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Protomagalhaensia cerastes n. sp. (Apicomplexa: Eugregarinida: Blabericolidae) Parasitizing the Pallid Cockroach, *Phoetalia pallida* (Dictyoptera: Blaberidae)

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ABSTRACT: *Protomagalhaensia cerastes* n. sp. is described from nymphs and adults of the Pallid cockroach, *Phoetalia pallida*. Gamonts of *Protomagalhaensia* species are elongate and serpentine in general shape, but associated gamonts of *P. cerastes* are considerably smaller than those of other species of *Protomagalhaensia*. Primites and satellites of *P. cerastes* average total lengths of 323.1 μm and 317.9 μm, respectively; whereas similar stages range from 400.0 μm to 650.0 μm in the other 4 species within the genus. All species of *Protomagalhaensia* possess dolioform oocysts. Oocysts of *Protomagalhaensia granulosae* and *Protomagalhaensia serpentula* also possess apical corner spines or knobs that are absent in the oocysts of *Protomagalhaensia wolfi*, *Protomagalhaensia blaberae*, and *P. cerastes*. The oocysts of *P. granulosae* possess a lateral depression unique among members of the genus, while *P. cerastes* and *P. wolfi* possess distinct polar plates absent in other members of the genus. Oocysts of *P. cerastes* are notably smaller than those of *P. wolfi* in both length (7.3 μm vs. 9.2 μm) and width (4.5 μm vs. 5.5 μm).

KEY WORDS: Apicomplexa, Blabericolidae, Blaberidae, Dictyoptera, Gregarine, Pallid cockroach, *Phoetalia pallida*, *Protomagalhaensia blaberae*, *Protomagalhaensia cerastes* n. sp., *Protomagalhaensia granulosae*, *Protomagalhaensia serpentula*, *Protomagalhaensia wolfi*, *Cardiohabitans attenuata* n. comb., *Protomagalhaensia attenuata*.

Gregarines of the genus *Protomagalhaensia* are characteristic parasites of blaberid cockroaches (Clopton, 2009). Pinto (1918) erected and refined the genus *Protomagalhaensia* through a series of preliminary meeting reports over the course of several months, establishing the genus to recognize differences in gametocyst dehiscence and oocyst morphology between *Protomagalhaensia serpentula* (de Magalhaes 1900) Pinto 1918 (=*Gregarina serpentula* de Magalhaes 1900), described from *Periplaneta americana* Linnaeus, 1758 (=*Blatta orientalis* Sulzer, 1776, but not *Blatta orientalis* Linnaeus, 1758) (Dictyoptera: Blattodea: Blattidae: Blattinae), and the remaining members of *Gregarina*. Théodoridès (1952) declared *Protomagalhaensia* a junior synonym of *Gregarina* based largely on the similar structure of trophozoites and gamonts among *Gregarina cavalierina* Blanchard 1905 and *Protomagalhaensia marottai* Filipponi 1952 (see Filipponi, 1952a–c, 1953), which he also synonymized as exemplar taxa of the putative genera. Although the species-level synonymy of Théodoridès (1952) appears valid, *P. marottai* parasitizes tenebrionid beetles and is clearly unrelated to members of *Protomagalhaensia* infecting cockroaches, and the pars pro toto synonymy of *Protomagalhaensia* and *Gregarina* was incorrect. Clopton and Hays (2006) resurrected and revised *Protomagalhaensia*, including only described species parasitizing cockroaches and clearly differentiating *Protomagalhaensia* from members of Hirmocystidae and Gregarinidae. Recognizing that gametocyst dehiscence characteristics precluded the inclusion of *Protomagalhaensia* within Gregarinidae, Clopton and Hays (2006) allied the genus with Hirmocystidae. Clopton (2009) demonstrated the monophyly and sisterhood of *Protomagalhaensia* and *Blabericola* (=*Leidyana* pro parte) parasitizing blaberid cockroaches and erected Blabericolidae to reflect this unique crown radiation within the septate gregarines, recognizing the family based on phylogenetic analysis of 18ssu rDNA data and diagnosing the group using association, gametocyst dehiscence, and oocyst liberation characters.

