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Short communication

Pandora bullata (Entomophthoromycota: Entomophthorales) affecting calliphorid flies in central Brazil

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ABSTRACT

Fungi are where one finds them, and if one seeks fungal pathogens affecting flies, then a garbage dump may be an ideal place to find both persistent, abundant fly populations and their fungal pathogens. An obvious fungal epizootic affecting the oriental latrine fly, *Chrysomya megacephala* (Diptera: Calliphoridae), was observed over several days in mid-February 2015 at the local garbage dump adjacent to the city of Cavalcante, northern Goiás. This site harbored large populations of both *C. megacephala* and a *Musca* sp. (Diptera: Muscidae) but only the population of oriental latrine fly was affected by any fungal pathogen and presented unusually dense populations of fresh cadavers. The fungus was identifiable as *Pandora bullata* (Entomophthorales: Entomophthoraceae) only after a very small number of characteristically decorated resting spores were found in these flies two months later; this represents the first Brazilian (and South American) record of this species. *P. bullata* is known previously from a small number of North American, European and Australian collections, all of which have included relatively abundant production of resting spores. We cannot dismiss the possibility that the extremely sparse formation of resting spores at this Brazilian site may be due to abiotic factors such as latitude (13°46′40.53″S), day length, ambient temperatures, or even the precipitation patterns in this mid-tropical montane site. Epizootic events affecting calliphorids in Brazil strengthen the interest in entomophthoran pathogens for biological control of flies.

1. Introduction

Blowflies (Diptera: Calliphoridae) play a crucial role in the balance of land ecosystems, however they are also responsible for great damage through the traumatic myiases that their larvae cause when burrowing the skin of domestic and wild animals, and even humans (Guimarães et al., 1982; Sukontason et al., 2005; Fernandes et al., 2009; Mello et al., 2009; Kuria et al., 2015). Among the many calliphorids affecting human and animal populations, the oriental latrine fly, *Chrysomya megacephala*, feeds and breeds in carrion, feces, and is an important indicator species in forensic entomology (Goff, 2001) because of its ready infestation of animal cadavers and carrion. Maggots of this species can, however, also serve positively in the treatment of cuticular wounds and lesions (Pinheiro et al., 2015). *C. megacephala* can carry and transfer the poliovirus, salmonellae, and other enteric pathogens among humans living under substandard conditions of sanitation and nutrition (Baumgartner and Greenberg, 1984; Olsen et al., 1993; Sukontason et al., 2007), as well as transferring helminth parasites to humans and poultry (Sulaiman et al., 1989; Avancini and Ueta, 1990; Monzon et al., 1991).

What little is yet known on the biodiversity of entomopathogenic fungi in Brazil (Sosa-Gómez et al., 2010) comes primarily from agricultural sites. The control of free-flying insects with entomopathogenic fungi has not been very widely considered because of the difficulty of applying the pathogen to the insects (Wright et al., 2004). Nevertheless, the development of trap-and-target technology, in which visual and semiochemical attractants are used to draw insects to a point source, makes such applications a realistic possibility (Muirhead-Thomson, 1991), and there can be no doubt that the populations of noxious and deleterious flies is a major concern that has received too little scientific scrutiny.

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The diversity of entomophthoran fungi in Brazil is vastly less well documented than the more numerous and taxonomically diverse entomopathogenic conidial and teleomorphic ascomycetes from the order Hypocreales. Not surprisingly, extremely little is known about the diversity of entomophthoran fungi from hosts in nonagricultural sites in Brazil (or most other parts of the world).

As a result of our seeking fungal pathogens of dipterans at a distinctly nonagricultural, fly-infested garbage dump in central Brazil, we are able here to report the first South American record of the entomophthoran fungus *Pandora bullata* (Thaxter & MacLeod ex Humber) Humber occurring in a natural epizootic affecting adults of the oriental latrine fly, *C. megacephala* (Diptera: Calliphoridae).

2. Materials and methods

Surveys of entomopathogenic fungi on dipterans were done in a garbage dump adjacent to the city of Cavalcante, State of Goiás, in Central Brasil (13°46′40.53″S; 47°27′08.71″W) on 12–14 February and 3–5 April 2015. Twigs and leaves bearing mycotized cadavers were carefully removed, put into paper bags, and transported in a polystyrene cooler at 20 °C (Benjamin et al., 2004) for later examination and laboratory processing. In the laboratory, all specimens were examined immediately or stored at 4 °C. Cadavers filled with vegetative cells were incubated at 25 °C in a moist chamber (a plastic bag with moistened filter paper) to induce external conidiogenesis. Semi-permanent slide mounts were prepared in lactophenol-aceto-orcein (LPAO) as described by Keller (1987). Fungal microstructures were examined by brightfield or phase contrast microscopy (Leica DMLS 020-518.500), measured microscopically (Nova 180i-T; Toupview), and documented with a digital camera (Leica UCMOS01300KPA). The micrographs of resting spores of this fungus are photomontages of four to eight focal planes of these structures taken with a compound microscope (Olympus BX-51, Olympus Corporation of America) with planapochromatic objectives to increase the apparent depth of focus, and were made using Helicon Focus Pro software (http://www.heliconsoft.com). Fifty measurements for any given structure usually came from fungus on several individual specimens.

