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A New Amoeba with Protosteloid Fruiting: *Luapeleamoeba hula* n. g. n. sp. (Acanthamoebidae, Centramoebida, Amoebozoa)

Lora L. SHADWICK[1], Matthew W. BROWN[2,3], Alexander K. TICE[2,3], Frederick W. SPIEGEL[1]

[1]Department of Biological Sciences, University of Arkansas, USA; [2]Department of Biological Sciences, Mississippi State University, USA; [3]Institute for Genomics, Biocomputing, and Biotechnology, Mississippi State University, USA

Abstract. We describe a new protosteloid amoeba, *Luapeleamoeba hula.* Protosteloid amoebae, sometimes called protostelids, are sporocarpic amoebae that make fruiting bodies that consist of a stalk and one to a few spores. This new taxon was cultured from dead leaves of mamaki (*Pipturus albidus*) from the Manuka Natural Area Reserve, Hawaii, USA. Light microscopic examination showed that this amoeba has a short, rigid stalk with a small apophysis and a spore that changes shape continuously until it is shed and crawls away from the stalk. In addition, this amoeba was initially observed to maintain a diurnal rhythm in which fruiting body formation occurred primarily in the late afternoon. This new species is unique in both its amoebal and fruiting body morphology. Spore deciduousness appears to be a result of shape changes in the spore itself. This is the fourth species of protosteloid amoeba described with a clearly described diurnal rhythm. In addition, previous molecular phylogenetic analyses suggested that this new species has SSU rRNA gene sequences that clearly separate it from any other protosteloid amoebae and place it as sister to *Protacanthamoeba bohemica* among the Acanthamoebidae family in Centramoebida of Amoebozoa. Because this new amoeba species does not fit into any of the centramoebid genera, we have proposed a new genus *Luapeleamoeba.*

Key words: Free-living amoeba, protostelid, centramoebid, acanthamoebid, stalk, taxonomy, terrestrial, Hawaii

INTRODUCTION

Protosteloid amoebae, often called protostelids, are amoebae that make simple fruiting structures comprised of a *de novo* formed acellular stalk supporting one to a few spores (Olive 1975, Spiegel 1990, Shadwick *et al.* 2009). They consume microbes that decompose plants and are observed in the laboratory by microscopically examining the edges of dead plant matter placed in primary isolation plates (Spiegel *et al.* 2004, Spiegel *et al.* 2007). In 2005, during an ongoing survey of Hawaiian protosteloid amoebae, we found a distinctive short-stalked species with spores that change shape constantly and that fruited heavily only in the late afternoon. At other times of the day, no fruiting bodies of this species were found in primary isolation plates, as all the spores had been shed. Because our new species fruited in abundance, it was easily brought into monoeukaryotic culture. At first, based on sporocarp devel-

Address for correspondence: Frederick W. Spiegel, Department of Biological Sciences, University of Arkansas, SCEN 601, Fayetteville, Arkansas 72701, USA; Tel.: +1 479 575 4248; E-mail: fspiegel@uark.edu

opment, we thought it was a species of protosteloid *Endostelium* in Pellitida (see Kudryavtsev *et al.* 2014). However, these amoebae were distinct from any pellitid taxon (Kudryavtsev *et al.* 2014) at both the light and ultrastructure levels. Also, phylogenetic analyses (Shadwick *et al.* 2009) showed that it did not branch with *Endostelium zonatum*. Rather, it branched as sister to *Protacanthamoeba bohemica* in Acanthamoebidae. Because its amoebal morphology was unlike that of *Protacanthamoeba* (Page 1981, Dyková *et al.* 2005) and because of its infrequent encystment, we describe this new species in a new genus in the Acanthamoebidae of the Centramoebida in Amoebozoa.

MATERIALS AND METHODS

1. Collections. We made collecting trips in late July and early August, 2005, around the island of Hawaii. At each collecting site a GPS reading was taken and samples of dead standing vegetation and ground litter were collected and placed in paper bags. The samples were air dried, then sent to the University of Arkansas. The new species was recorded only in a collection from the Manuka Natural Area Reserve, Hawaii, USA. GPS: 19.110217° N, 155.825600° W.

2. Primary isolation plates. Subsets of the samples were plated out with standard techniques (Spiegel *et al.* 2004). Briefly, eight pieces of substrate were placed on a primary isolation plate (PIP) of wMY agar (Spiegel *et al*. 2004; 0.002 g malt extract, 0.002g yeast extract, 0.75 g K_2HPO_4 and 15 g agar / L deionized H_20) then saturated with sterile, distilled water. After three to five days of culture, the edges of substrates in PIP were scanned for protosteloid fruiting bodies using the 10× objective of a compound microscope.

3. Monoeukaryotic culture. The new species was isolated into mono-eukaryotic culture by picking spores from fruiting bodies observed in a PIP with a sterile glass needle and placing them onto a streak of the bacterial food organism *Sphingomonas* sp. strain "FLAVO" ATCC BAA-1467 on wMY agar plates, or by cutting an agar block full of fruiting bodies from the primary isolation plate and allowing spores to drop onto a wMY plate containing the food organism. To assure that no other eukaryotes contaminated the cultures, early cultures were used as sources for the spore dropping technique and spore drops were repeated several times. Once dropped spores had germinated and begun to grow, they were cut out and sterilely transferred to fresh culture plates to establish stock cultures. Subsequently, cultures were passed to fresh media every month to two months.

4. Strains examined. We have isolated and examined two independent cultures of this species from dead leaves of mamaki (*Pipturus albidus* [Hook and Arn.] A. Gray ex. H. Mann), and from ground litter samples, collected by Lora L. Shadwick, John D. L. Shadwick, and Frederick W. Spiegel from the Manuka Natural Area Reserve. The type specimen, LHI05-M5g-1, from mamaki ground litter was isolated by F. W. Spiegel on August 29th, 2005. An isotype, LHI05-M5g-2, was isolated from the same collection by L. L. Shadwick on the same day. This species was previously observed and recorded in his collecting notes by F. W. Spiegel as "new species T" from the same location, collection HI98-81a on October 14, 1998 from standing dead fronds of *Nephrolepus* sp., but it was not cultured.

5. Light microscopy. The new species was observed repeatedly in culture under a compound light microscope using 10 × brightfield (BF), 20 × BF, 40 × dry differential interference contrast (DIC) and phase-contrast (PC), and 63 × oil immersion DIC optics, and digitally photographed using Auto-Montage software (Syncroscopy, Frederick, Maryland, USA), which allows for in-focus images of three-dimensional objects by combining a series of through-focus images. It was observed subaerially directly on agar plates, and immersed in liquid on agar-coated, culture slides under cover slips (see Spiegel *et al.* 2007, Kudryavstev *et al.* 2014).