Cockroaches and their gregarines are readily and inexpensively maintained in laboratory culture (Clopton, 1995). They have proven admirable experimental systems for chemotherapeutic targeting (Clopton and Smith, 2002; Smith and Clopton, 2003), as well as empirical tests of host–parasite specificity (Smith and Cook, 2008), and they can serve as informative models for phylogenetically relevant analyses of host–parasite specificity, host-induced morphometric variation, and gregarine speciation. The primary impediments to large-scale empirical work remain the relatively poor taxonomic state of many known species (Clopton and Hays, 2006), and more broadly, the largely unknown biodiversity of gregarines infecting cockroaches. Although the immediate phylogenetic relationship of *Blabericola* and *Protomagalhaensia* has been resolved, the potential species

diversity of these genera remains unknown. Herein, we describe a new species of *Protomagalhaensia* parasitizing the Pallid cockroach, *Phoetalia pallida* (Brunner von Wattenwyl 1865) Princis, K. 1967 (=*Nauphoeta pallida* Brunner von Wattenwyl 1865) (Dictyoptera: Blattodea: Blaberidae: Blaberinae), and distinguish the new species from known members of *Protomagalhaensia*.

MATERIALS AND METHODS

Phoetalia pallida breeding colonies were established using stock originally obtained from Key West, Monroe County, Florida, U.S.A. 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 & Balanced; Nestle Purina PetCare Company, St. Louis, Missouri, U.S.A.) and water were provided ad libidum. Adult or late-instar nymphal *P. pallida* individuals were removed from the colony and examined for gregarine parasites. 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 a generalized blaberid cockroach saline (pH 7.5–7.8) (BCS) designed using data from Yeager (1939), Griffiths and Tauber (1943), Wood (1961), and Usherwood (1963), and containing the following millimolar (mM) salt concentrations: NaCl, 172 mM; KCl, 20 mM; $CaCl_2$, 7 mM; $MgCl_2$, 6 mM; NaH_2PO_4, 6 mM; and $NaHCO_3$, 4 mM.

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-xylol and mounted in Damar balsam (see Clopton [2000] and 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 lined with a triple-layer filter paper absorbent ring saturated with 0.1% aqueous methylparaben solution to provide 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 were noted.

Oocyst structure and dimensions were taken from fresh preparations of oocysts in wet mounts and agar monolayer mounts (Clopton, 1999) prepared as follows. Molten agar (1.5% solution) was pipetted onto a clean glass slide, allowed to drain, and the slide was quickly chilled on a cold aluminum block to produce a thin, uniform layer of agar. Oocysts were placed in a small (~7 µl) drop of water on a 12-mm round cover glass (#0 thickness), and the agar slide was inverted to pick up the cover glass. The resulting preparation produced a monolayer of oocysts trapped between the agar layer and the cover glass, providing a uniform object plane for light microscopy.

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 *Protomagalhaensia* 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 (NSD), 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), polar plate length (PPL), polar plate width (PPW), total length of primite (PTL), width of protomerite at equatorial axis (PWE), maximum width of protomerite (PWM), and total length of satellite (STL).

The shape and relative proportion 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 description of primite and satellite ontogenic stages are provided to account for the sexual dimorphism (Filipponi, 1947, 1951, 1952c, 1954, 1955). Measurements are presented in µm 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 cerastes n. sp. (Figs. 1–13)

