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The captured launch of a ballistospore

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Abstract: Ballistospore discharge is a feature of 30 000 species of mushrooms, basidiomycete yeasts and pathogenic rusts and smuts. The biomechanics of discharge may involve an abrupt change in the center of mass associated with the coalescence of Buller's drop and the spore. However this process occurs so rapidly that the launch of the ballistospore has never been visualized. Here we report ultra high-speed video recordings of the earliest events of spore dispersal using the yeast *Itersonilia perplexans* and the distantly related jelly fungus *Auricularia auricula.* Images taken at camera speeds of up to 100 000 frames/s demonstrate that ballistospore discharge does involve the coalescence of Buller's drop and the spore. Recordings of *I. perplexans* demonstrate that although coalescence may result from the directed collapse of Buller's drop onto the spore, it also may involve the movement of the spore toward the drop. The release of surface tension at coalescence provides the energy and directional momentum to propel the drop and spore away from the fungus. Analyses show that ballistospores launch into the air at initial accelerations in excess of 10 000 *g*. There is no known analog of this micromechanical process in animals, plants or bacteria, but the recent development of a surface tension motor may mimic the fungal biology described here.

Key words: Buller's drop, fungal biomechanics, surface tension catapult

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INTRODUCTION

In the absence of musculature, a remarkable array of dispersal devices has evolved among the fungi. These include the jet-propelled canon of *Pilobolus,* the spore gun, or ascus, of the Ascomycota, and a miniature springboard in the artillery fungus *Sphaerobolus* (Ingold 1971). In each of these cases the force that propels the spores (or spore-containing sporangia) into the air is derived from osmotically generated hydrostatic pressure, or turgor. The mechanism of spore discharge in the majority of the basidiomycete fungi is fundamentally different (Webster and Chen 1990, Money 1998).

Ballistospores (or ballistosporic basidiospores) are generated by basidia. Each spore is situated at the tip of a sterigma (Fig. 1). The launch is initiated when a fluid, named Buller's drop, grows at the base of the spore, and a separate body of fluid accumulates on the side of the spore. Buller's drop grows for a few seconds, and then the drop and spore simultaneously disappear from the sterigma. Buller's drop and the fluid on the spore contain sugars and polyhydric alcohols, including mannitol (Turner and Webster 1995, Webster et al 1995). These compounds appear to drive the condensation of water on the spore surface by lowering its water potential (Webster et al 1989). The hydrophobicity of portions of the spore surface and details of spore morphology are thought to maintain the gap between Buller's drop and the fluid on the spore, until they reach a critical size and make contact (Money 1998).

A variety of mechanisms have been proposed to launch ballistospores, including the explosive flexing of a double membrane separating the spore and sterigma (Buller 1909, Prince 1943), the bursting of a gas bubble or blister (Ingold 1971), and electrostatic repulsion (Webster et al 1984). Most of these ideas have been rejected and the model currently favored by mycologists involves an abrupt shift in the center of spore mass caused by the growth and collapse of Buller's drop. This model is termed a surface tension catapult because the surface tension of Buller's drop is hypothesized to provide the energy necessary to launch the spore (Turner and Webster 1991). The catapult model is supported by theoretical calculations, but the predicted motion of Buller's drop and the launch of the spore have never been visualized.