6. Fixation. Because amoebae: 1) often rounded up quickly when exposed to the microscope light, 2) contained highly mobile granuloplasm, and 3) were quite three dimensional, some amoebae were gently fixed on a glass slide to maintain their locomotive form when photographed under the light microscope and stacks of those images were combined using the program Automontage (Syncroscopy) (Figs 7, 9, 10). We have used this fixation method for several decades in the Spiegel lab, and cells were carefully monitored to assure that they were not being distorted by fixation (compare to unfixed amoebae shown in Figs 2, 6, 8, 19–21, and in supplemental videos). An agar block containing as many amoebae as possible was cut from the culture with a flamed spear-point needle and gently inverted onto a clean glass slide. Liquid wMY (all ingredients of wMY agar except the agar) was added drop wise to the edge of the block so that it could wick under the block. This was allowed to nearly dry for approximately 10 min so that the amoebae would settle onto the glass. A cocktail of 1% OsO_4 in liquid wMY was added, and was followed immediately with enough glutaraldehyde to reach a final concentration of roughly 1%. Additional wMY was wicked under the agar block to rinse the cells and to float the agar block off of the cells so that the block could be replaced with a clean cover slip. Amoebae were then viewed under the compound microscope, and photographed as described above (Figs 7, 9, 10).

7. Transmission electron microscopy. For transmission electron microscopy (TEM), amoebae were grown on wMY agar dishes. The cells were suspended in liquid by flooding the plate with 5 mL of liquid wMY and then scrapping the agar surface. The cell suspension was collected in a 15 mL conical tube and cells were pelleted by centrifugation at 2000 × g for 2 min. The supernatant was poured off and the cell pellet resuspended in a cocktail of wMY culture media containing 2.5% v/v glutaraldehyde, 1% OsO4, and 0.2 M sodium cacodylate and fixed for 30 min on ice. The fixed cells were then centrifuged at 2000 × g for 1 min and the supernatant was removed. Cells were then washed once in wMY liquid medium. This process was repeated twice more, with ultrapure H_2O washes. Cells were again concentrated by centrifugation, then enrobed in 2.0% (w/v) agarose. Agarose blocks were dehydrated in a graded series of ethanols up to absolute ethanol, and then embedded in SPI-Pon 812 Epoxy resin (SPI Supplies, West Chester, Pennsylvania, U.S.A.). Serial sections (50–70 nm) were cut with a diamond knife on a Reichert-Jung Ultracut E ultramicrotome (Reichert-Jung [Leica Biosystems], Wetzlar, Germany), mounted on pioloform film on grids using the technique of Rowley and Moran (1975), and were subsequently stained with saturated uranyl acetate in 50% ethanol and with lead citrate. Sections were observed using a JEOL 1230 120kV transmission electron microscope (JOEL, Tokyo, Japan).

8. Epiflourescence microscopy. For fluorescent staining of the actin cytoskeleton and genomic DNA, amoebae were grown in liquid wMY on Nunc™ Lab-Tek™ II Chamber Slide™ System (Thermo-Fisher, Waltham, MA, USA) on 2 chamber slides in 1 mL medium. Fresh medium was innoculated with a block of densely growing amoebae from wMY agar dishes. Agar blocks were removed from the liquid medium after 4 hours. Cells were allowed to grow on the glass slide for another 12 hours. Subsequently, 1 volume of 8% paraformaldehyde solution in wMY was added and removed from the slide chamber after 7 min. Paraformaldehyde was then inactivated in 50 mM NH_4Cl solution in wMY for 5 min and removed from the chamber. Cells were then permeabilized in 1mL 0.1% Triton x-100 wMY for 5 min and then washed 3 times in phosphate buffered saline. Then 1 mL of PBS was added to the chamber with 2 drops ActinRed 555 (Thermo-Fisher, Waltham, MA, USA) and cells were incubated for 30 min in the dark. The PBS solution was then removed and a drop of Fluoroshield with DAPI (Sigma F6057-20ML) was added and covered with a cleaned coverslip. Slides were viewed under a AxioSkop 2 Plus (Zeiss, Peabody, MA, USA) equipped with a DAPI (Zeiss filter 02 [350 nm, 250 nm]), Rhodamine filter (Zeiss filter 15 [546/12 nm, 590 nm]) and imaged with a Canon 5DS camera (Canon, Melville, NY, USA). Subsequently in Fiji (http://imagej.net), images were converted to 8-bit monotone images merged using the merge color channels function within the image color menu.