Generic diagnosis

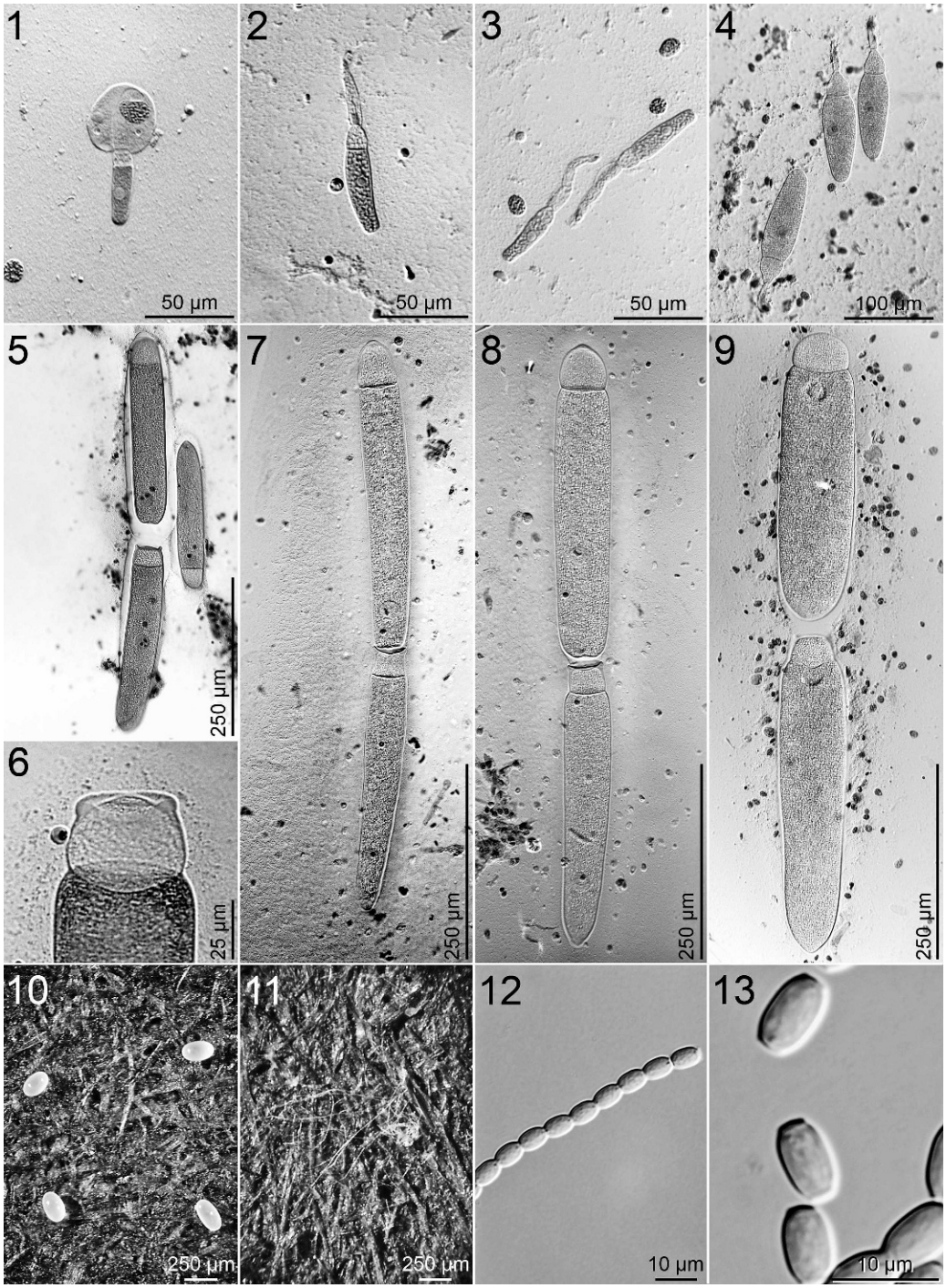
Order Eugregarinida Léger, 1892, sensu Clopton (2002); Suborder Septatina Lankester, 1885, sensu Clopton (2002); Superfamily Gregarinoidea, Chakravarty, 1960 sensu Clopton (2009); Family Blabericolidae Clopton (2009); Genus *Protomagalhaensia* Pinto, 1918 sensu Clopton (2009): epimerite shallowly obovoid to shallowly obdeltoid, developed intracellularly within a single host intestinal epithelial cell, not retained in gamonts; trophozoites becoming elongate after association; association gamontic, caudofrontal, association interface a shallowly semi-obpanduriform interlock in which the posterior end of the primite'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. 1–4): Young trophozoites solitary, extracellular forms attached to host ventricular epithelium. Holdfast a simple epimerite developing intracellularly in a single host epithelial cell (Fig. 1). Epimerite gladiate to ensiform solitary trophozoites (Figs. 1–4). Protomerite broadly ovoid, becoming markedly constricted at protomerite–deutomerite septum in older solitary trophozoites. Deutomerite narrowly obovoid in young solitary trophozoites, becoming spatulate in older solitary trophozoites. Nucleus orbicular with 1 distinct, large, smooth-margined karyosome.

Association (Figs. 5–9): Presyzygial, gamontic; gamonts anisomorphic primarily due to structures involved in association interface; association interface a shallowly semi-obpanduriform interlock in which the posterior end of the primite's deutomerite is constricted and clamped by an acetabulum formed from the anterior membranes of the satellite's protomerite (Fig. 6). Indices: PTL/STL 1.0 (0.9–1.2, ±0.09, 30), PPL/SPL 1.5 (1.0–2.1, ±0.29, 30), PPWM/SPWM 1.0 (0.8–1.2, ±0.11, 30), PDL/SDL 1.0 (0.8–1.2, ±0.10, 30), PDWM/SDWM 1.1 (0.9–1.3, ±0.09, 30), PDWE/SDWE 1.1 (0.9–1.4, ±0.11, 30).

