

## Redescription of *Protomagalhaensia granulosa* Peregrine, 1970 (Apicomplexa: Eugregarinida: Blabericolidae) Parasitizing the Discoid Cockroach, *Blaberus discoidalis* (Dictyoptera: Blaberidae)

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**ABSTRACT:** *Protomagalhaensia granulosa* Peregrine, 1970, is redescribed from the type host, the discoid cockroach, *Blaberus discoidalis*. Complete morphometric data on all life cycle stages is presented, and *P. granulosa* is distinguished from other species in the genus and stabilized with the deposition of new voucher specimen material. Species of *Protomagalhaensia* are distinguished by differences in relative metric ratios, morphology of oocysts, and by relative metric ratios of mature gamonts in association.

**KEY WORDS:** Apicomplexa, Blabericolidae, Gregarine, *Protomagalhaensia blaberae*, *Protomagalhaensia cerastes*, *Protomagalhaensia granulosa*, *Protomagalhaensia serpentula*, *Protomagalhaensia wolffi*.

The cockroaches (Dictyoptera: Blattaria) are a cosmopolitan insect group comprising 4,337 described species constituting 515 genera (Roth, 1983). The genus *Blaberus* (Blattaria: Blaberidae: Blaberinae) comprises 17 named species of large, New World cockroaches distributed from southern Florida throughout the West Indies and across Central and South America (Princis, 1963, 1971; Roth, 1969, 1983; Jurberg et al., 1977; Rocha e Silva and Aguiar, 1977; Lopes and de Oliveira, 2000). Gregarines of the genus *Protomagalhaensia* (Apicomplexa: Eugregarinida: Blabericolidae) are characteristic gregarine parasites of cockroaches (Clifton, 2009) but have been reported only sporadically over the last century.

Pinto (1918) erected the genus *Protomagalhaensia* through a series of preliminary meeting reports describing and refining the diagnosis of *Protomagalhaensia serpentula* (de Magalhaes 1900) Pinto 1918 (= *Gregarina serpentula* de Magalhaes 1900) parasitizing *Periplaneta americana* (Linnaeus, 1758) (= *Stylopyga americana* Fischer, 1846) (Dictyoptera: Blattodea: Blattidae: Blattinae) in Rio de Janeiro, Brazil (Pinto, 1918, 1922). This is the same host and locality originally reported by de Magalhaes (1900). No new report of *P. serpentula* has arisen in the intervening 92 years, although the original reports of de Magalhaes (1900) and Pinto (1918, 1922) are listed variously by Watson (1916, 1922) and Roth and Willis (1960). There has been some confusion regarding the type host of *P. serpentula*. Watson (1916, 1922) incorrectly reports the type host as "*Periplaneta orientalis* (L.)" in 1916 but as "*Blatta orientalis*" in 1922, presumably a misnomer based on the *B. orientalis* homonym (*Periplaneta americana* Linnaeus, 1758 [= *Blatta orientalis* Sulzer, 1776 but not *Blatta orientalis*

Linnaeus, 1758]). The confusion may have been initiated by de Magalhaes himself. Although the work of de Magalhaes (1900) is clearly based on *P. americana*, he mentions the rumor of a second, protomagalhaensid-like gregarine infection in *B. orientalis* but cannot recall the source: "J'ai lu quelque part, sans pouvoir préciser en quel ouvrage, qu'au moins deux espèces de Grégarines habitent en parasites le corps de la *P. orientalis*. Ce serait un nouveau trait de ressemblance avec la *P. americana*." I can find neither the report of gregarines in *B. orientalis* mentioned by de Magalhaes (1900) nor a new report of a protomagalhaensid gregarine from *P. americana* despite this host's cosmopolitan distribution as a domiciliary pest.

*Protomagalhaensia granulosa* Peregrine, 1970, and *Protomagalhaensia blaberae* Peregrine, 1970, were the second and third species described in the genus, originally reported from *Blaberus discoidalis* Serville, 1839 (Dictyoptera: Blattaria: Blaberidae: Blaberinae) and *Blaberus boliviensis* Princis, 1946 (Dictyoptera: Blattaria: Blaberidae: Blaberinae), respectively (Peregrine, 1970). More recently, Clifton and Hays (2006) redescribed *Protomagalhaensia wolffi* (Geus, 1969) Clifton and Hays, 2006 (= *Gregarina wolffi* Geus, 1969) from *Nauphoeta cinerea* (Olivier, 1789) (Dictyoptera: Blattaria: Blaberidae: Oxyhalinae: Nauphoetini). *Protomagalhaensia cerastes* Clifton (2010) is described from *Phoetalia pallida* (Brunner von Wattenwyl 1865) Princis 1967 (= *Nauphoeta pallida* Brunner von Wattenwyl 1865) (Dictyoptera: Blattaria: Blaberidae: Blaberinae) (Clifton, 2010), bringing to 5 the number of described species of *Protomagalhaensia*.

Species of *Protomagalhaensia* are recognized in part by separation of morphometric centroids.

Relatively poor character descriptions and scant data sets are available for *P. serpentula*, *P. granulosa*, and *P. blaberae*. No type material is known for *P. serpentula*, and the existing type material for *P. granulosa* and *P. blaberae* exhibits drastic fixation artifacts rendering it inadequate for morphometric analysis. Taxonomic progress in the genus requires redescription and stabilization of these preliminary taxa. Herein I begin this process by redescribing *P. granulosa* parasitizing *B. discoidalis*, differentiating it from existing species in the genus, and depositing new voucher material to stabilize the taxon.

