using the 'standard' pollen preparation

247 method (Faegri and Iversen 1989; Moore et al. 1991). However, a range of alternative

248 preparation techniques are also available. Several studies have tested the effect of a number of

249 chemicals and preparation techniques on the survival and preservation of fungal remains.

250   Clarke (1994) processed samples from a variety of substrates with three different

251 techniques:

13

252   A. boiling in KOH (potassium hydroxide), sieving (150 μm mesh), HF (hydrofluoric

253   acid), acetolysis, mounting using TBA (tertiary butyl alcohol) (similar to the 'standard'

254   pollen preparation method; Faegri and Iversen 1989; Moore et al. 1991);

255   B. boiling in NH_4OH (ammonium hydroxide), sieving (150 and 10 μm mesh), swirling,

256   mounting using TBA;

257   C. boiling in KOH, sieving (150 μm mesh), heavy liquid separation with $ZnCl_2$ (zinc

258   chloride), mounting using TBA.

259 Her results indicated that small round to oval fungal spores behave in a similar way as pollen in

260 terms of survival. Treatment A led to a loss of large, buoyant forms, whilst these were the only

261 forms consistently present in samples treated with method B. Thick-walled forms were lost in

262 treatment C. None of the treatments led to significant preservation issues.

263   In a study focusing specifically on spores of coprophilous fungi recovered from dung

264 samples incubated in the lab, Van Asperen et al. (2016) tested five preparation methods:

265   A. boiling in NaOH (sodium hydroxide), sieving (125 and 6 μm mesh), treatment with

266   HCl (hydrochloric acid), acetolysis, mounting using TBA.

267   B1. boiling in NaOH, sieving, treatment with HCl, mounting using TBA.

268   B2. boiling in KOH, sieving, treatment with HCl, mounting using TBA.

269   C. boiling in KOH, sieving, density separation by swirling, treatment with HCl, mounting

270   using TBA.

271   D. sieving, mounting using TBA.

272 This allowed them to tease out the effects of the different chemicals used in standard pollen

273 preparation procedures on coprophilous fungi recovery and preservation. The use of corrosive

274 chemicals, such as NaOH, KOH and acetolysis, led to a significant loss of hyaline spores (e.g.

275 *Cheilymenia*, *Coprotus*, *Iodophanus*, *Peziza* and immature *Cercophora* spores), as well as spores

276 that lose their epispores over time (e.g. *Ascobolus* and *Saccobolus*). Such spores are unlikely to

277 be preserved in sediments, but since these spores dominate certain dung types (Lundqvist 1972;

278 Richardson 1972), this can significantly bias spore counts. Hyaline appendages were also lost

279 (*Sordaria* and *Cercophora/Podospora*). Spores with thicker, pigmented spore walls (e.g.

280 *Sordaria*, *Sporormiella* and *Cercophora/Podospora*, as well as basidiomycete spores) were more

281 resistant to chemical degradation. *Sordaria* spores deteriorated and *Sporormiella* spores tended

282 to swell when acetolysis was used. All spores that were large enough to be retained in the mesh

283 were recovered when samples were sieved but not submitted to any other treatment. This

284 includes the vulnerable spores that were lost when chemicals were used. Spores small enough to

285 pass through the mesh (e.g. small basidiomycete spores and single cells of *Saccobolus*) were lost,

286 which is significant considering that *Sporormiella* spores often break up into their constituent

287 cells (Ahmed and Cain 1972). The single cells of some *Sporormiella* species are so small that

288 they would not be retained in a 10 or 6 μm mesh, leading to a potential loss of information.

289   Other alternative pollen preparation techniques are also available, although these have not

290 been tested explicitly on spores of coprophilous fungi. Riding and Kyffin-Hughes (2004) used a

291 treatment with sodium hexametaphosphate followed by density separation by means of swirling

292 and centrifuging. With this simple method, they achieved equal or better palynomorph recovery

293 than with the standard preparation method for most lithologies, although they did not test their

294 method on sediments high in organic material.