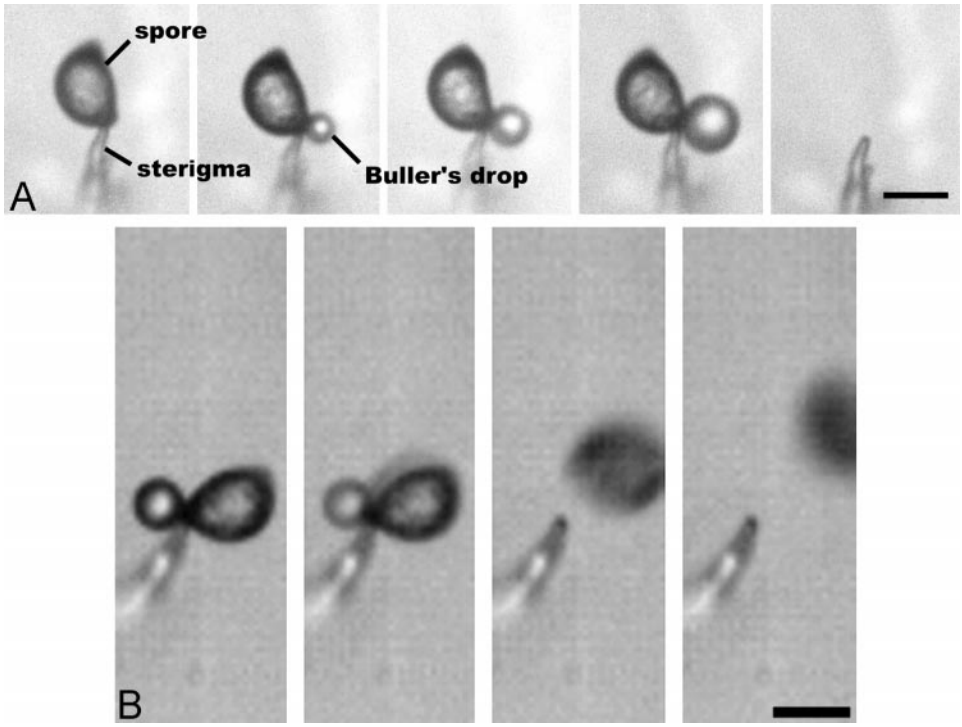

FIG. 1. a. Mechanism of ballistospore discharge in *I. perplexans* as captured using conventional still photomicroscopy. Successive images separated by 10 s show the growth of Buller's drop and simultaneous disappearance of drop and spore from sterigma in final frame (scale bar 10 μm). Photographs courtesy of John Webster. b, Images of Buller's drop and the ballistospore using ultra high speed video. Successive images separated by 10 μs (scale bar 10 μm).

Earlier attempts to capture discharge with high-speed ciné-cameras, running at speeds of up to 500 frames/s, were unsuccessful (Webster et al 1984). Like conventional video recordings, these showed only the growth of Buller's drop followed by multiple frames of the sterigma without its spore (FIG. 1a).

MATERIALS AND METHODS

Fungi.—The basidiomycete yeast *Itersonilia perplexans* was ordered from the Centraalbureau voor Schimmelcultures (culture CBS 363.85) and subcultured on cornmeal agar (CMA) at room temperature for several months before use. Fruiting bodies of *Auricularia auricularia* were collected from the woods surrounding Miami University in Oxford, Ohio, and air dried. To prepare *I. perplexans* for filming, small blocks of 1 wk old cultures were excised from CMA and transferred to Petri dishes with sterile distilled water agar (DWA) poured to a depth of 5 mm. Ballistospore discharge was filmed by placing these dishes face down on the stage of an inverted microscope (Model IX70, Olympus, Japan) fitted with 20× and 40× long working-distance objectives. The use of CMA blocks on DWA limited the distance between the objective lenses and the culture while maintaining a high humidity within the closed Petri dish. Ballistospores were shot downward, toward the microscope objective. To prepare *A. auricularia* for filming, fruiting bodies were rehydrated by incubation on wet filter paper. Thin slices (<1 mm wide) were first cut from the rehydrated fruiting bodies using a scalpel, then placed sideways on DWA blocks, transferred onto fresh DWA in Petri dishes and viewed lid down with the inverted microscope. This technique allowed the spore-producing surfaces of *A. auricularia* to be oriented vertically and the spores shot horizontally.

Video microscopy.—Ballistospore discharge and spore trajectories were recorded with a monochrome Ultima APX high speed digital video system (Photron, San Diego, California). Videos are available as supplementary material (http://www.mycologia.org/cgi/content/full/97/4/866/DC1). Camera speeds of 10 000–100 000 frames/s were used in conjunction with shutter speeds of 10–100 μs. A total of 25 videos were taken and the parameters unique to each video are available either with the supplementary material or from A. Pringle.