## MATERIALS AND METHODS

*Blaberus discoidalis* breeding colonies were established using stock obtained from commercial sources. Colonies were maintained in 22-liter polycarbonate containers with coir bedding and cardboard egg-crate roosting habitat. Food (Purina® Dog Chow® brand dog food complete and balanced [Nestle Purina Pet Care Company, St. Louis, Missouri, U.S.A.]) and water were provided ad libitum. Adult or late-instar nymphal *B. discoidalis* were examined for gregarine parasites as follows. Cockroaches were held overnight in stacked 250-ml glass Carolina culture dishes (Carolina Biological Supply Company, Burlington, North Carolina, U.S.A.) each containing 15–20 individuals to collect shed feces for gametocyst studies. Cockroaches were eviscerated and their alimentary canals dissected in the generalized blaberic cockroach saline (BCS) described by Clopton (2010) (NaCl, 86 mM; KCl, 10 mM; CaCl<sub>2</sub>, 3.5 mM; MgCl<sub>2</sub>, 3 mM; NaH<sub>2</sub>PO<sub>4</sub>, 3 mM; NaHCO<sub>3</sub>, 2 mM), diluted to half strength. This more dilute saline allows extended dissection times while inducing minimal osmotic artifacts in protomogalgaensid gregarines.

Permanent microscope slide preparations were made using wet smears of gregarines and host gut tissues fixed by flotation on hot AFA (ethanol, formalin, and acetic acid), stained with either Semichon's acetocarmine (Semichon, 1924) or Harris' hematoxylin and eosin-xytol and mounted in Damar balsam (see Clopton and Hays, 2006, and references therein).

Gametocysts were isolated from collected feces, triple-rinsed in BCS, and transferred to 7-mm black cardstock disks saturated with a 0.1% aqueous methylparaben (methyl parahydroxybenzoate) solution, photographed for morphometric analysis, and placed in the well of a 60-mm center-well organ culture dish (BD Falcon, Franklin Lakes, New Jersey, U.S.A.). The outer trough of the dish was filled with a hydrating gel of finely milled cross-linked polyacrylamide (SoilMoist Granules® [JRM Chemical, Inc., Cleveland, Ohio, U.S.A.]) saturated with 0.1% aqueous methylparaben solution. The hydrating gel provides consistent high humidity for gametocyst development and dehiscence. Each dish was covered and placed inside a 100-mm glass Petri dish to reduce desiccation. Gametocysts were observed daily, and any changes in structure, maturation, or dehiscence noted. Oocyst structure and dimensions were taken from fresh preparations of oocysts in wet mounts and agar monolayer mounts as described by Clopton (2010). Oocysts were used to establish new infections to confirm the

association of gametocysts, oocysts, and gamonts by gregarine species. These techniques were used to collect new, complete oocyst and mature gamont data for *P. granulosa* and *P. wolfi*. Morphometric data sets from the original description of *P. cerastes* were used for comparative analyses.

Gregarine DNA samples were prepared and stored for future genomic analysis using a procedure similar to that described by Clopton (2009). Individual gametocysts were washed by transfer through 3 changes of BCS and 3 changes of distilled water and transferred to individual microcentrifuge tubes. A hypodermic needle was used to rupture each gametocyst, and individual microcentrifuge tubes were incubated in a 60°C hot block to dry liberated gametocyst contents before capping the microcentrifuge tube. Dried gametocyst samples were extracted using the PureLink genomic DNA mini kit (Invitrogen, Carlsbad, California, U.S.A.) and accompanying FTA protocol. Isolated DNA samples were resuspended in NE buffer (USB Corporation, Cleveland, Ohio, U.S.A.) and stored by aliquot at 4°C for future genomic analysis.

Observations were made using an Olympus B-Max 50 compound microscope with ×10, ×20, ×40, and ×60 universal planapochromatic objectives with either phase contrast condensers or differential interference contrast prisms and an infinity-optics turret doubler. Digital photographs were taken with an Olympus DP-70 digital camera through the aforementioned microscope. Measurements were taken from the digitized images of preserved specimens using Image-Pro Discovery® v 4.0 image analysis software (Media Cybernetics, L.P., Silver Spring, Maryland, U.S.A.). Photographic plates were processed and assembled using Adobe® PhotoShop® 7.0.1 software (Adobe Systems Inc., San Jose, California, U.S.A.).

The extended morphometric character set for *Protomogalgaensia* delineated by Clopton and Hays (2006) is used herein, including the following metric characters and abbreviations: satellite acetabulum depth (AcD), satellite acetabulum width (AcW), length of deutomerite (DL), distance from protomerite–deutomerite septum to deutomerite axis of maximum width (DLAM), distance from posterior end of deutomerite to deutomerite axis of maximum width (DLPM), dehiscence plate length (DPL), dehiscence plate width (DPW), width of deutomerite at equatorial axis (DWE), maximum width of deutomerite (DWM), diameter of major karyosome (KD1), distance from nucleus to protomerite–deutomerite septum (NDS), length of nucleus (NL), width of nucleus (NW), interior oocyst length (OLI), maximum exterior oocyst length (OLM), oocyst width (OW), width of protomerite–deutomerite septum (PDSW), length of protomerite (PL), distance from anterior end of protomerite to protomerite axis of maximum width (PLAM), distance from protomerite–deutomerite septum to protomerite axis of maximum width (PLPM), total length of primate (PTL), width of protomerite at equatorial axis (PWE), maximum width of protomerite (PWM), total length of satellite (STL).

The shape of structures in mature trophozoites, particularly the epimerite, comprise an important diagnostic character suite, but significant developmental variation within taxa precludes the use of absolute metrics taken from trophozoites (Filipponi, 1951; Watwood et al., 1997; Clopton, 1999). Separate descriptions of primate and satellite ontogenetic stages are provided to account for the sexual

dimorphism (Filipponi, 1947, 1951, 1952, 1954, 1955). Measurements are presented in micrometers as mean values followed by range values, standard deviations, and sample sizes in parentheses. Terminology for parasite ontogenetic stages and anatomy largely follows that proposed by Levine (1971). Terminology for shapes of planes and solids follows Clopton (2004).