RESULTS

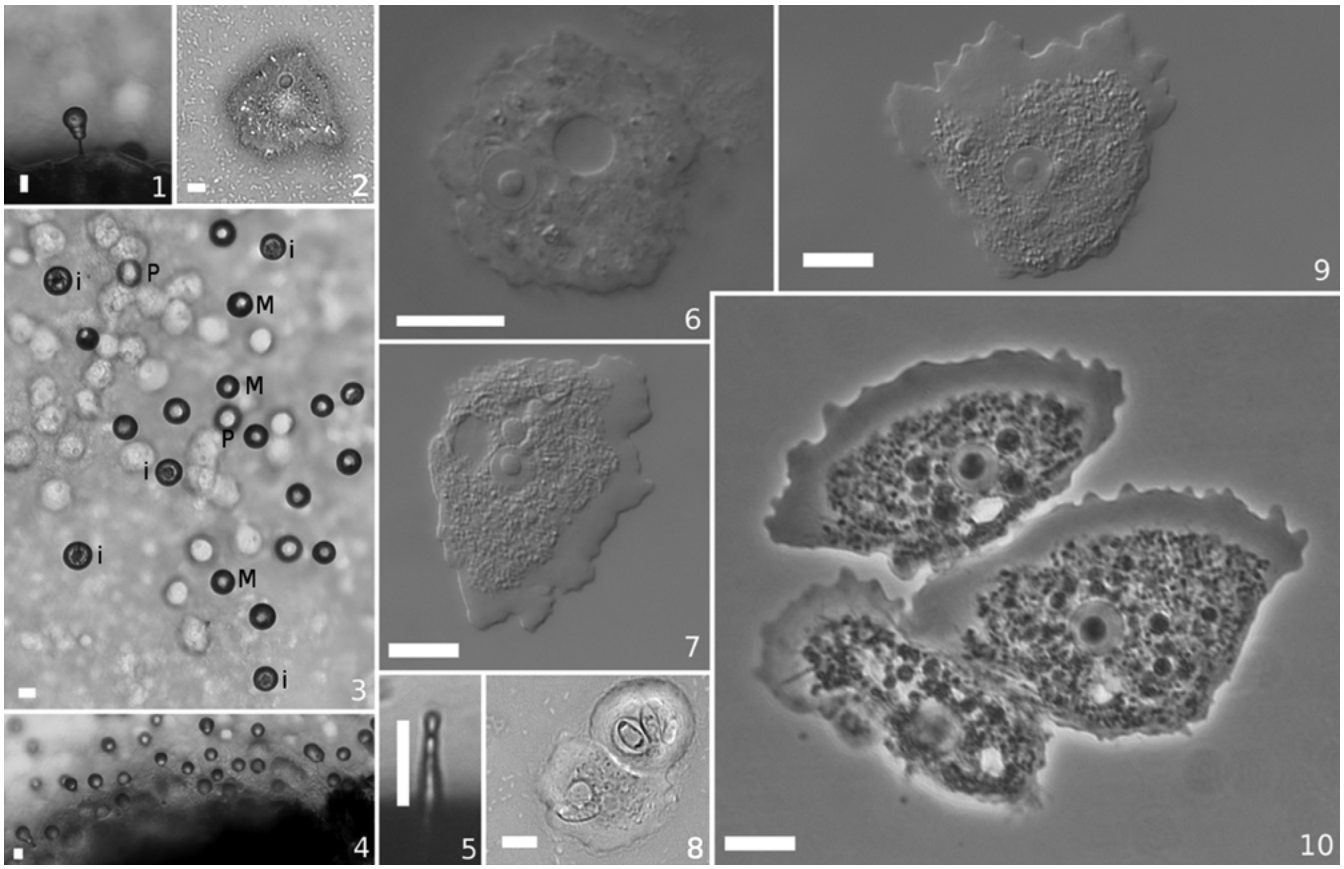
The unique protosteloid amoeba, which we propose to call *Luapeleamoeba hula* n. g. n. sp., was found fruiting in abundance between the afternoon hours of 15:00 and 18:00 on native substrates in a primary isolation plate (PIP). Developing fruiting bodies, or sporocarps, viewed from the side were distinct from all described protosteloid fruiting bodies in that they had a rigid stalk and a spore the shape of an upside down pear (Fig. 1). Amoebae on an agar surface were also distinctive in that they often had the shape of a shield volcano with a conspicuous contractile vacuole near the thickest part of the cell (Fig. 2).

Fruiting has a diurnal rhythm that was initially maintained in culture such that the bulk of fruiting starts in the late afternoon and ends by early evening. Spores disappear rapidly and few fruiting bodies are seen during other times of the day. Both cultures we established fruit consistently from one transfer to the next, and cultures that are stored in stasis in liquid nitrogen fruit readily upon being plated out; however, the diurnal pattern of fruiting is not as pronounced as when the culture was originally established.

Fruiting body development starts when an amoeba rounds up and becomes a refractile prespore cell (Figs 3, 4) that is circular to slightly ellipsoid. Maturing fruiting bodies have a portion of the forming stalk embedded within the developing, obpyriform spore (Fig. 3, viewed from top, and Fig. 4, viewed from side). The internally developing stalk is clearly visible as it is refracted through developing spores, or sporogens, when viewed subaerially from above (Fig. 3) while fully mature spores are more rounded so that the stalk is not clearly visible through the spore (Figs 3, 4). Within an hour or so of maturation, the spores disappear suddenly from the stalk. Stalks sometimes remain intact and upright on the substrate and can sometimes be found lying down on the agar surface. The spores on many sporocarps would jerk suddenly and land on the substrate adjacent to the base of the stalk. When that happened it was easy to see that the spore assumed an amoeboid morphology and moved off within a minute or so, suggesting that the spore is very thin walled or lacks a wall altogether (see supplemental video SV1). Sometimes amoebae drag other fruiting bodies around on the agar, and sometimes the stalk remains attached to the trailing posterior region of the amoeba for a few minutes after it begins to crawl away, eventually leaving the stalk lying behind on the agar surface (see supplemental video SV1).

Fruiting bodies viewed from the side range in size from 14.1 to 28.8 μm tall averaging 23.7 μm (n = 7) when measured from the base of the stalk to the top of the spore (Figs 1, 3, 4). Spores, when viewed and measured from the top, are generally round with an average diameter of 14.75 μm which ranges from a minimum of 11.3 μm to a maximum of 18.11 μm (n = 83). Spores viewed from the side are generally more irregular in shape and are often longer than they are wide (Figs 1, 4). Spore length measured from the side ranges from 11.5 to 18.4 μm, and maximum width measured from the side ranges from 8.9 to 17 μm. An apical apophysis of the stalk is embedded within the spore when the spore is attached and is only visible when the spore has been shed (Fig. 5). Spores are deciduous. Stalks sometimes remain standing after the spores disappear. Stalks are rigid and do not change shape or bend (Fig. 5). Total stalk length, including the apophysis, without the spore present, ranges from 5.9 to 9.8 μm averaging 7.7 μm (n = 17). The length of the apophysis ranged between 1.0 and 2.71 μm with an average of 1.92 μm (n = 10).

Amoebae of the new species are usually uninucleate with a single, central, round nucleolus (Figs 6–11) and pseudopodia and subpeudopodia are supported by an actin cytoskeleton (Fig. 11). Living amoebae viewed subaerially in their locomotive form in both monoeu-



Figs 1–10. Light micrographs of *Luapeleamoeba hula* n. g. n. sp. strain LHI05M-5a-1. **1.** Side view of fruiting body on native substrate in primary isolation plate (PIP). **2.** Living amoeba on agar surface in monoeukaryotic culture. The cell is moving in the direction of the bottom of the image. **3.** Amoebae and fruiting bodies in various stages of development on agar surface viewed subaerially from top in a PIP during the late afternoon. i – immature sporocarp, M – mature sporocarp, P – prespore cell. Note the apparent thickness of the amoebae. **4.** Fruiting bodies in various stages of development viewed subaerially on native substrate in PIP during late afternoon. Amoebae seen obliquely are obviously dome shaped. **5.** Stalk with apophysis viewed from side after spore has discharged. **6.** Floating form of a living amoeba in liquid media slightly flattened with cover slip under 63 × oil differential interference contrast microscopy (DIC). **7.** Amoeba gently fixed on slide to maintain locomotive form, 40 × dry DIC. **8.** Two living amoebae in PIP digesting fungal spores. **9.** Amoeba gently fixed on slide to maintain locomotive form showing blunted triangular subpseudopodia extending from broad hyaline lamellipodium, 40 × dry DIC. **10.** Three amoebae gently fixed on slide to maintain locomotive form 40 × dry phase contrast. Scale bars: 10 μm throughout.