Analyses.—We measured the displacement of the spore with digital imaging techniques implemented in Matlab v.7.0.1 (The Mathworks, Natick, Massachusetts) and VideoPoint v.2.5 (Lenox Softworks Inc, Lenox, Massachusetts). The precise timing of the launch could not be measured from the first video frame showing the spore in flight because this captured information from the entire period the shutter was open (i.e. a spore might have left its sterigma 6 μs after the beginning of a 10 μs opening). Instead initial speed was calculated with a two-step process. First, the time at which

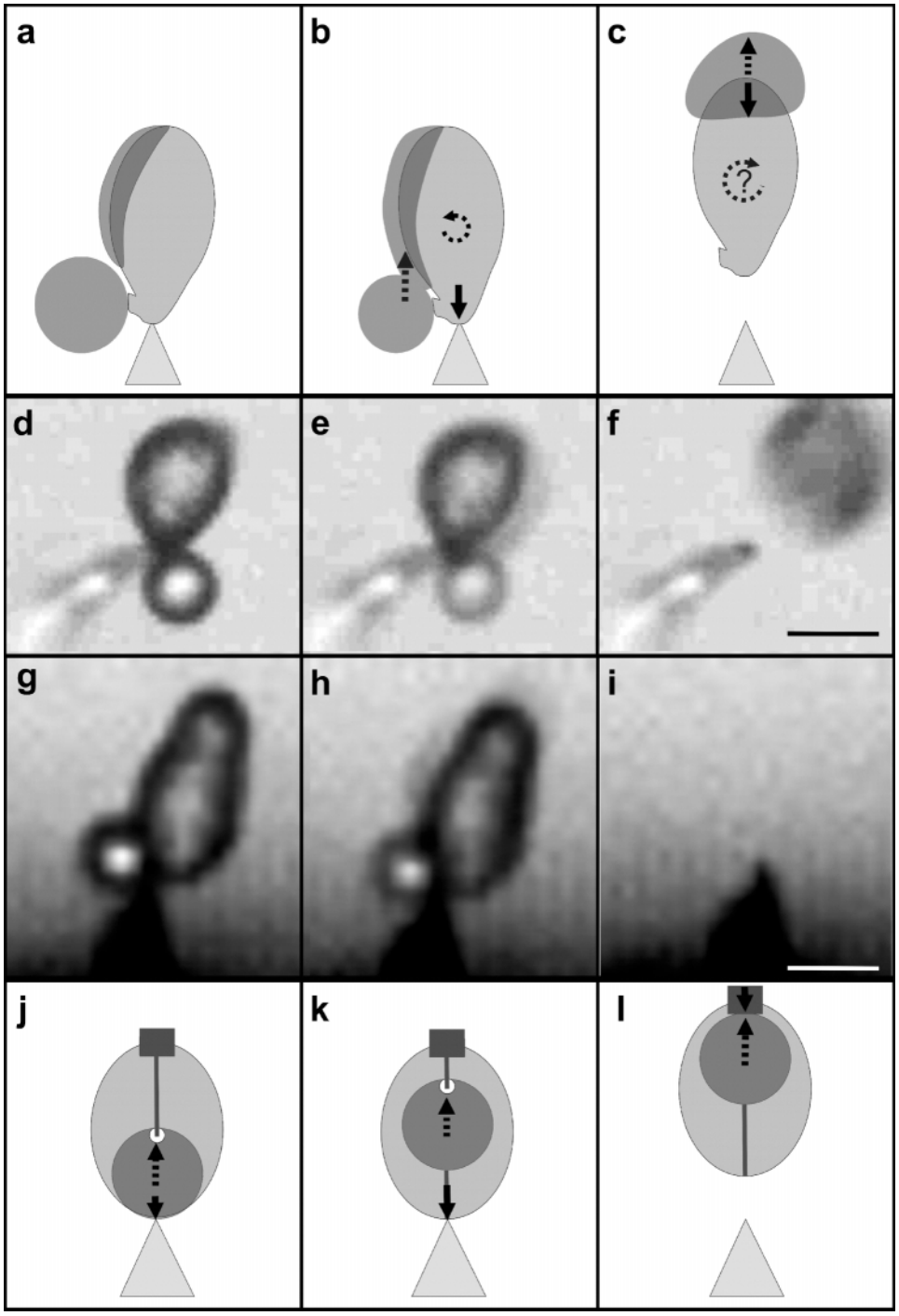

FIG. 2. An illustration of the surface tension catapult (a–c), video (d–f and g–i) and a model of spore release (j–l). a, Buller's drop contains energy in the form of surface tension forces. b, When Buller's drop touches the fluid on the side of the spore (the adaxial drop), the merging fluid flows rapidly toward the distal end of the spore (dashed arrow), exerts force (black arrow) on the sterigma (triangle), and generates a slight rotation of the spore toward the drop (curved dashed arrow). c, The collapsed drop and spore launch from the sterigma. The spore may rotate counterclockwise, a result of the off-axis loading of the spore's tip, or clockwise, a result of the distal, off-axis momentum of the drop as it traverses the spore. d–e, An oblique view of the spore and Buller's drop of *I. perplexans* (10 μs between each frame, scale bar 10 μm). A shadow of either the drop or spore is visible in e. The shadow may indicate the movement of fluid over the spore's surface, the snapping together of drop and spore, or the movement of the spore toward the drop. g–i, A lateral view of *A. auricularia* during ballistospore launch (50 μs between each frame, scale bar 10 μm). Again, a shadow of fluid and/or spore movement is visible in h. j–l, A mechanical diagram of the forces that may cause the spore to eject from the sterigma. j, the drop begins to collapse and surface tension energy is released, the fluid (dashed arrow) moves toward the tip of the spore because of

the spore left its sterigma was calculated by extrapolating backward on a plot of spore position as a function of time. Second, initial speed was determined from a plot of the speed as a function of time, assuming a linear increase in speed from launch to the first video frame containing a moving spore. Because motion was blurred throughout the trajectory, the position of the spore was set as the center point along the blurred trajectory in each frame. Our approach provides a conservative estimate of speed. An estimate of acceleration was obtained by measuring the initial speed from the video frames divided by the time necessary to achieve this speed. Because of the uncertainty of the start of the launch, this time was conservatively estimated as equal to the 10 μs shutter period during which the launch occurred.

RESULTS