3. Results and discussion

In February, it was impossible to overlook a very active fungal epizootic affecting oriental latrine flies, *C. megacephala*, when huge numbers of cadavers were visible on plants, fence wires and other surfaces in the local garbage dump (Fig. 1). The outbreak was observed over three consecutive days. This site also included very large populations of both *C. megacephala* and a *Musca* species (Diptera: Muscidae), but only the calliphorids were found to be mycotized and their cadavers were present in unusually dense populations (Fig. 1). During the survey in April, very few calliphorid individuals were found at the site; similarly small populations of the muscid flies were present. We are unable to account for the decreases in observable populations of both fly species as a matter of seasonality or some other physical factor although it appears that populations of this host may be positively correlated with temperature (Seolin Dias et al., 2009; Ngoen-klan et al., 2011). We do not know whether the calliphorid population was largely eliminated by the action of the *Pandora* species, but no mycotized individuals of *Musca* were found on this site during any of the surveys. A total of 261 mycotized calliphorid cadavers were collected in February, but only five individuals in April.

When the cadavers were put into high relative humidity, the further development of the fungus was stimulated, and characteristic light brown to white bands and clusters of actively sporulating conidiophores emerged through abdominal and other intersegmental membranes (Fig. 2). The bitunicate, uninucleate primary conidia of this pathogen, $29.6 \pm 0.4 \times 13.8 \pm 0.2$ μm [23.2–34.8 × 11.5–16.3 μm] (Fig. 3), were formed singly and forcibly discharged from conidiogenous cells formed singly at the apices of the digitately branched conidiophores typical of *Pandora* species (Humber, 2012a). Rhizoids with large, discoid holdfasts emerged singly and in small bundles from the host's abdomen and served to anchor the cadavers to twigs, leaves, and wires on which the host died (Fig. 4). *Chrysomya* cadavers collected in early April included extremely small numbers of resting spores, 43.1 ± 1.5 μm [37.3–53.5 μm] overall diameter (including the superficial decorations), with prominent bullate (rounded, bump-like) projections of the epispore's surface (Fig. 5). The combination of the host identity and the broadly rounded (bullate) decorations of these resting spores are unambiguously diagnostic for *P. bullata*. Humber (1981) described this fungus as *Erynia bullata* Thaxter & MacLeod by combining two earlier but nomenclaturally invalid descriptions of *Entomophthora bullata* by Povah (1935) who transcribed Roland Thaxter's herbarium notes about a set of infected flies, and by MacLeod et al. (1973) who provided a thorough characterization of this fungus. The species was later transferred to the newly recognized genus *Pandora* (Humber, 1989). The infected calliphorids in these collections were deposited in the Herbarium of the Federal University of Goiás, Goiânia, Brazil (UFG 50067, UFG 50069 and UFG 50071).

P. bullata was known previously from few collections in the northern states of the United States and adjacent areas of Canada (MacLeod et al., 1973) and, less prominently, from Australia, the United Kingdom, and Spain (Glare and Milner, 1987; Bałazy, 1993; Niell and Santamaria, 2001). This is also the first record of *P. bullata* from any tropical location. The ARSEF culture collection (Ithaca, NY) lost its only isolate of *P. bullata* long ago before ARSEF acquired more specialized equipment for controlled cryopreservation of its isolates; the only available culture of *P. bullata* might be the American Type Culture Collection's 24298 (which was derived from the now defunct ARSEF 116).

As with the great majority of entomophthoran taxa, few gene sequence data are yet available to suggest or to verify individual species identifications, and since we were not able to isolate *P. bullata* in culture, we have not attempted to add any sequences for the Cavalcante fungus. For most of these fungi there is no option except to use traditional morphological, developmental and pathobiological characters as the bases for species identifications. In the case of *P. bullata*, the morphology of the resting spores coupled with the host identity is unambiguously diagnostic.

We attempted to culture this fungus in the field on quarter-strength Sabouraud dextrose agar amended with 0.2% yeast (SDAY/4) (Inglis et al., 2012). None of these attempts was successful using the media available to us at that place and time. The greatest likelihood of isolating a culture of this fungus would have been to use vegetative (hyphal or conidiophore) inoculum and any of a wide range of liquid culture media. Because the vegetative cells of many entomophthoraceous fungi such as this grow in the hosts as wall-less protoplasts, culture isolations are often more successful in liquid media than on solid culture media (Humber, 2012b), and then many (but not all) can be transferred to grow successfully as walled hyphae on solid media.

The resting spores of entomophthoran fungi often do not form until the end of a disease outbreak, and when these spores are formed the host cadavers are almost always substantially filled with resting spores in temperate regions. That so few resting spores were recovered from this site literally in the middle of the southern tropic zone raises interesting questions that, while unanswered, need to be asked: Were these few flies with resting spores collected too late in the disease cycle? Might some cadavers with resting spores have fallen off the substrates or been eaten by birds, rodents, or