karyotic culture (Fig. 2) and PIP (Fig. 8) on an agar surface are usually triangular to nearly circular, with a broad, lamellipodium at the leading edge and a large central cytoplasmic region that contains the nucleus and a conspicuous contractile vacuole (Figs 2, 8). When amoebae are observed subaerially on agar, they are very thin at the cell margins and rise to form a shield-shaped dome that is thickest just over the area of granuloplasm that contains the nucleus and contractile vacuole. This shape is most evident when using bright field microscopy and by focusing up and down at higher power. The locomoting amoebae frequently quickly round up when exposed to bright light (Fig. 6, the upper cell in Fig. 8) though they stay motile and able to ingest prey (Figs 15–17). Therefore, our detailed observations and measurements were taken from lightly fixed cells (Figs 7, 9, 10). The breadth of locomotive form averages 38.4 μm, but ranges from 32.9 to 51.1 μm (n = 10), with an average length/breadth ratio of 1.15 ranging from 0.61 to 1.64 (n = 10). The lamellipodium has an average length of 7.3 μm ranging from 4.4 to 9.5 μm, so that the average fraction of frontal hyaloplasm is 0.17, ranging from 0.08 to 0.24 (n = 10). Migrating amoebae typically have blunt, triangular, subpseudopodial

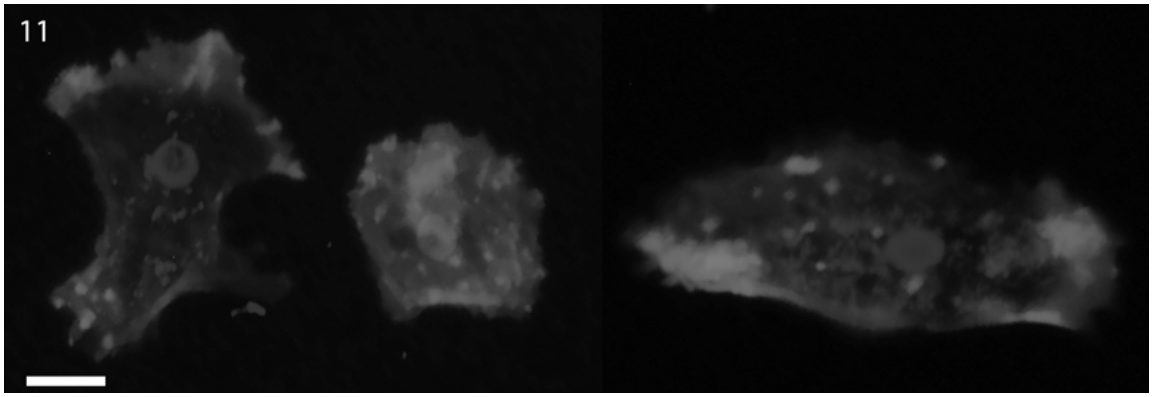
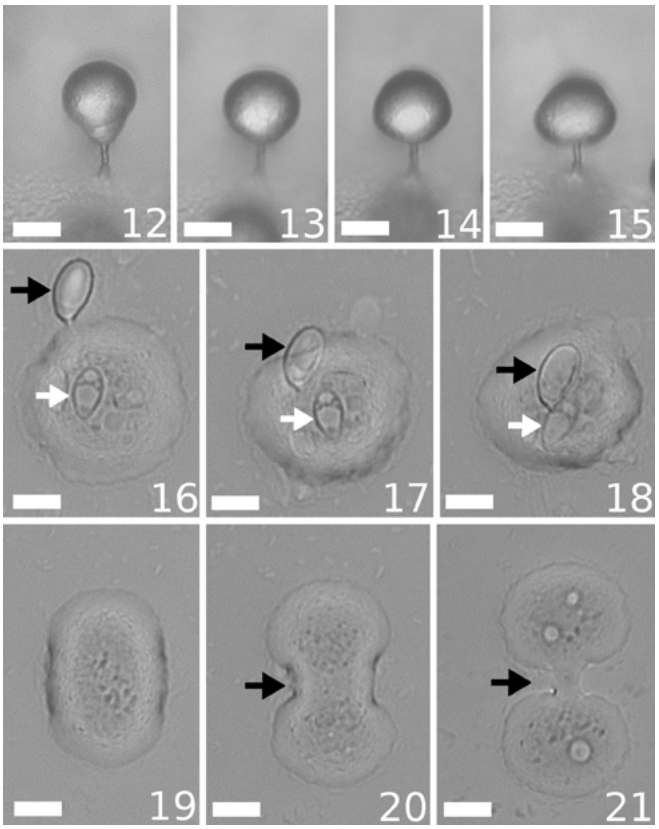


Fig. 11. Fluorescence image of fixed *Luapeleamoeba hula* n. g. n. sp. amoebae. Red is actin stain and blue is DNA stain. The amoebae on the left and right are motile, the one in the center is stationary. Small areas of DNA fluorescence outside the nuclei are from undigested bacteria in the food vacuoles. Scale bar 10 μm.

extensions on the leading edge (Figs 2, 9). The large contractile vacuole (Fig. 7) continuously builds in size averaging 5.5 μm at diastole (n = 11) with a maximum of 7.1 μm until its contents are expelled (Fig. 9), then smaller vacuoles migrate together and coalesce to form a new conspicuous contractile vacuole – the contents of which will eventually be expelled (data not shown). The contractile vacuole is usually found just posterior to the nucleus, within a distance equal to approximately one half of the diameter of the nucleus. The nucleus ranges in length from 5.1 to 8.1 μm with an average of 6 μm (n = 12), and breadth averaging 5.5 μm and ranging from 4.5 to 6.5 μm, containing a single, central, round nucleolus with a diameter ranging from 2.02 to 2.82 μm, average 2.4 μm (n = 12). The amoebae were seen digesting a variety of bacteria and fungal spores on primary isolation plates (Fig. 8) but grew and sporulated well in monoeukaryotic culture on *Sphingomonas* sp. Although amoebae are often triangular in outline, they vary greatly in size (Fig. 10) and shape especially when changing directions (bottom left amoeba in Fig. 10). The motion of the motile amoebae may be seen in the Supplemental Videos SV1, SV2.

Time-series images, approximately 30 seconds apart, of the new species on primary isolation plates (PIP) revealed several interesting behaviors. Spores were frequently observed to change shape atop the fully formed stalk (Figs 12–15). Amoebae ingested fungal spores including basidiospores (Figs 16–18). Amoebae were observed undergoing cytokinesis (Figs 19–21).