Primite (Figs. 7–9): Epimerite absent; protomerite deltoid to very broadly ovoid, often with equatorial constriction; PL 51.4 (36.0–64.9, ±7.91, 30), PWE 49.5 (41.2–62.1, ±5.24, 30), PWM 56.2 (46.7–67.6, ±5.23, 30), PLAM 36.8 (22.9–44.7, ±5.43, 30), PLPM 14.9 (1.3–38.6, ±6.52, 30), PDSW 55.8 (47.2–63.9, ±4.29, 30), PL/PWE 1.0 (0.7–1.4, ±0.14, 30), PL/PWM 0.9 (0.6–1.3, ±0.14, 30), PL/PDSW 0.9 (0.6–1.2, ±0.13, 30), PLAM/PL 0.7 (0.4–0.9, ±0.10, 30), PLAM/PLPM 3.5 (0.6–26.5, ±4.47, 30), PWM/PWE 1.1 (1.0–1.3, ±0.07, 30). Deutomerite elongated, very narrowly dolioform or elliptoid, often with slight posterior depression to accommodate anterior margin of satellite's protomerite (Fig. 8.); DL 278.3 (224.0–312.0, ±20.89, 30), DWE 76.7 (58.8–94.4, ±8.24, 30), DWM 80.2 (65.6–103.0, ±8.16, 30), DLAM 117.3 (55.1–189.0, ±32.63, 30), DLPM 160.5 (94.6–223.0, ±31.85, 30), DL/DWE 3.7 (2.7–5.1, ±0.51, 30), DL/DWM 3.5 (2.5–4.6, ±0.46, 30), DLAM/DL 0.4 (0.2–0.6, ±0.11, 30), DLAM/DLPM 0.8 (0.3–1.5, ±0.36, 30), DWM/DWE 1.1 (1.0–1.1, ±0.04, 30), PTL 323.1 (274.0–371.0, ±22.96, 30). Indices: PTL/PL 6.4 (4.4–8.7, ±0.99, 30), DL/PL 5.5 (3.6–7.7, ±0.93, 30), DWM/PWM 1.4 (1.2–1.6, ±0.09, 30), PTL/DL 1.2 (1.1–1.2, ±0.02, 30). Nucleus roughly orbicular with multiple eccentrically placed orbicular karyosomes; NL 28.0 (21.1–33.7, ±2.73, 30), NW 25.7 (19.8–35.3, ±3.33, 30), NDS 31.2 (3.6–154.0, ±31.62, 30), KD1 8.0 (5.7–10.8, ±1.27, 30), NL/NW 1.1 (0.9–1.4, ±0.13, 30), NDS/NL 1.1 (0.1–4.8, ±1.10, 30), DL/NDS 17.8 (1.9–84.2, ±17.84, 30), NL/KD1 3.6 (2.4–4.8, ±0.59, 30).

Satellite (Figs. 6–9): Protomerite shallowly to very broadly orbicular, anterior membranes forming a cup-shaped acetabulum; PL 34.4 (27.8–42.7, ±4.01, 30), AcW 45.5 (29.3–59.2, ±5.77, 30), AcD 7.5 (4.0–10.9, ±1.67, 30), PWE 51.5 (41.5–63.2, ±5.80, 30), PWM 56.8 (45.0–67.6, ±5.80, 30), PLAM 22.7 (17.3–30.4, ±3.00, 30), PLPM 11.4 (5.3–21.1, ±3.66, 30), PDSW 56.3 (46.3–66.4, ±5.89, 30), AcW/AcD 6.4 (3.4–10.5, ±1.79, 30), AcW/PWM 0.8 (0.7–1.0, ±0.07, 30), AcD/PL 0.2 (0.1–0.4, ±0.05, 30), PL/PWE 0.7 (0.5–1.0, ±0.11, 30), PL/PWM 0.6 (0.5–0.8, ±0.09, 30), PL/PDSW 0.6 (0.5–0.8, ±0.09, 30), PLAM/PL 0.7 (0.4–0.8, ±0.09, 30), PLAM/PLPM 2.3 (1.1–5.0, ±0.98, 30), PWM/PWE 1.1 (1.0–1.2, ±0.05, 30). Deutomerite elongated, very narrowly dolioform; DL 286.8 (244.0–330.0,