295   Given the clear adverse effect of some of the chemicals used in standard pollen

296 preparation methods on coprophilous fungi recovery and preservation, it is highly advisable to

297   test and use alternative, non-chemical, techniques wherever possible (cf. Van Asperen et al.

298   2016).

299   3.5 Analysis and Quantification

300   Typically, spores of coprophilous fungi are counted alongside pollen. In some

301   environments, however, it may be beneficial to count spores in relation to tracer spores. Etienne

302   and Jouffrey-Bapicot (2014) suggest counting 300-350 *Lycopodium* tracer spores to obtain an

303   accurate evaluation of *Sporormiella* in a sample, but the number of tracer spores and sample

304   sizes in this study were not reported. The amount of tracer spores counted should depend on the

305   environment, the size of the sample, the concentration of pollen within a sample, and how many

306   tracer spores are added.

307   Examining pollen samples for spores of coprophilous fungi using different approaches

308   can produce discrepancy in results between sites. The threshold for "background" levels of

309   spores of coprophilous fungi has been consistently suggested to be below 2% of the total pollen

310   assemblage various modern environments (Baker et al. 2016; Gill et al. 2013; Raczka et al.

311   2016). It was first suggested by Davis (1987), in his work in (mostly arid) western North

312   America, that functional extinction of megaherbivores can be observed when fungal spores fall

313   below 2% of the total pollen assemblage (TPA). However, it is not likely that this threshold can

314   truly be extrapolated between sites and environments. In the southeastern United States, for

315   example, two different methods for quantifying spores of coprophilous fungi may have yielded

316   differing results. To our knowledge, recent research at Cupola Pond, Missouri (Jones et al. 2017)

317   and Page-Ladson, Florida (Perrotti 2018) are the only two published palynological studies that

318   attempt to incorporate spores of coprophilous fungi within the analysis from that region. These

319   sites are similar in age and both occur within spring-fed ponds in areas with carbonate rich

320   bedrock. In contrast to Page-Ladson, no evidence of *Sporormiella* spores were found in pollen

321   samples at Cupola Pond, leading Jones et al. (2017) to conclude that herbivory was not a key

322   factor in ecosystem regulation around the site. The discrepancy between the observation of dung

323   fungal at Cupola Pond and Page-Ladson deserves further exploration.

324   Though late Pleistocene vegetation at the two locations does differ, the inconsistency in

325   fungal spore records may have resulted from differences in noting and quantifying spores of

326   coprophilous fungi. Perrotti et al. (2018) counted spores of coprophilous fungi separately from

327   pollen rather than noting them only when encountered during pollen counting. Most North

328   American coprophilous fungi studies, including that by Jones et al. (2017), have used the latter

329   method (Davis 1987; Davis and Shafer 2006; Gill et al. 2009). At Page-Ladson, Florida,

330   *Sporormiella* spores never constitute more than 2% of the TPA (Perrotti 2018) due to a high

331   concentration of arboreal pollen at the site. If *Sporormiella* was tallied as a percentage of that

332   total pollen assemblage, spores of coprophilous fungi would have been far less represented than

333   those from arid environments as in the desert west, where this method was first conceived.

334   Pollen concentrations at Cupola Pond (Jones et al. 2017) are similar to those at Page-Ladson,

335   suggesting that a different method of searching for spores of coprophilous fungi may have

336   resulted in the recovery of more spores.

337   Analyzing and quantifying spores of coprophilous fungi as %TPA can also produce

338   fluctuations within a spore record that are not representative of herbivore abundance. This

339   method is vulnerable to fluctuations in pollen accumulation rates. In sediments with high pollen

340   concentrations, fungal spores are often represented by lower %TPA values. Parker and Williams

341   (2012) found a negative relationship between mean annual precipitation and spore abundance in

342   lake-center sediments that they attributed to a higher influx of arboreal pollen during wet years,

343   which would drive down relative *Sporormiella* abundance. Wood and Wilmshurst (2013)

344   confirm that *Sporormiella* as %TPA is subject to fluctuations in pollen accumulation that may

345   skew spore data, even when the spores are being consistently deposited. Moreover, in contrast to

346   the sporopollenin walls of pollen grains, the cell walls of fungal spores are composed mainly of

347   chitin and glucans (Deacon 2006), and therefore will degrade in response to different

348   environmental factors.