Figs 12–21. Time series of events in *Luapeleamoeba hula* n. g. n. sp. life cycle. **12–15.** Changes in spore shape. **16–18.** Ingestion of basidiospore. **19–21.** Cytokinesis. All images taken with 20 × dry lens bright field microscopy on agar surface in primary isolation plate (PIP). Approximately 30 sec elapsed between images. Scale bars: 10μm throughout.

The floating form (Fig. 6) is simply round with no distinct polarity of any kind. Floating cells often contain a conspicuous contractile vacuole (Supplemental Fig. S1). The margins of the floating cells are not smooth, but rather rough and irregular (Supplemental Figs S1, S2, Supplemental Video SV3). Floating cells quickly settle on glass slides and resume a more flattened appearance (Compare Figs S1 to S2).

Cysts (data not shown) differ from spores in that they are never stalked. Cysts have been observed only rarely even though we have continuously maintained the cultures for many years (data not shown). Cysts, when they appear, are spherical and extremely thin-walled unless what we took to be cysts were aborted attempts at fruiting or recently fallen "spores".

The method of preparation of amoebae for fixation for ultrastructure was such that it stimulated the cells to round up into floating forms (Fig. 22). However, we are primarily interested in demonstrating their main ultrastructural features (Fig. 22).

Amoebae are rounded with an irregular outline (Fig. 22). There is a central nucleus with a relatively large, relatively homogeneous nucleolus that may vary in electron density (Figs 22, 23). The granular cytoplasm is full of food vacuoles (Figs 22, 23) and mitochondria with tubular branched cristae (Figs 22, 24). The cortical portions of the amoebae are devoid of major organelles and rich in microfilaments (Figs 22, 25), and the plasma membrane is not covered with any obvious cell coat (Figs 22, 25). There is a centrosomal region adjacent to the nucleus that contains a very small microtubule organizing center (MTOC) that is surrounded by Golgi and other vesicles (Figs 26, 27). MTOC are small and only visible in one section or in two adjacent sections. We interpret them to be roughly discoid in shape and the focus of no more than about one dozen microtubules (Figs 26, 27). In some views they appear to be lamellate with a very thin electron dense layer on each surface with an electron lucent layer subtending each surface layer, and a thicker electron dense layer midmost (Fig. 26).

DISCUSSION

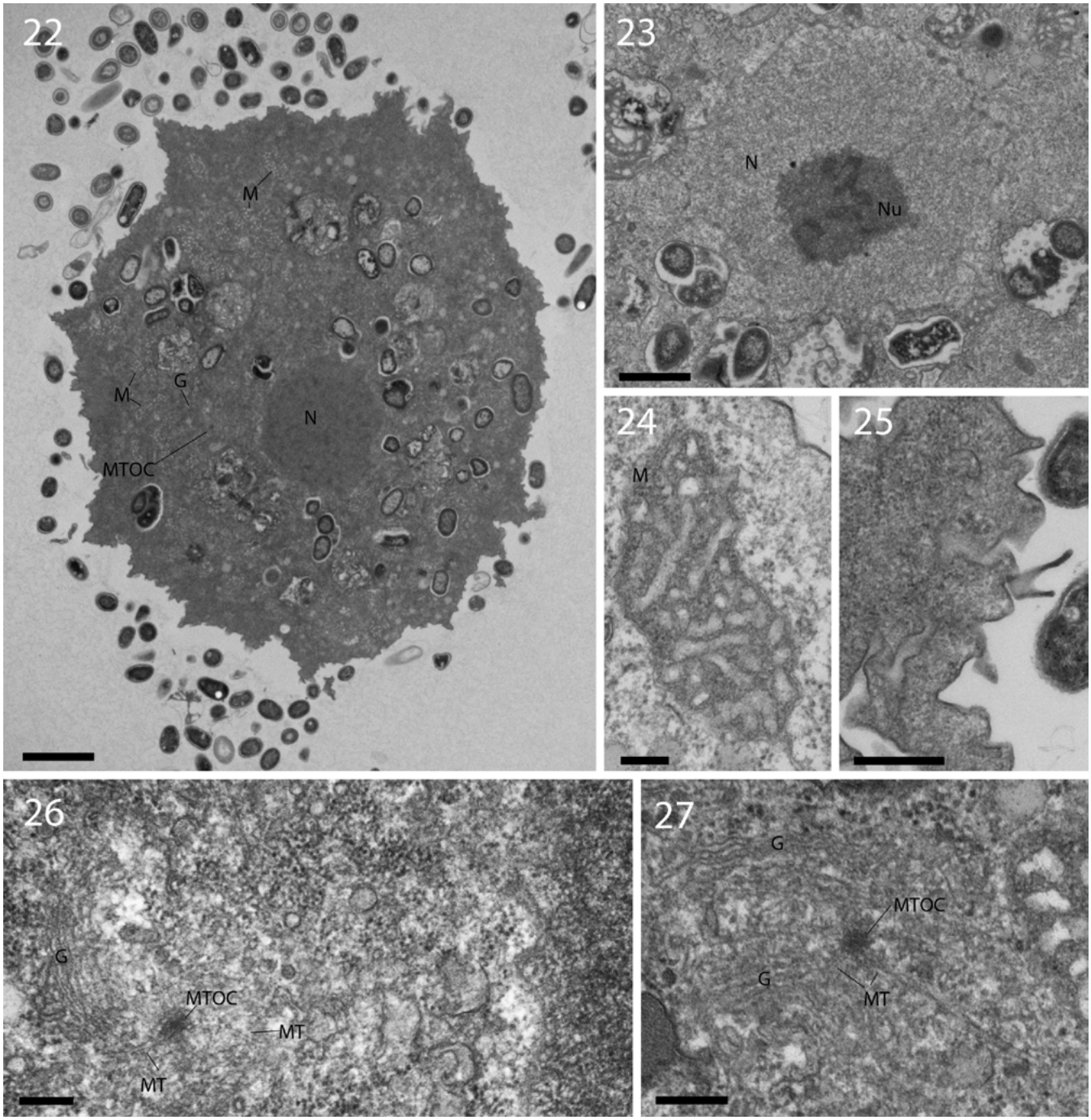
Most protosteloid amoebae have been placed into morphological and phylogenetic groups in which the amoebae and prespore cells share strong similarities, but the fruiting bodies are unique and thus often delineate the species (Olive 1975, Spiegel 1990, Shadwick *et al.* 2009, Adl *et al.* 2012). This new species, *Luapeleamoeba hula* n. g. n. sp., does not fit into any of the morphological groups of protosteloid amoebae with respect either to fruiting or amoebal morphology, and its molecular placement has previously been shown to be within the centramoebids, closely related to *Protacanthaomeba bohemica* (Shadwick *et al.* 2009).