Does ballistospore discharge involve the directed collapse of Buller's drop onto the spore?—The videos referenced in the following sections are available as described in Materials and Methods. The coalescence of Buller's drop and the ballistospore was filmed at 100 000 frames/s using *I. perplexans* (videos 5–9). Each of these videos has three key frames: a frame with the expanded Buller's drop and spore, a frame showing movement, and an additional frame showing Buller's drop and the spore as a single unit launched from the sterigma. Each video captured a slightly different sequence of images. The reader is encouraged to view the actual videos at http://www.mycologia.org/cgi/content/full/97/4/866/DC1.

Videos confirmed that Buller's drop and the ballistospore coalesce (FIG. 2). Furthermore the sterigma did not appear to move in any of the images. However the second frame can be interpreted variously in the different videos: one video (7) appeared to show the very clear "ghost" of Buller's drop and suggested that Buller's drop collapses onto the ballistospore. In contrast a second video (6) suggested movement of the spore to the drop (FIG. 2). Finally a third video (5) appeared to show the movement of both Buller's drop and the ballistospore.

Spore launches of *A. auricula* were filmed at slower speeds with the goal of describing the flight pattern of an individual ballistospore, but two videos provided further insight into the earliest events of ballistospore discharge. One (15) clearly showed Buller's drop moving while the spore and sterigma remained stationary. When frames were viewed in succession, a second video (4) suggested that Buller's drop was racing over the spore surface; Buller's drop appeared to drag the spore from the fungus. However the individual frames (of video 4) once again were ambiguous (FIG. 2).

Using these data we propose a more detailed model of spore release (FIG. 2). The mechanism described in the currently accepted model is based on shifts in the center of spore mass (induced by the growth of Buller's drop), which moves the spore's center of mass toward the drop, and a more rapid shift when the drop collapses onto the spore (Webster et al 1984, Money 1998). Here we propose that several additional factors may drive spore release. In our model momentum is generated as Buller's drop traverses the length of the spore and simultaneously increases in speed. The force vector that propels the spore from the sterigma is generated by an abrupt halt in the drop's movement when it reaches the tip of the spore. This model differs from Webster's model because the force vectors are not derived solely from the short shifts in center of mass caused by the drop's growth and subsequent collapse upon the spore. Instead substantially more momentum is developed as the drop travels along and increases in speed over the spore length, and a greater shift in center of mass is explained by the longer distance movement of the drop to the tip of the spore. Most important the braking of the drop at the spore's tip provides the necessary force vector to pull the spore from the sterigma.