## RESULTS

### *Protomagalhaensia granulosa* Peregrine, 1970 (Figs. 1–19)

#### Generic diagnosis

Order Eugregarinida Léger, 1892, sensu Clopton (2002); suborder Septatina Lankester, 1885, sensu Clopton (2002); superfamily Gregarinoidea, Chakaravarty, 1960, sensu Clopton (2009); family Blabericolidae Clopton (2009); genus *Protomagalhaensia* Pinto, 1918 sensu Clopton (2009): epimerite ovoid to deltoid, developed intracellularly within a single host intestinal epithelial cell, not retained in gamonts; trophozoites becoming elongate with maturity; association gamontic, caudofrontal, association interface a shallowly semiobpanduriform interlock in which the posterior end of the primate's deutomerite is constricted and clamped by an acetabulum formed from the anterior membranes of the satellite's protomerite; oocysts dolioform with or without spines or knobs at terminal apices, released in monete chains from gametocyst by extrusion.

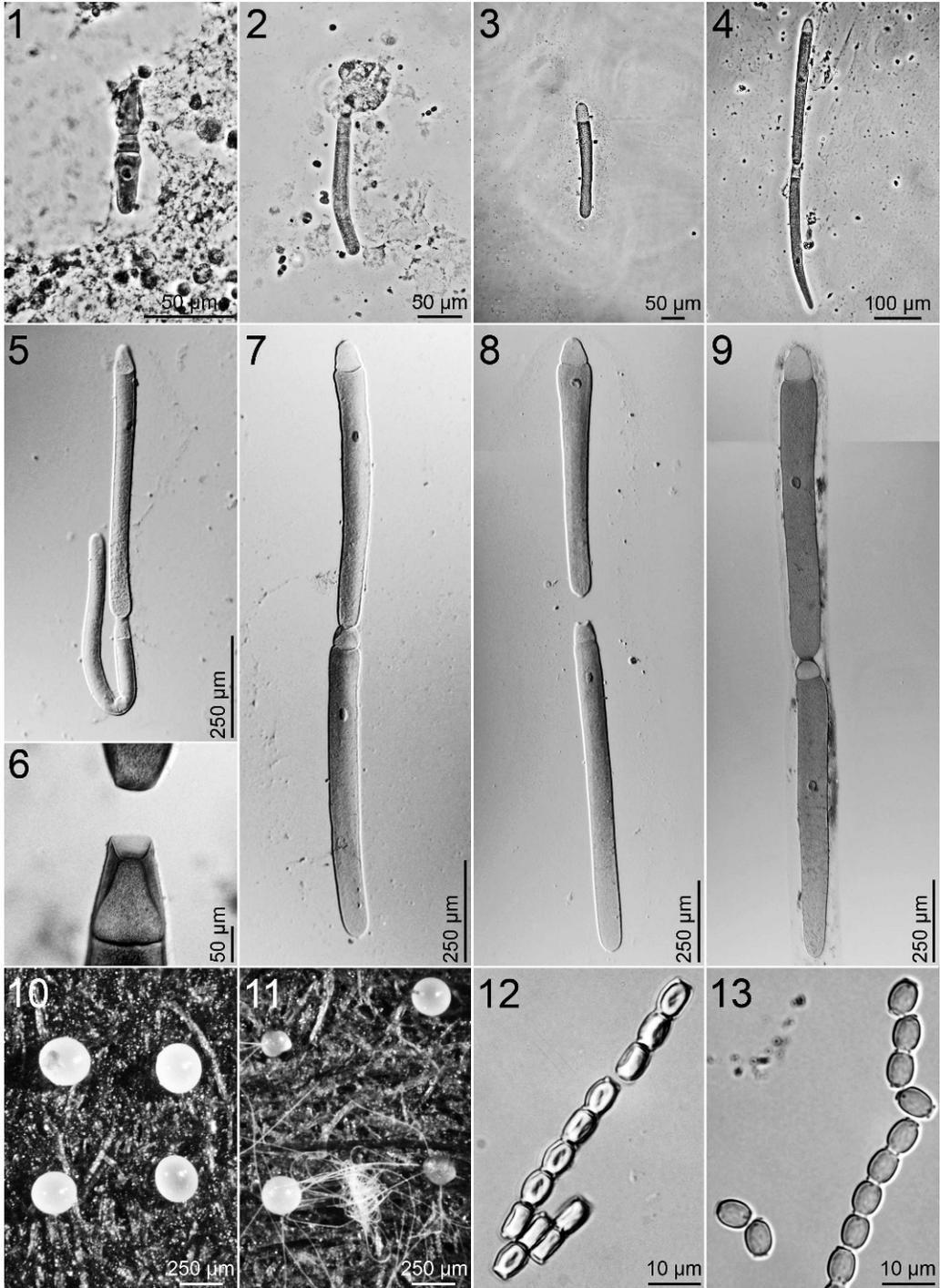
*Young solitary trophozoites* (Figs. 1–3): Young trophozoites solitary, extracellular forms attached to host ventricular epithelium. Holdfast a simple (i.e., lacking a diamerite) epimerite developing intracellularly in a single host epithelial cell (Fig. 1). Epimerite gladiate to deltoid in solitary trophozoites (Figs. 1–2). Protomerite broadly to shallowly ovoid in young trophozoites, becoming oblong in older solitary trophozoites, markedly constricted at protomerite–deutomerite septum (cf. Fig. 1 and Figs. 2–3). Deutomerite obovoid in young solitary trophozoites, becoming very narrowly to linearly oblong in older solitary trophozoites (cf. Fig. 1 and Figs. 2–3). Nucleus orbicular with 1 distinct, large, smooth-margined karyosome.