This species' sporocarps are distinct from all presently described protosteloid amoebae (Spiegel *et al.* 2007) with respect to their short, rigid, apophysate stalks where the apophysis appears to be completely embedded in what we assume is an invagination at the base of the spore. Other similar species have longer, more flexuous stalks, e.g., the pellitid *Endostelium* spp. and *"Protostelium" pyriformis* of undetermined affinity have apophyses that insert fully into the base of the spore (Olive 1975, Spiegel 1990, Spiegel *et al.* 2007), but neither species has spores which continuously change shape atop the stalk as dramatically as our new species does. Both also have stalks that develop while surrounded by an invagination of the developing fruiting body (Olive 1975, Spiegel *et al.* 2007), but so does the myxogastrid *Echinostelium bisporum* (Spiegel and Feldman 1989). Neither *Endostelium* nor *E. bisporum* are closely related in molecular phylogenetic analyses (Shadwick *et al.* 2009, Kudryavtsev *et al.* 2014); thus, internal stalk development likely arose independently more than once. Additionally, spores of our new species do not wave around or "flag" on top of the stalk in the way that is characteristic of either *Endostelium zonatum* or *"Protostelium" pyriformis*.

Our new species is distinct from other medium to short stalked protosteloid amoebae (see Spiegel *et al.* 2007 for illustrations of these). Several have two or more spores. *Echinostelium bisporum* and *Echinosteliopsis oligospora* both have multiple spores atop their stalks. This characteristic also separates it from all of the protosporangiids, including *Clastostelium recurvatum* and all members of the genus *Protosporangium* (Shadwick *et al.* 2009, Spiegel *et al.* in Press) and from *Microglomus paxillus* (Olive 1975).

It is distinct from *"Protostelium" arachisporum* in that *"P." arachisporum* has a longer more flexuous stalk and a more elongate to peanut-shaped spore. However, there is a knob-like apophysis embedded in a shallow invagination of the spore in *"P." arachisporum* (Olive and Stoianovitch 1969, Spiegel *et al.* 2007).

Fruiting bodies can be distinguished from all species of cavosteliids, including *Cavostelium apopysatum, Tychosporium acutostipes,* and all members of



Figs 22–27. Ultrastructure of the amoeba of *Luapeleamoeba hula* n. g. n. sp. **22.** General ultrastructure showing nucleus (N) with homogeneous central nucleolus, mitochondria (M), and centrosomal region with Golgi n. g. n. sp. (G) and MTOC. Note lack of an obvious cell coat. Scale bar – 2.0 µm. **23.** Detail of nucleus (N) and portion of the nucleolus (Nu). Scale bar – 1.0 µm. **24.** Detail of mitochondrion (M). Scale bar – 500 nm. **25.** Detail of cell surface showing microfilament-rich cortex and lack of cell coat. Scale bar – 500 nm. **26, 27.** Detail centrosomal region, Golgi (G), MTOC, and microtubules (MT on figures). Fig. 26 is an enlargement of the centrosomal region of Fig. 22 and views the MTOC from a perspective that shows its lamellate structure. Scale bars – 200 nm.

the genus *Schizoplasmodiopsis* (Shadwick *et al.* 2009, Spiegel *et al.* in Press), because none of those have deciduous spores. It can be distinguished from all species of protosteliids including *Protostelium mycophaga, Protostelium okumukumu, Protostelium nocturnum*, and *Planoprotostelium aurantium* (Shadwick *et al.* 2009, Spiegel *et al.* in Press), because all of those have much more flexuous stalks which are longer relative to the size of the spore. In addition, *Protostelium sensu stricto* species have orange pigmentation, and spores with cell walls that do not change shape on top of the stalk (Spiegel *et al.* 1994).

The fruiting bodies of the new species could possibly be confused with some of the short stalked schizoplasmodiids, *Schizoplasmodium seychellarum* and *S. obovatum* (Olive and Stoianovitch 1966, 1976), but can be easily distinguished by looking closely at the spores which continuously change shape in the new species, but are much more static in the schizoplasmodiids. Also the apophysis on the stalks of schizoplasmodiids is more robust and cup-like (Spiegel 1990). The short stalked schizoplasmodiids are all ballistosporous. These ballistosporous species include *Schizoplasmodium cavostelioides*, *S. obovatum*, and *S. seychellarum* (Olive and Stoianovitch 1966, 1976) and use a Buller's drop-like spore dispersal mechanism (Pringle *et al.* 2005; van Niel *et al.*1972; Olive and Stoianovitch 1966, 1976). The new species does not have a Buller's drop-like spore dispersal mechanism. In addition, the plasmodia of the schizoplasmodiids are so morphologically distinct from the amoebae of our new species that they would not be confused.

L. hula can easily be distinguished from *Soliformovum irregularis* and *Soliformovum expulsum* because its stalk is much shorter than *S. irregularis,* and it never has a bent stalk like that of *S. expulsum* (Olive and Stoianovitch 1981). Its amoebae are much thicker than those of *Soliformovum* and completely lack the fine, filose subpseudopodia that are characteristic of the genus *Soliformovum.*

Initially we thought that *L. hula* was ballistosporous because of the speed with which the spores disappeared from the stalks and the apparent lack of a spore directly below on the agar. We now think that the new species is simply deciduous and the "spore" germinates more or less immediately when it touches the agar surface (Supplementary Video S1). This is somewhat similar to what is seen in *"Protostelium" arachisporum* which is reported sometimes to crawl down its own stalk onto the agar surface (Olive and Stoianovitch 1969).

Diurnal patterns of fruiting are often noted in protosteloid amoebae (Olive 1975). Like *L. hula* n. g. n. sp., both *Protostelium nocturnum* and *Clastostelium recurvatum* maintain a strong diurnal rhythm, in which all fruiting is limited to certain parts of the day (Olive and Stoianovitch 1977, Spiegel 1984). However, both *Protostelium nocturnum* and *Clastostelium recurvatum* generally fruit heavily late at night into early morning (Olive and Stoianovitch 1977, Spiegel 1984). *"P" arachisporum* was reported to fruit at noon (Olive and Stoianovitch 1969). Unlike these protosteloid amoebae, *L. hula* generally fruits in the mid to late afternoon (between 15:00 and 18:00). Since the culture has been cultivated in the laboratory for years and has been revived from liquid nitrogen storage, the diurnal rhythm has become less pronounced. Also, this may vary from isolate to isolate, so we cannot say with certainty that all strains of this species will fruit as does LHI05-M5g-1.