349   Furthermore, expressing fungal spore presence as %TPA can mask low spore counts,

350   making it difficult to assess the reliability of the conclusions drawn from such percentages. In

351   pollen analyses, it is common, for instance, to count up to 200-400 pollen grains. In such

352   assemblages, the 2% TPA "background" level of fungal spore presence translates into 4-8 spores

353   counted, with each 1% increase representing 2-4 extra spores counted. In most cases, even where

354   herbivores are present, the coprophilous fungi counts are not higher than 10%TPA, with counts

355   for most Pleistocene (predating megafaunal extinctions) samples below 5% (e.g. Davis and

356   Shafer 2006; Parker and Williams 2012; Gill et al. 2013; Johnson et al. 2016). Such low spore

357   count fluctuations are unlikely to be statistically significant and should be interpreted with

358   caution.

359   Whenever possible, reporting coprophilous fungi abundance using both accumulation or

360   concentration and %TPA could be beneficial. Total pollen production will vary greatly between

361   different ecosystems and fungal spores and pollen have differing reproductive strategies and cell

362   wall compositions, so future studies should avoid quantifying spores solely as %TPA.

363   Calculating spore accumulation requires a well-dated core but can be a useful illustration of how

364   changes in sediment deposition at a site can affect the accumulation of fungal spores (Figure 2).

365   3.6 Current Limitations

366   Typically, North American researchers focus on *Sporormiella* as an indicator of

367   megaherbivore abundance without including other coprophilous fungi. Perhaps due to its distinct

368   morphology or presence in many North American pollen samples, it has become the sole taxon

369   reported in many studies (Gill et al. 2009, 2014; Halligan et al. 2016; Perrotti 2018). Though

370   *Sporormiella* alone has been used as a proxy for herbivore abundance, it is becoming more

371   apparent that noting other coprophilous fungi can increase the robustness of interpretations

372   regarding megaherbivore abundance.

373   Johnson et al. (2015) found that the commonly encountered coprophilous and semi-

374   coprophilous ascomycetes *Cercophora, Coniochaeta, Podospora,* and *Sordaria* contributed to a

375   better overall understanding of dung fungal abundance. In addition, Van Asperen (2017) notes

376   that *Sporormiella* can be rare on the dung of some modern large herbivores whilst other

377   coprophilous fungi are found in abundance. Therefore, a lack of *Sporormiella* in sediments does

378   not always indicate that herbivores were not present at the site, and as a consequence, counting

379   *Sporormiella* only may prevent the recognition of herbivore presence (e.g. Jones et al. 2017).

380   Incorporating all dung fungal counts better indicates herbivore presence and abundance (Baker et

381   al. 2016; Johnson et al. 2015; Van Asperen et al. 2016, 2017), but the dependence on dung of

382   each taxon must be considered (Table 1).

383   In addition to a lack of herbivore activity, an absence of dung fungi at a site may be the

384   result of a number of factors. First, as previously discussed, little is known about the

385   environmental preferences of each dung fungus and the effect of varying environmental

386   conditions on dung fungal reproduction and dispersal. More research is needed on the topic.

387   Second, most dung fungi inhabit many different types of herbivore dung, with many genera also

388   utilizing other substrates. It is possible that noting particular species of fungi that are associated

389   with dung of specific animal species could contribute to the understanding of extinctions.

390   However, species-level dung fungus identification relies on the fruiting body in addition to the

391   spores. Though van Geel et al. (2011) recovered complete fruiting bodies from inside a

392   mammoth dung ball, fruiting bodies are rarely preserved in sediment samples and would

393   typically be destroyed during laboratory procedures. Third, little is known about how spores

394   preserve. It is widely assumed that they are more durable than pollen because of their thick,

395   chitinous walls and frequent presence in pollen samples exhibiting poor pollen preservation.