*Association* (Figs. 4–9): Presyzygial, gamontic; gamonts anisomorphic because of structures involved in association interface; association interface a shallowly semiobpanduriform interlock in which the posterior end of the primate's deutomerite is constricted and clamped by an acetabulum formed from the anterior

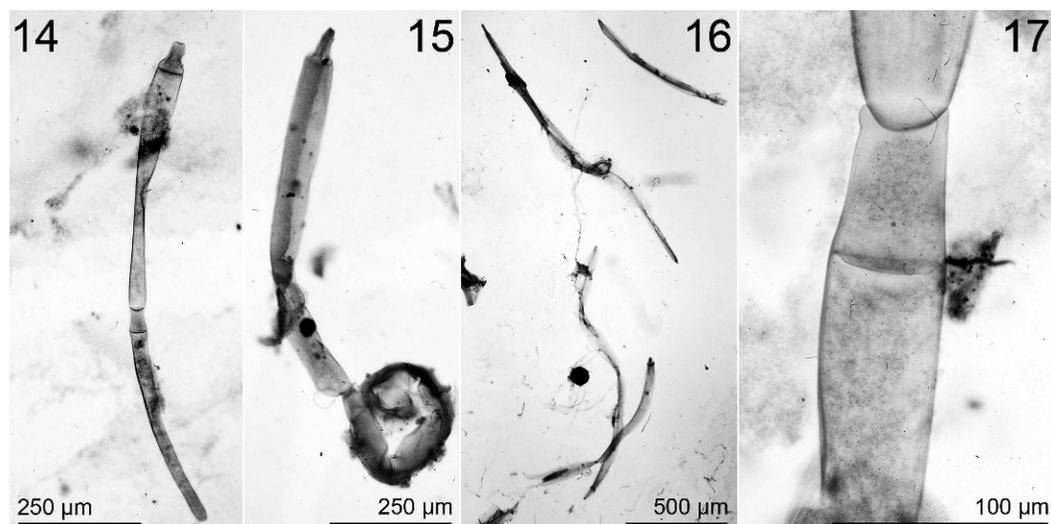
membranes of the satellite's protomerite (Figs. 6, 8). Measurements taken from mature associations only (cf. immature association of Fig. 4 and mature associations of Figs. 7–9). Indices: PTL/STL 0.9 (0.8–1.1,  $\pm 0.06$ , 60), PPL/SPL 1.2 (0.8–1.6,  $\pm 0.17$ , 60), PPWM/SPWM 1.2 (0.9–1.5,  $\pm 0.12$ , 60), PDL/SDL 0.9 (0.8–1.0,  $\pm 0.06$ , 60), PDWM/SDWM 1.2 (0.9–1.4,  $\pm 0.13$ , 60), PDWE/SDWE 1.0 (0.8–1.3,  $\pm 0.10$ , 60).

*Primate*: Observations and data taken from mature associations only (cf. immature association of Fig. 4 and mature associations of Figs. 7–9). Epimerite absent; protomerite pyriform to deeply deltoid; PL 86.1 (58.0–115.0,  $\pm 11.85$ , 60); PWE 56.2 (37.1–95.5,  $\pm 10.49$ , 60); PWM 69.8 (50.4–102.0,  $\pm 10.10$ , 60); PLAM 72.1 (51.3–93.2,  $\pm 8.98$ , 60); PLPM 15.8 (3.8–29.5,  $\pm 6.04$ , 60); PDSW 66.2 (47.7–89.4,  $\pm 8.69$ , 60); PL/PWE 1.6 (1.2–2.4,  $\pm 0.23$ , 60); PL/PWM 1.2 (1.0–1.6,  $\pm 0.14$ , 60); PL/PDSW 1.3 (1.0–1.6,  $\pm 0.15$ , 60); PLAM/PL 0.8 (0.7–1.0,  $\pm 0.07$ , 60); PLAM/PLPM 5.6 (2.0–19.9,  $\pm 3.28$ , 60); PWM/PWE 1.3 (1.1–1.5,  $\pm 0.10$ , 60). Deutomerite elongated, very narrowly to linearly obpanduriform; DL 763.8 (635.5–861.0,  $\pm 57.51$ , 60); DWE 69.7 (55.3–103.0,  $\pm 10.33$ , 60); DWM 88.8 (63.4–133.0,  $\pm 13.67$ , 60); DLAM 86.6 (18.9–221.0,  $\pm 39.85$ , 60); DLPM 679.0 (474.3–802.0,  $\pm 64.97$ , 60); DL/DWE 11.1 (7.8–14.0,  $\pm 1.36$ , 60); DL/DWM 8.7 (6.3–11.0,  $\pm 1.10$ , 60); DLAM/DL 0.1 (0.0–0.3,  $\pm 0.05$ , 60); DLAM/DLPM 0.1 (0.0–0.5,  $\pm 0.07$ , 60); DWM/DWE 1.3 (1.1–1.7,  $\pm 0.13$ , 60); PTL 845.3 (708.9–960.0,  $\pm 62.66$ , 60). Indices: PTL/PL 10.0 (7.6–12.8,  $\pm 1.22$ , 60); DL/PL 9.0 (6.8–11.7,  $\pm 1.18$ , 60); DWM/PWM 1.3 (1.1–1.4,  $\pm 0.08$ , 60); PTL/DL 1.1 (1.1–1.2,  $\pm 0.01$ , 60). Nucleus broadly elliptoid with a single, concentric broadly elliptoid karyosome; NL 35.8 (25.3–51.1,  $\pm 5.71$ , 60); NW 31.4 (19.3–47.3,  $\pm 6.04$ , 60); NDS 144.5 (13.2–407.9,  $\pm 86.31$ , 60); KD1 9.3 (3.8–16.2,  $\pm 2.30$ , 60); NL/NW 1.2 (0.9–1.6,  $\pm 0.17$ , 60); NDS/NL 4.1 (0.4–9.9,  $\pm 2.34$ , 60); DL/NDS 8.3 (1.9–56.6,  $\pm 8.03$ , 60); NL/KD1 4.0 (2.4–7.8,  $\pm 1.02$ , 60).