Our initial impression, based on the shape of the developing sporocarp and its internal stalk production, was that this species would be related to *Endostelium*, a taxon of pellitid in which most described species produce sporocarps (Olive *et al.* 1984, Kudryavtsev *et al.* 2014, Spiegel 1990, Spiegel *et al.* 2007). However, *L. hula* n. g. n. sp. lacks the thick cell coat and punctate ventral subpseudopodia of pellitids (Kudryavtsev *et al.* 2014, Bennett, 1986a). It also lacks the extensive centrosomal MTOC that is reported in some pellitids (Kudryavtsev *et al.* 2014, Bennett 1986a).

Our earlier molecular phylogenetic work indicated that *L. hula*, reported as LHI05, is a member of the Acanthamoebidae Sawyer and Griffin 1975, a family that includes the genera *Acanthamoeba* and *Protacanthamoeba* (Shadwick *et al.* 2009). Shadwick *et al.* 2009 show *L. hula* in a monophyletic clade with *Protacanthamoeba bohemica,* making Acanthamoebidae paraphyletic. Subsequent work on the acanthamoebids and centramoebids shows this same relationship using a phylogenomic approach (Tice *et al.* 2016; Tice *et al.* unpublished). Because of the method by which LHI05-M5g-1 was isolated, we are certain that it was in monoeukaryotic culture. The subsequent unpublished molecular data and the fact that only one type of amoeba has been seen in both light microscopic and ultrastructural work also make us confident that our placement of *L. hula* with the acanthamoebids is accurate.

Nonetheless, the amoeba of *L. hula* is very distinct from typical acanthamoebids. Its subpseudopodia are shorter and blunter that the acanthapodia characteristic of *Acanthamoeba* and *Protacanthamoeba* (Volkonsky

1931; Page 1967, 1981; Sawyer and Griffin 1975; Dyková *et al.* 2005). In subaerial view on PIP and culture plates, typical acanthamoebids appear to be more uniformly flattened (unpublished observations) than do the amoebae of *L. hula*, which are distinctly dome shaped with a profile similar to a shield volcano. The nucleoli of typical acanthamoebids are proportionally smaller within the nucleus than those of *L. hula* (see Page 1983 for good examples in *Acanthamoeba*). Like typical acanthamoebids, *L. hula* has a juxtanuclear centrosomal region with Golgi apparatus and a distinct MTOC (Page 1967, 1981; Sawyer and Griffin 1975, Dyková *et al.* 2005, see also Adl *et al.* 2012). However, the MTOC of *L. hula* is smaller, and less obviously laminate. It is also smaller than the laminate MTOC of *"Protostelium" pyriformis* (Bennett 1986b), a protosteloid amoebae we suspect may be an acanthamoebid (Adl *et al.* 2102). Also, we have noticed in subaerial view that typical acanthamoebids often have proportionally large contractile vacuoles that are juxtanuclear (personal observations, see also Page 1983 for good examples). Unlike typical acanthamoebids (Page 1967, 1981, 1983; Sawyer and Griffin 1975; Dyková *et al.* 2005), amoebae of *L. hula* rarely encyst, and if/when they do, their cysts appear almost to be without cell walls.

Although the morphology of the amoebae of *L. hula* does not conform with that of either *Acanthamoeba* or *Protacanthamoeba*, molecular phylogenetic data completely support it as a sister group to *Protacanthamoeba* and that *Protacanthamoeba* + *Luapeleamoeba* form a sister group to *Acanthamoeba* (Shadwick *et al.* 2009, Tice *et al.* 2016, Tice *et al.* unpublished). Exclusion of *L. hula* from Acanthamoebidae would make the taxon paraplyletic. Therefore, the new species must be included in Acanthamoebidae, and an understanding of the diversity of amoebal forms must be addressed in further work.

As a result of our observations of both fruiting characters and amoebal characters, and in light of our data suggesting a phylogenetic position with the acanthamoebids, we feel confident that LHI05-M5g-1 is a species new to science that belongs in a genus new to science.

To our knowledge, this amoeba has been recorded only four times, and two records are from the same site on the Big Island of Hawai`i. The first record is a drawing of two fruiting bodies and an amoeba made by F. W. Spiegel from substrates collected at Manuka Natural Area Reserve in 1998, which he labeled "new species T" in his unpublished notes. For the second record, both L. L. Shadwick and F. W. Spiegel established monoeukaryotic cultures of this new species from substrates they collected together at the same site in 2005. The third and fourth recording of the species was as Proto-DRC-Sp2 = LHI05 from two sites in the Democratic Republic of the Congo (de Haan *et al.* 2014).

TAXONOMIC SUMMARY

urn:lsid:zoobank.org:pub:ABD2943C-C4E9-44F0-9B55-177E35E77E13

Eukaryota Chatton, 1925
Amoebozoa Lühe, 1913, *sensu* Cavalier-Smith, 1998
Centramoebida Rogerson et Patterson, *sensu* Cavalier-Smith, 2004
Acanthamoebidae Sawyer and Griffin, 1975
Luapeleamoeba n. g. Shadwick et Spiegel
urn:lsid:zoobank.org:act:AEB79E11-1B42-49E5-A852-2559706EE22B

Diagnosis: During locomotion, these flabellate, uninucleate amoebae have a single broad, hyaline lamellipodium with blunted triangular subpseudopodia at the leading edge. Following the lamellipodium is a thick granuloplasmic region containing a single nucleus and a conspicuous contractile vacuole that is usually posterior to the nucleus and no more than one half the diameter of the nucleus away from the nucleus. The diameter of the single, homogeneous nucleolus is at least half the diameter of the nucleus. There is a juxtanuclear centrosomal region with Golgi, other vesicles, and an electron dense MTOC that appears to be lamellate. This MTOC is smaller and less obviously lamellate than those seen in other acanthamoebid centramoebids. The actively moving amoeba may have subpseudopodia at its trailing edge, but a well formed uroid is not present. In addition, the amoeba is thickest near the contractile vacuole and nucleus, tapering gradually toward the edges, thus the amoeba has the overall appearance of a minute shield volcano. The floating form is round but not smooth. No flagellate form is known. The cysts are rare and thin-walled in the type species. Subaerial fruiting bodies have been observed in the type species.

Etymology: Derived from the Hawaiian word *luapele*, which means volcanic crater, for the conspicuous contractile vacuole in the amoeba and its overall resemblance to a Hawaiian shield volcano (Fig. 2).

Type species: *L. hula* Shadwick et Spiegel.