Speeds.—The speeds of *A. auricula* spores were 0.87–1.57 m s^{-1} with a mean speed of 1.20 m s^{-1} (0.05 SE; n = 13). Speeds of *I. perplexans* spores were more difficult to calculate because the spores moved swiftly beyond the focal plane of the microscope. Nonetheless it was possible to estimate the speed of two spores of *I. perplexans* based on flight paths of 18–20 μm. These spores traveled at an average initial speed of 0.67 m s^{-1}. These data confirm previous estimates of velocity that were based on spore size and mass and

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an attraction to the adaxial drop. This initial movement exerts force on the sterigma (solid arrow). The directed motion of the drop collapse is represented by a gray "trackway" along the length of the spore. k, the drop gains momentum as it accelerates along the length of the spore. l, surface tension forces cause the drop to stop abruptly and prevent the fluid from flying off of the spore. The abrupt deceleration delivers a directed and substantial force to the spore, and causes the spore and drop to eject away from the sterigma. The increasing drop momentum during its directed distal movement is critical for directing the spore away from the sterigma, and for delivering sufficient force for spore release.

the measured range of the discharge mechanism (Buller 1909, Webster et al 1984).

Predicting the path of a ballistospore.—The flight of individual ballistospores was filmed with speeds of 10 000–40 000 frames/s should be in *A. auricula* (videos 4, 13–18). With a model developed by Fischer et al (2004) we used one video (13) to successfully predict the ballistospore trajectory (FIG. 3). Predictions were made by modeling the spore as a sphere with aerodynamic radius (r) = 4.15 μm, density 1000 kg m^{-3}, and mass (m) = 3.7×10^{-13} kg. Mass was estimated from the projectile size in the videos. Air viscosity (η) was estimated as 18.27×10^{-6} Pa s. The force due to viscous drag was modeled by $F = -(6\pi r\eta/m)v$, where v equals the time-dependent projectile velocity. The model predicted that an initial velocity of 1.25 m s^{-1} would propel the spore over a distance of 0.4 mm. The trajectory is dominated by the viscosity of the air, which causes a rapid deceleration after launch. The spore brakes abruptly whether it is launched horizontally or vertically and gravity assumes control over its descent after a flight time of less than 2 milliseconds, according to our calculations. A more complete description of the mathematical model is given by Fischer et al (2004).

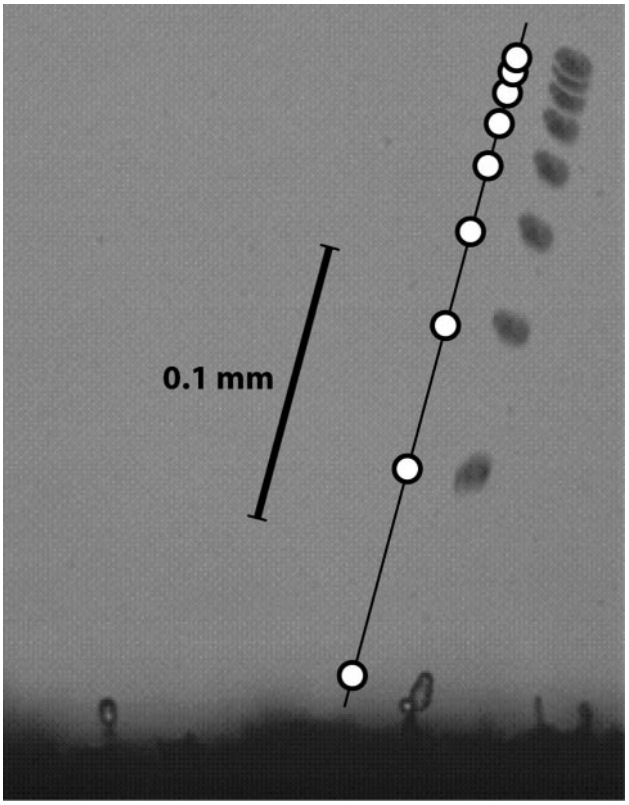

FIG. 3. Composite image of the flight of an *A. auricula* spore. White dots are from predictions made using a model developed by Fischer et al (2004).