*Satellite*: Observations and data taken from mature associations only (cf. immature association of Fig. 4 and mature associations of Figs. 7–9). Protomerite finely to deeply deltoid, anterior membranes forming a cup-shaped acetabulum (Figs. 6, 8); PL 75.0 (57.9–93.3,  $\pm 8.59$ , 60); AcW 40.9 (29.9–58.7,  $\pm 5.33$ , 60); AcD 13.4 (7.7–20.9,  $\pm 2.93$ , 60); PWE 51.5 (33.8–71.8,  $\pm 7.92$ , 60); PWM 58.7 (44.1–83.4,  $\pm 8.54$ , 60); PLAM 60.7 (39.7–78.7,  $\pm 7.88$ , 60); PLPM 14.9 (8.1–23.7,  $\pm 3.68$ , 60); PDSW 55.0 (39.0–75.7,



**Figures 1–13.** *Protomagalhaensia granulosa*. 1. Young trophozoite with epimerite fully embedded within a host cell, protomerite and deutomerite extracellular. 2. Young elongate trophozoite with epimerite still fully embedded in host cell. 3. Young elongate trophozoite free in the intestinal lumen but without epimerite. 4. Young gamonts in association. 5. Immature gamonts in association. 6. Acetabular association interface of satellite. 7–9. Mature gamonts in association. 10. Gametocysts. 11. Monete oocyst chains extruded from mature gametocyst. 12. Monete oocyst chains in thick water mount (surface focal plane), note corpuscular surface of oocysts. 13. Monete oocyst chains in agar mount (frontal focal plane), note residua, apical corner knobs and polar dehiscence plates.



Figures 14–17. *Protomagalhaensia granulosa*, representative syntypes (The Natural History Museum, London, England, U.K. NHM registration numbers 1970:3:3:3 and 1970:3:3:4). Note severe fixation artifacts of syntypes. 14–15. Mature gamonts in association. 16. Associations of varying maturity. 17. Acetabular association interface of satellite.

$\pm 6.70$ , 60); AcW/AcD 3.2 (1.9–5.5,  $\pm 0.72$ , 60); AcW/PWM 0.7 (0.5–1.0,  $\pm 0.10$ , 60); AcD/PL 0.2 (0.1–0.3,  $\pm 0.04$ , 60); PL/PWE 1.5 (1.1–2.1,  $\pm 0.26$ , 60); PL/PWM 1.3 (0.9–1.9,  $\pm 0.23$ , 60); PL/PDSW 1.4 (1.0–2.2,  $\pm 0.23$ , 60); PLAM/PL 0.8 (0.7–0.9,  $\pm 0.05$ , 60); PLAM/PLPM 4.3 (2.1–7.9,  $\pm 1.30$ , 60); PWM/PWE 1.2 (1.0–1.4,  $\pm 0.09$ , 60). Deutomerite elongated, linearly oblong; DL 856.2 (728.0–964.7,  $\pm 61.63$ , 60); DWE 67.9 (50.7–99.7,  $\pm 11.08$ , 60); DWM 77.7 (60.8–115.0,  $\pm 11.11$ , 60); DLAM 150.7 (52.4–353.0,  $\pm 62.75$ , 60); DLPM 692.2 (174.0–854.1,  $\pm 115.28$ , 60); DL/DWE 12.9 (8.7–15.2,  $\pm 1.74$ , 60); DL/DWM 11.2 (7.8–13.8,  $\pm 1.45$ , 60); DLAM/DL 0.2 (0.1–0.4,  $\pm 0.07$ , 60); DLAM/DLPM 0.2 (0.1–0.7,  $\pm 0.14$ , 60); DWM/DWE 1.2 (1.0–1.4,  $\pm 0.08$ , 60); STL 925.6 (785.9–1,038.0,  $\pm 63.01$ , 60). Indices: STL/PL 12.5 (9.2–15.4,  $\pm 1.29$ , 60); DL/PL 11.5 (8.3–14.4,  $\pm 1.27$ , 60); DWM/PWM 1.3 (1.1–1.6,  $\pm 0.10$ , 60); STL/DL 1.1 (1.1–1.1,  $\pm 0.01$ , 60). Nucleus broadly elliptoid with a single, concentric broadly elliptoid karyosome; NL 35.1 (23.5–53.7,  $\pm 6.34$ , 60); NW 29.6 (20.0–44.1,  $\pm 4.99$ , 60); NDS 278.6 (29.5–592.3,  $\pm 132.27$ , 60); KD 9.8 (5.4–15.0,  $\pm 2.31$ , 60); NL/NW 1.2 (0.8–1.6,  $\pm 0.18$ , 60); NDS/NL 8.0 (1.0–15.9,  $\pm 3.86$ , 60); DL/NDS 4.3 (1.4–25.8,  $\pm 3.70$ , 60); NL/KD 3.8 (2.3–6.9,  $\pm 0.94$ , 60).