396   Spores of many fungi can remain in soil for extended periods in a dormant state (Lockwood

397   1977; Deacon 2006). Fourth, because spores of coprophilous fungi typically represent a very

398   local proxy due to their limited dispersal distances, it likely not possible to draw any conclusions

399   regarding the demise of wide-ranging megafauna from spore abundance in one location alone.

400   Finally, hydrological factors also influence where spores, if present in the environment,

401   ultimately enter the palaeoecological record. For example, spores of coprophilous fungi records

402   from cores taken in lakes at different distances from the shoreline (Raper and Bush 2009) or

403   from the nearest stream discharge (Etienne et al. 2013) show different relationships to animal

404   abundance in the area around the lake.

405   Because of the factors discussed above, it is possible that an absence of dung fungi from

406   a palaeoecological record may not always indicate a decline in herbivores. However, by

407   understanding the past environment, particularly in regard to hydrologic factors, more weight can

408   be placed on the interpretation of the abundance or lack of spores of coprophilous fungi. Overall,

409   the effects of these factors on spore records may be minimized with the incorporation of multiple

410   fungal taxa. Ultimately, spores of coprophilous fungi alone likely cannot be used to infer an

411   absence of herbivores. When using spores to infer extinction or regional disappearance of large

412   herbivores, it is best practice to utilize this proxy alongside other lines of evidence, such as

413   faunal remains or macrofossils from herbivore dung (i.e. Halligan et al. 2016; Perrotti 2018).

414   **4. Future Directions and Conclusions**

415   The methods for analysis and research of analogs for the interpretation of dung fungal

416   records are improving, but additional research is still needed. First, more modern experiments

417   need to be conducted to understand the relationship between dung fungal abundance, herd size,

418   and other geographical and environmental factors. Much research has been devoted to this topic

419   recently and studies have established correlations between cattle (Wood and Wilmhurst 2012)

420   and bison (Gill et al. 2013) herd size and *Sporormiella* abundance. Baker et al.'s (2016)

421   informative study conducted in The Netherlands was an excellent demonstration of the

422   correlation between spore accumulation, taphonomic processes and herd size, but more studies

423   incorporating a wider variety of large herbivores would be valuable. Because spore reproduction

424   and deposition differ between environments, a wider array of modern environments should also

425   be explored. If a clearer correlation between coprophilous fungi abundance and herbivore

426   diversity and abundance could be established, dung fungal data could be incorporated into

427   dynamic vegetation models, strengthening our interpretation of the effects of grazing on

428   vegetation systems.

429   Second, still more research is needed on laboratory recovery and identification of spores

430   of coprophilous fungi. Certain laboratory recovery procedures can alter the size of dung fungi,

431   further inhibiting species identification (van Asperen et al. 2016). Minimizing harsh chemical

432   extraction procedures could be made possible by the implementation of techniques such as

433   sonication-assisted sieving through <5 micron mesh (i.e. Perrotti et al. 2018). Regardless, more

434   research is needed on the effects of standard palynological procedures on the recovery of spores

435   of coprophilous fungi. By developing more standardized processing and extraction, comparisons

436   between studies would be made easier.

437   Although additional research is still needed before researchers can fully rely on the

438   application of dung fungi to questions raised in archaeology, we believe that this type of research

439   has proven its potential as a valuable tool for understanding past herbivore abundances.

440   Thorough consideration of the limitations of the method, particularly through engagement with

441   the mycological literature, as well as through carefully designed actualistic experiments, greatly

442   increases the reliability and applicability of dung fungal data to archaeology.

443

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Figure and Table Captions

Fig. 1 A generalized depiction of the lifecycle of *Sporormiella*. The spores are inadvertently consumed and pass through the herbivore's gut and are then discharged as a part of the feces. Although some spores may be carried away by water or work their way into terrestrial sediments, many germinate in the feces and the mycelium produces the perithecia (or fruiting bodoies). These perithecia contain the individual ascospores. Figure courtesy of Chase W. Beck.