Luapeleamoeba hula n. sp. Shadwick et Spiegel.

urn:lsid:zoobank.org:act:E7D6D3C3-9164-4DDE-9010-9E39B3BBA2A1

Diagnosis: *L. hula* n. sp., Sporocarps average 23.7 μm tall with stalk. Stalk 8.97 μm in total length; with an apophysis at the tip of the stalk, embedded within the spore. Stalk is inflexible along its whole length including the articulation below the apophysis. Spores, single, uninucleate, shape continuously variable, but typically in the shape of an upside-down pear when viewed from the side and round when viewed from the top. Spores germinate as uninucleate, nonflagellate amoebae that are characteristic of the genus. Amoebae are often flabellate on agar surface, with broad, hyaline, anterior lamellipodium with an average length of 7.3 μm with short rounded triangular subpseudopodia. The average fraction of frontal hyaloplasm is 0.17. The length of the locomotive form averages 42 μm, breadth of locomotive form averages 38 μm with an average length/breadth ratio of 1.15. Cysts are rare and thin-walled.

Etymology: The specific epithet is from the Hawaiian *hula* which means dance, for the spore of this species continuously changes shape as if it were dancing.

Type locality: Manuka Natural Area Reserve, Hawai`i USA. GPS: 19.110217° N, 155.825600° W, a native upland dry/mesic forest, elevation 547 meters above sea level.

Prevalence: This species has been recorded twice at the type locality on the Big Island of Hawai`i and twice in the Democratic Republic of the Congo.

Ecology/Habitat: This species has been isolated in association with decaying plant matter. It has been shown to eat both fungi and bacteria associated with decaying plants.

Specimens examined: We have isolated and examined two cultures of this species from dead leaves of mamaki (*Pipturus albidus* [Hook and Arn.] A. Gray ex. H. Mann) and ground litter samples from the type locality the Manuka Natural Area Reserve on the Big Island of Hawaii in the state of Hawaii, USA. The type specimen, LHI05-M5g-1, from mamaki ground litter was isolated by F. W. Spiegel. The isotype, LHI05-M5g-2, was isolated from the same collection by L. L. Shadwick, Collection Date August 26, 2005. This species had previously been observed and recorded from a Manuka Natural Area Reserve collection HI98-81a on October 14, 1998, from standing dead fronds of *Nephrolepus* sp.

Type material: A fixed and embedded resin TEM block of the type isolate LHI05-M5g-1 was deposited in the Smithsonian Museum under accession number 1416887. This permanent physical specimen is considered the hapantotype (name-bearing type) of the species. The type culture (LHI05-M5g-1) has been deposited with the American Type Culture Collection Accession # ATCC PRA-198 in the Eumycetozoan Project Special Collection.

Type strain sequence data: Two clones of the partial SSU of the type strain has been deposited on NCBI GenBank accession numbers FJ792702 and FJ794612.

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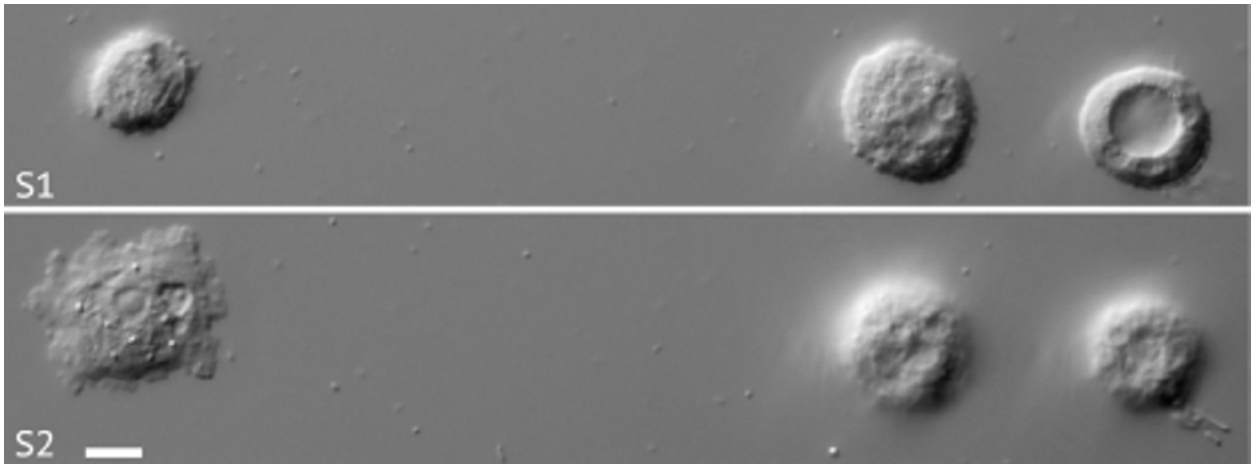
SUPPLEMENT



Supplemental Figs S1–S2. Three floating forms of *Luapeleamoeba hula.* **S1.** The amoeba on the left attaches to the surface of a glass slide. **S2.** Image of attached amoeba, taken approximately 3 minutes after S1. Scale bar – 10 μm.

Suplemental videos available at: http://www.ejournals.eu/Acta-Protozoologica/2016/Volume-55-Issue-3/art/8259/

Supplemental Video. SV1. Lh_SV1.mp4. *Luapeleamoeba hula* amoebae crawling on an agar surface. Spores move continuously on top of their stalks. One spore jerks suddenly, then crawls off as an amoeba with its stalk trailing behind. An amoeba begins to make a prespore cell on the top right corner. Images were taken every 5 seconds. This video is compiled at 30 images per second. Magnification at 10 × on a Zeiss AxioVert 135 equipped with a Canon T1i camera.

Supplemental Video. SV2. Lh_SV2.mp4. *Luapeleamoeba hula* amoebae crawl over an agar surface. The spores on top of three fruiting bodies move around constantly until they each germinate as amoebae. Images were taken every 30 seconds. This video is compiled at 30 images per second. Magnification at 10 × on a Zeiss AxioSkop 2 Plus equipped with a Canon 5DS camera.

Supplemental Video. SV3. Lh_SV3.mp4. Real-time video of floating forms of *Luapeleamoeba hula.* Floating forms are round with margins that are not rough and irregular. They sometimes have a conspicuous contractile vacuole. A drop of liquid WMY with amoeboid cells were deposited on a glass slide and a coverslip was added. Immediately the slide was viewed and videoed to show that rapid transition from floating to adhered. The video starts with a 20 × objective and then the objective was switched to 40 ×. The three cells are videoed at the end of the video are the same cells that are depicted in Figs S1 and S2. Magnification at 20 × and 40 × on a Zeiss AxioSkop 2 Plus equipped with a Canon 5DS camera.