**Gametocysts** (Figs. 10–11): Opaque, irregularly orbicular to elliptoid in outline, length (GL) 273.2 (243.0–305.0,  $\pm 16.49$ , 45); width (GW) 259.5 (229.0–293.0,

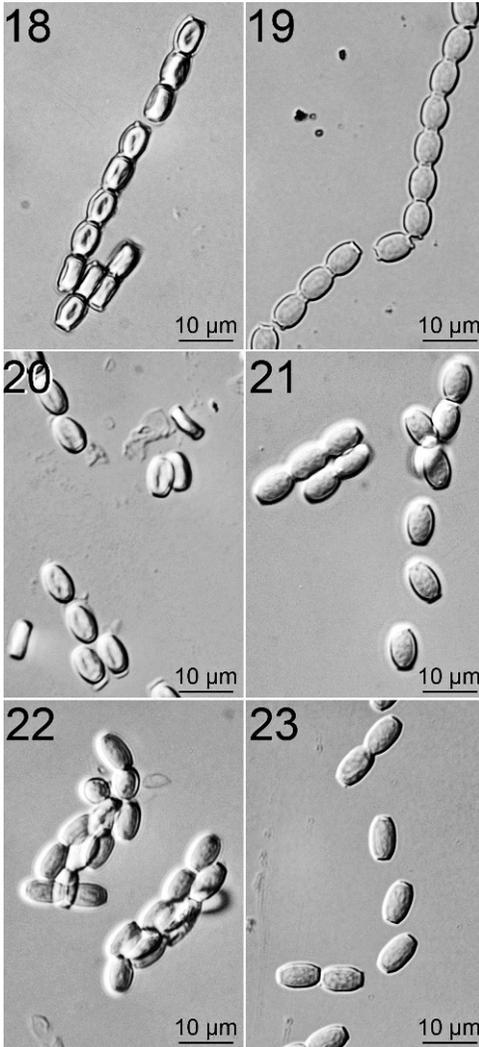
$\pm 18.07$ , 45); GL/GW 1.1 (1.0–1.1,  $\pm 0.03$ , 45). Of 60 gametocysts collected and stored under moist conditions, most dehisced within 60–72 hr, releasing oocysts in monete chains by extrusion (cf. Fig. 10 and Fig. 11).

**Oocysts** (Figs. 12–13, 18–19): Dolioform to broadly dolioform with terminal dehiscence plate, oocysts broadly dolioform in outline without dehiscence plate; presenting as dolioform in outline with apical corner knobs with dehiscence plate (Figs. 13, 19); sagittally flattened, corpuscular with dorsal surface depression (Figs. 12, 18); OLM 6.9 (6.6–7.2,  $\pm 0.18$ , 60); OLI 6.1 (5.5–6.5,  $\pm 0.23$ , 60); OW 5.2 (5.0–5.3,  $\pm 0.08$ , 60); DPW 2.8 (2.5–3.1,  $\pm 0.14$ , 60); DPL 0.5 (0.3–0.7,  $\pm 0.10$ , 60); ResDia 0.8 (0.5–1.0,  $\pm 0.12$ , 60); OLM/OLI 1.3 (1.2–1.4,  $\pm 0.04$ , 60); OLM/OW 1.3 (1.2–1.4,  $\pm 0.04$ , 60); OLI/OW 1.2 (1.1–1.3,  $\pm 0.05$ , 60); DPW/DPL 6.4 (4.1–11.7,  $\pm 1.56$ , 60); OW/DPW 1.9 (1.7–2.1,  $\pm 0.10$ , 60); OLM/DPL 15.8 (9.5–26.3,  $\pm 3.68$ , 60); OLM/ResDia 8.9 (6.5–13.6,  $\pm 1.41$ , 60); OW/ResDia 6.7 (5.0–10.0,  $\pm 1.03$ , 60).

### Taxonomic summary

**Type host:** *Blaberus discoidalis* Serville, 1839 (Dictyoptera: Blattaria: Blaberidae: Blaberinae), nymphs and adults.

**Type locality:** Laboratory cultures, Department of Zoology, University College Cardiff (Cardiff University), Cardiff, Wales, United Kingdom.



**Figures 18–23.** Oocyst morphology of 3 species of *Protomagalhaensia*. **18.** *Protomagalhaensia granulosa* oocysts in thick water mount. Note sagittal compression of oocysts in lateral view and corpuscular depression of oocysts in dorsal view. **19.** *Protomagalhaensia granulosa* oocysts in thin agar mount. Note acute apical spines created by terminal dehiscence plate. **20.** *Protomagalhaensia wolfi* oocysts in thick water mount. Note sagittal compression of oocysts in lateral view, corpuscular depression of oocysts in dorsal view, and friable nature of terminal dehiscence plate. **21.** *Protomagalhaensia wolfi* oocysts in thin agar mount. Note abaxial apical spines created by terminal dehiscence plate. **22.** *Protomagalhaensia cerastes* oocysts in thick water mount. Note lack of sagittal compression and corpuscular depression. **23.** *Protomagalhaensia cerastes* oocysts in thin agar mount. Note that the terminal dehiscence plate does not create substantial apical spines.

*Types examined:* Full syntype series, 2 slides containing multiple trophozoites, gamonts, and associations, registration numbers 1970:3:3:3 and 1970:3:3:4, The Natural History Museum, London, England, United Kingdom.

*Host vouchers:* Twelve symbiotype specimens are deposited in the Sam Houston State University Insect Collection (SHSUI), Department of Biological Sciences, Sam Houston State University, Huntsville, Texas, U.S.A.

*Site of infection:* Trophozoites were collected from ventricular caeca and postintercecal region. Associations were collected from the ileum. Gametocysts were collected from host feces.

*Prevalence:* Prevalence in colony approaches 100%.

*Records:* Laboratory cultures, nymphs, and adults, Peru State College, Peru, Nebraska, U.S.A.

*Specimens deposited:* The voucher slide series for this redescription is deposited in the Harold W. Manter Laboratory for Parasitology (HWML), Division of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska, U.S.A. and comprises 45 hapantotype slides containing multiple trophozoites, gamonts, and associations accessioned as HWML100042 (45 slides, author's slide numbers REC090016h–y, REC090056a–b, REC090057a–h, REC090060a–c, REC090061a–n).

## Remarks

The syntypes of *P. granulosa* (Registration numbers 1970:3:3:3 and 1970:3:3:4, The Natural History Museum, London, England, United Kingdom) were examined, and although the general gamontic and association features of *Protomagalhaensia* are confirmed, distortion artifacts in the syntype specimens preclude their use in assembling a complete morphometric data set (Figs. 14–17). Despite preservation artifacts, the syntype series does illustrate the range of maturity included in the original gamontic data of Peregrine (1970). Data presented in the redescription herein is consistent with that of Peregrine (1970) and the syntype series but reflects the larger, mature end of the gamontic range.