Fig. 2 *Sporormiella* data from Page-Ladson, Florida (Halligan et al. 2016; Perrotti 2018). Spore accumulation is the most accurate way to understand coprophilous fungi abundance in a sedimentary record.

Fig. 3 Commonly encountered coprophilous and semi-coprophilous fungi. a) *Arnium sp.* (from modern context-pollen trap); b) *Arnium imitans* (from modern context-pollen trap); c) *Apiosordaria sp.* (from modern context-pollen trap); d) *Cercophora sp.* (from Pleistocene sediment) e) *Coniochaeta sp.* from Pleistocene sediment); f) *Podosopora sp.* (from Pleistocene sediment); g) *Sordaria sp.* (from modern context-directly from dung); h;i) *Sporormiella sp.* (from Pleistocene sediment); j) *Trichodelitschia sp.* (from modern context-soil).
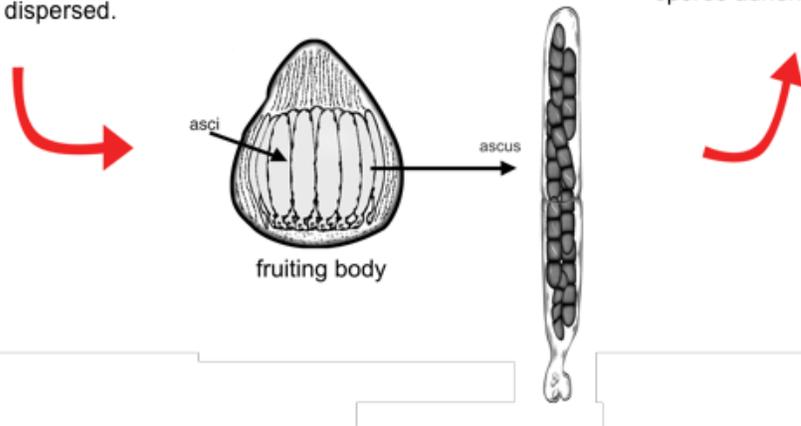
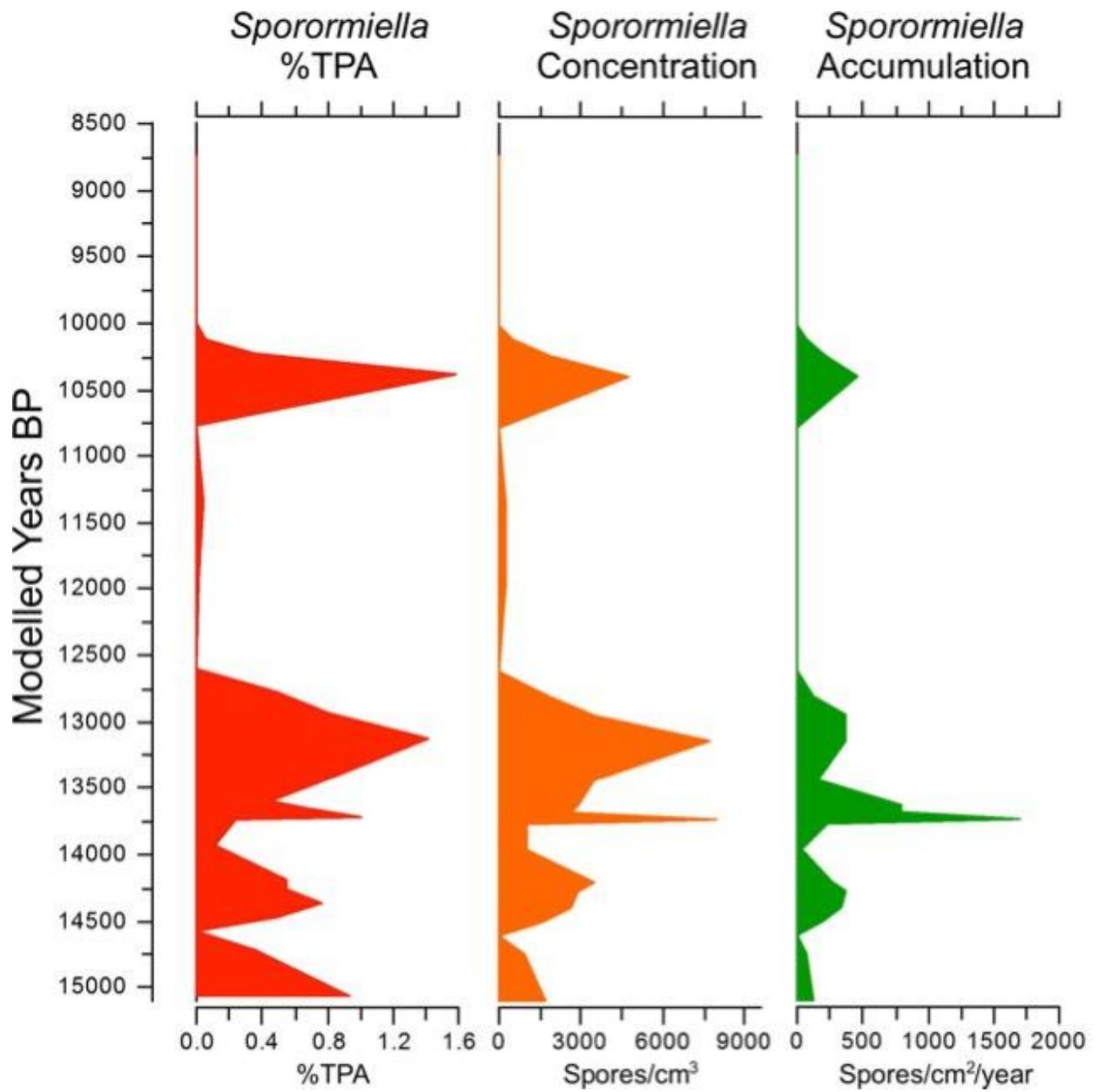**Table 2.** Substrates of commonly encountered spores of coprophilous fungi in sedimentary sequences.
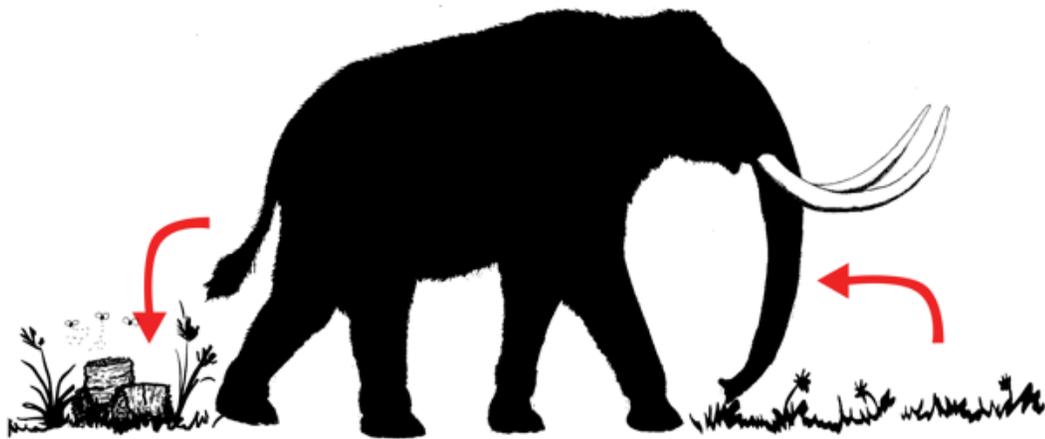
Animal excretes spores in feces.
Spores germinate, produce fruiting bodies,
and spores are dispersed.