DISCUSSION

In these experiments we used a pair of distantly related basidiomycete fungi and extreme high-speed video (10 000–100 000 frames/s) to visualize the coalescence of Buller's drop and ballistospore and the subsequent flight of a ballistospore (FIGS. 2, 3). Despite differences in spore shape and size, the mechanism of discharge appeared similar in *I. perplexans* and *A. auricula*. The data confirm some aspects of the surface tension catapult, especially the stationary sterigma (all videos) and the movement of Buller's drop (video 15, perhaps videos 4, 5, 6 and 7). However it appears that the spore also may move (videos 5, 6 and 7). The movement of the spore suggests that rotational forces may play a role in ballistospore discharge. With these data we refined the surface tension catapult model (FIG. 2) and by following the trajectory of Buller's drop over the spore we provide both a more powerful mechanism for the launch of the ballistospore, and a consideration of the rotational forces involved. We also matched the observed trajectories of the spores to flight paths predicted with a mathematical model (FIG. 3).

We do not know whether Buller's drop moves independently of the spore, or whether the drop and spore move toward each other simultaneously. Current high-speed video technology is unlikely to resolve the timing and path of the drop's movement along the spore surface because of the time scales involved. Alternative approaches include the use of multiple lasers to follow the movements of the drop and spore or experimental manipulations (i.e. the use of heavy water to form Buller's drop and influence the momentum of the ballistospore, or the use of fluorescent dyes to distinguish Buller's drop from the spore).

Ballistospore discharge may rank among the fastest cellular motions of fungi, but animals and plants often are faster. The launch of an *A. auricula* ballistospore takes no more than 10 μs, with accelerations in excess of 12 000 *g* required to achieve the initial velocity of 1.20 m s^{-1}. This eclipses the acceleration of an arthropod, the recently reported 10 000 *g* spring-loaded strike of a mantis shrimp (*Odontodactylus scyllarus*) (Patek et al 2004) but is surpassed by the 40 000 *g* pressure-based discharge of a nematocyst cell (*Hydra attenuata*) (Holstein and Tardent 1984) and the 3.1 m s^{-1} opening of the bunchberry dogwood pollen catapult (*Cornus canadensis,* Edwards et al 2005).

The recent development of a surface tension oscillator (Regan et al 2005) provides an intriguing

mimic of the surface tension catapult. This electric motor uses surface tension to control the release of energy between two drops of liquid metal. As one drop reaches a critical size it touches the second drop and then energy is released as metal flows from the larger to the smaller drop. The engineering is an obvious analog to the use of surface tension to store energy in Buller's drop, and the release of energy when Buller's drop and the ballistospore coalesce.

Although the formation of Buller's drop has been described in diverse basidiomycetes with a remarkable array of natural histories, including ectomycorrhizal species that form mushrooms, basidiomycete yeasts, and phytopathogenic rusts and smuts, the comparative biomechanics of ballistospore discharge is poorly studied. Ballistospory may have appeared early in the evolution of basidiomycetes, and phylogenetic data suggest that ballistospory has been lost repeatedly during the subsequent evolutionary history of this phylum (Hibbett 2004). Morphological details are likely correlated with the particular biology of each species, but little is known about differences in trajectories, accelerations or the effects of variation in Buller's drop size and spore shape (Money 1998). In yeast species, spores must be propelled far enough to escape the motionless air at the surface of the colony. By contrast, in mushroom species, spores must navigate tightly packed gills and narrow tubes (Ingold 1992). To avoid hitting the opposing gill surface, ballistospores of these species are discharged horizontally for approximately 0.1 mm before they fall between the gills and are dispersed in the turbulent air around the cap. Future work on the comparative biology of the discharge mechanism will reveal how biomechanical variation can control the intriguing reproductive diversity of basidiomycete fungi.

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