### *Protomagalhaensia wolfi* (Geus, 1969) Clopton and Hays, 2006 (Figs. 20–21)

*Oocysts* (Fig. 20–21): Dolioform to broadly dolioform with terminal dehiscence plate, oocysts broadly

dolioform in outline without dehiscence plate; presenting as dolioform in outline with terminal bars with dehiscence plate (Fig. 21); sagittally flattened, corpuscular with dorsal surface depression (Fig. 20); OLM 7.6 (7.2–8.0,  $\pm 0.18$ , 60); OLI 6.7 (6.1–7.1,  $\pm 0.21$ , 60); OW 5.3 (5.1–5.6,  $\pm 0.13$ , 60); DPW 2.6 (2.0–3.0,  $\pm 0.22$ , 60); DPL 0.5 (0.3–0.8,  $\pm 0.11$ , 60); ResDia 0.9 (0.6–1.2,  $\pm 0.11$ , 60); OLM/OLI 1.1 (1.1–1.2,  $\pm 0.03$ , 60); OLM/OW 1.4 (1.3–1.5,  $\pm 0.04$ , 60); OLI/OW 1.3 (1.1–1.3,  $\pm 0.04$ , 60); DPW/DPL 5.5 (3.3–8.1,  $\pm 1.11$ , 60); OW/DPW 2.1 (1.8–2.5,  $\pm 0.18$ , 60); OLM/DPL 16.0 (9.4–24.2,  $\pm 3.36$ , 60); OLM/ResDia 8.5 (6.3–12.1,  $\pm 1.15$ , 60); OW/ResDia 6.0 (4.5–8.0,  $\pm 0.74$ , 60).

## Remarks

Clopton and Hays (2006) described the oocysts of *P. wolfi* and *Blabericola haasi*, a second gregarine that cooccurs in *N. cinerea*. In that manuscript, we associated gamonts and oocysts among species based on gametocyst shape. During the reevaluation of oocysts reported herein, a larger study of gametocysts reveals that while gametocyst size differs among *P. wolfi* and *B. haasi*, (*P. wolfi* gametocysts are smaller) gametocyst shape is not diagnostic. These observations were confirmed by establishing new infections using confirmed oocysts from isolated gametocysts. The oocyst data reported herein conforms to that for *B. haasi* as reported by Clopton and Hays (2006); thus the oocyst associations we reported therein are incorrect. The Clopton and Hays (2006) oocyst data for *P. wolfi* are actually oocyst data for *B. haasi* and vice versa.

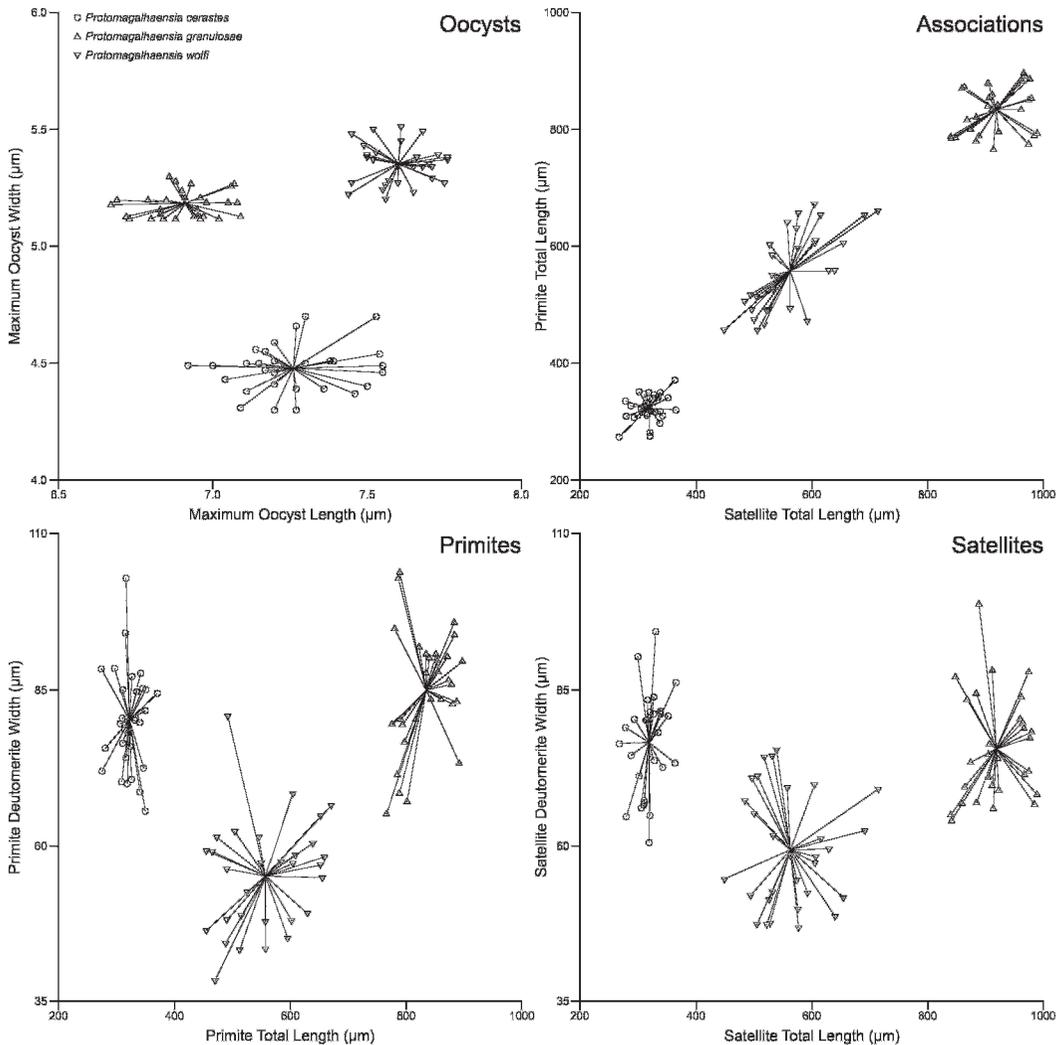
## DISCUSSION

Five species constitute *Protomagalhaensia*: the type species, *P. serpentula*, described from *P. americana*; *P. wolfi* described from *N. cinerea*; *P. blaberae* described from *B. boliviensis*; *P. cerastes* described from *P. pallida*; and, *P. granulosa*, redescribed herein from the type host *B. discoidalis*.