Animal inadvertently eats fungal
spores adhering to vegetation

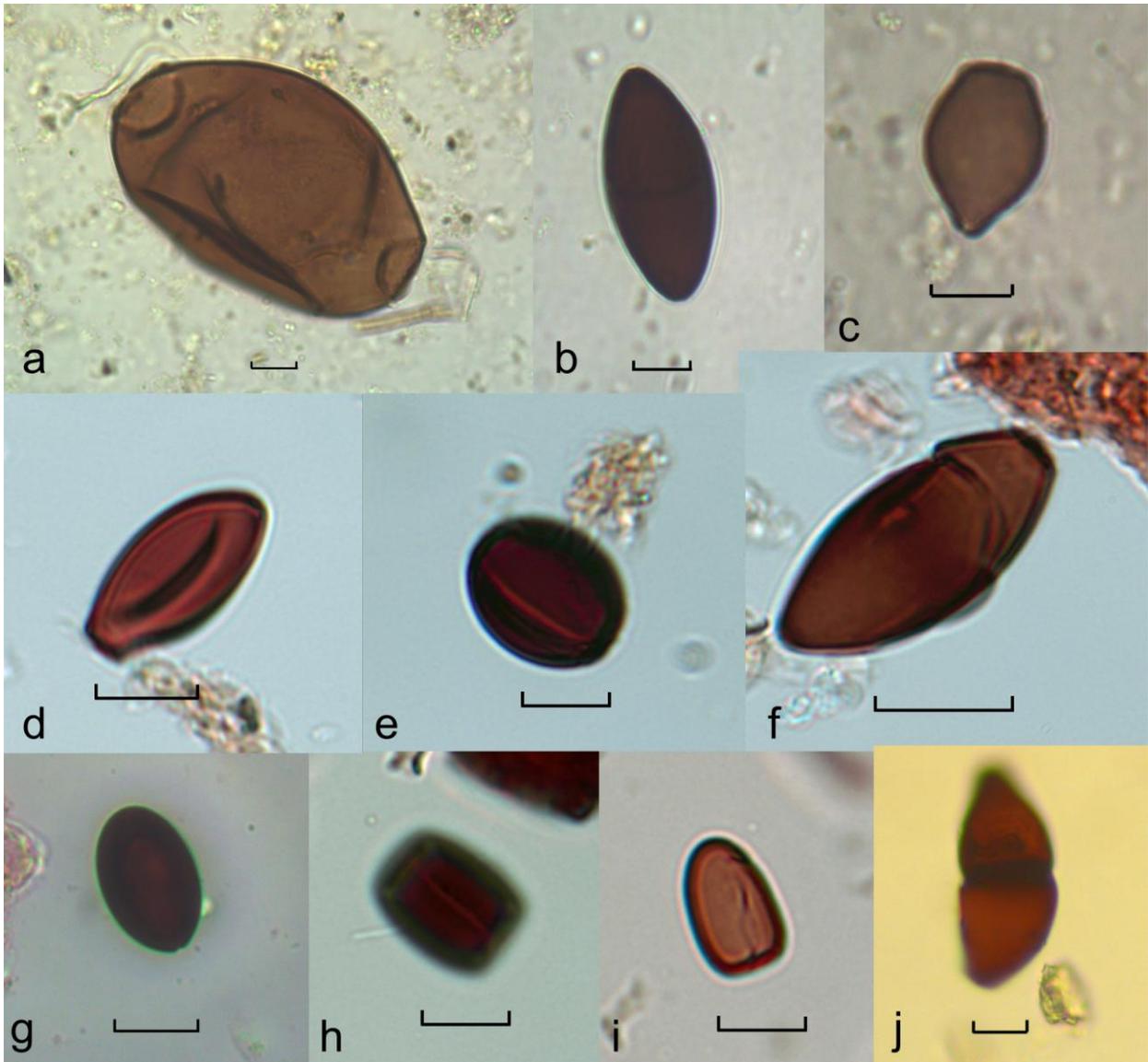asci

ascus

fruiting body

782

783   Figure 1

784

785   Figure 2

786

Figure 3

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