Epimerites are relatively consistent in form. They are simple gladiate to deltoid holdfasts that lack a diamerite. Clopton and Hays (2006) observed differences in epimerite structure among species, but these differences are neither substantial nor stable enough to allow confident species diagnosis. However, the general epimerite structure does have utility as a shared character uniting the genus. Species of *Protomagalhaensia* are most clearly distinguished by differences in relative metric ratios and morphology

of oocysts and by relative metric ratios of mature gamonts in association.

All 5 species possess dolioform to broadly dolioform oocysts that differ primarily in size, morphometric ratio, surface ornamentation, and the shape of the terminal dehiscence plate. Oocysts of *P. serpentula* are not well known, and data are limited to those reported in the original and supporting descriptions of the species: oval to dolioform in outline, possessing apical corner spines; OLM, 7 to 8  $\mu\text{m}$ ; OW, 2.5  $\mu\text{m}$  (de Magalhaes, 1900; Pinto, 1918, 1922). No photograph of a *P. serpentula* oocyst exists, but the illustration of de Magalhaes (1900) indicates the presence of a dehiscence plate with substantial axial corner spines. Oocysts of *P. blaberae* are known only from the brief original description of Peregrine (1970): “dolioform but lacking the dorsal depression and apical corner spines of *P. granulosa*,” OLM, 8.0; OW, 5.0. No photograph or illustration of these oocysts exists. Oocysts of *P. granulosa* and *P. wolfi* are distinctly dolioform and possess distinct polar dehiscence plates. These plates form distinct, acute corner spines in *P. granulosa* oocysts but only small, abaxial corner spines in *P. wolfi* oocysts (cf. Figs. 18–19 vs. 20–21). When observed in a thick water mount, oocysts of both species exhibit distinct sagittal compression with a dorsal surface depression, making the oocysts distinctly corpuscular in appearance (Figs. 18, 20). Neither sagittal compression nor the corpuscular depression is evident in a thin layer agar mount (Figs. 19, 20). Peregrine (1970) noted the dorsal depression in the oocysts of *P. granulosa* as a unique feature of the species, but it occurs in at least 1 other member of the genus. Reevaluation of the oocysts of *P. granulosa* also reveals that the terminal dehiscence plates of *P. wolfi* occur in other members of the genus and appear to form the apical spines characteristic of the taxon. Although similar in overall appearance, the oocysts of *P. granulosa* and *P. wolfi* differ in size (OLM and OW,  $6.9 \times 5.2 \mu\text{m}$  vs.  $7.6 \times 5.3 \mu\text{m}$ , respectively). Oocysts of *P. cerastes* (Figs. 22–23) are also dolioform with depressed terminal dehiscence plates that do not form apical corner spines (Fig. 23). Unlike the oocysts of *P. granulosa* and *P. wolfi*, the oocysts of *P. cerastes* do not appear sagittally flattened or corpuscular in a thick water mount but appear to take the shape of a uniform solid (Fig. 22). Although similar in overall length, the oocysts of *P. cerastes* are notably narrower than those of *P. granulosa* and *P. wolfi* (OLM, 7.3  $\mu\text{m}$ ; OW, 4.5  $\mu\text{m}$ ). Differences in overall morphometric size and shape of oocysts



**Figure 24.** Centroid clustering of *Protomagalhaensia granulosa*, *Protomagalhaensia wolfi*, and *Protomagalhaensia cerastes* based on differences in overall morphometric size and shape of oocysts and mature gamonts in association. Centroid clusters illustrate the population variation and central morphometric tendency of each species within a 95% confidence interval ( $n = 30$  individuals/species randomly chosen from population members less than 2 standard deviations from the mean).

among *P. granulosa*, *P. wolfi*, and *P. cerastes* are illustrated by centroid cluster diagram in Figure 24. Centroid clusters illustrate the population variation and central morphometric tendency of each species and clearly distinguish *P. granulosa*, *P. wolfi*, and *P. cerastes*. No population datum is available for *P. serpentula*, but the data reported by de Magalhaes (1900) place the centroid point for the species below the abscissa in Figure 24. Likewise, available data for *P. blaberae* place the centroid for this species well outside the oocyst centroid clusters illustrated in Figure 24.

Differences in overall morphometric size and shape of mature gamonts in association among *P. granulosa*, *P. wolfi*, and *P. cerastes* are illustrated by centroid cluster diagrams in Figure 24. Again, centroid clusters illustrate the population variation and central morphometric tendency of each species and clearly distinguish *P. granulosa*, *P. wolfi*, and *P. cerastes*. Although no population datum is available for *P. serpentula*, the data reported by de Magalhaes (1900) place the centroid point for associations of the species at a midpoint between *P. wolfi* and *P.*

*cerastes*. Similarly, the summary data of Peregrine (1970) for *P. blaberae* place the centroid point of the species between *P. wolffi* and *P. cerastes*. Final analysis and discrimination of taxa within the genus must await rediscovery and full morphometric redescription of *P. blaberae* and *P. serpentina*.

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