Figures 1–13. *Protomagalhaensia cerastes* n. sp. **1.** Young trophozoite with epimerite fully embedded within a host cell, protomerite and deutomerite extracellular. **2–4.** Trophozoites displaying typical lability of the epimerite. **5.** Small gamonts in broken association. **6.** Acetabular association interface of satellite. **7–9.** Gamonts in association. **10.** Gametocysts. **11.** Monete oocyst chains extruded from mature gametocyst. **12.** Monete oocyst chain. **13.** Single oocysts showing polar plates and distinct, spherical polar residua.

±22.71, 30), DWE 71.0 (54.0–94.2, ±9.12, 30), DWM 76.6 (60.5–94.4, ±8.05, 30), DLAM 99.0 (40.8–225.0, ±33.08, 30), DLPM 189.3 (88.6–252.0, ±33.38, 30), DL/DWE 4.1 (3.1–5.2, ±0.57, 30), DL/DWM 3.8 (2.9–4.6, ±0.42, 30), DLAM/DL 0.3 (0.2–0.7, ±0.11, 30), DLAM/DLPM 0.6 (0.2–2.5, ±0.41, 30), DWM/DWE 1.1 (1.0–1.2, ±0.05, 30), STL 317.9 (267.0–365.0, ±23.53, 30). Indices: STL/PL 9.3 (7.5–11.1, ±0.95, 30), DL/PL 8.4 (6.7–10.3, ±0.91, 30), DWM/PWM 1.4 (1.2–1.6, ±0.12, 30), STL/DL 1.1 (1.1–1.1, ±0.02, 30). Nucleus roughly orbicular with multiple eccentrically placed orbicular karyosomes; NL 28.3 (22.8–37.1, ±3.70, 30), NW 24.1 (16.1–31.8, ±4.04, 30), NDS 67.1 (5.1–239.0, ±80.03, 30), KD 7.0 (5.3–9.7, ±1.13, 30), NL/NW 1.2 (0.8–1.7, ±0.23, 30), NDS/NL 2.5 (0.2–8.4, ±3.00, 30), DL/NDS 16.3 (1.2–59.1, ±17.48, 30), NL/KD 4.1 (2.8–5.4, ±0.68, 30).

Gametocysts (Fig. 10): Opaque, pearlescent in color, elliptoid in outline, length (GL) 253.4 (234.0–276.0, ±9.87, 30), width (GW) 155.5 (140.0–173.0, ±7.89, 30), GL/GW 1.6 (1.5–1.8, ±0.08, 30). Thirty-eight gametocysts were collected and stored under moist conditions, dehiscing by simple rupture in 18–24 hr.

Oocysts (Figs. 11–13): Dolioform with depressed oblong polar plates, without spines or knobs at terminal apices; OLM 7.3 (6.9–7.6, ±0.17, 30), OLI 6.6 (6.3–7.0, ±0.18, 30), OW 4.5 (4.3–4.7, ±0.10, 30), PPW 2.1 (1.8–2.4, ±0.15, 30), PPL 0.4 (0.2–0.6, ±0.09, 30), ResDia 0.7 (0.5–0.9, ±0.10, 30), OLM/OLI 1.1 (1.1–1.1, ±0.02, 30), OLM/OW 1.6 (1.5–1.7, ±0.05, 30), OLI/OW 1.5 (1.4–1.6, ±0.05, 30), PPW/PPL 6.4 (4.0–13.1, ±1.99, 30), PPW/OW 0.5 (0.4–0.5, ±0.03, 30), PPL/OLM 0.0 (0.0–0.1, ±0.01, 30), OLM/ResDia 10.7 (7.5–13.0, ±1.44, 30), OW/ResDia 6.6 (4.8–8.2, ±0.88, 30); released in monete chains from gametocyst by extrusion.

Taxonomic summary

Type host: Phoetalia pallida (Brunner von Wattenwyl 1865) Princis, K. 1967 (=*Nauphoeta pallida* Brunner von Wattenwyl 1865) (Dictyoptera: Blattodea: Blaberidae: Blaberinae), nymphs and adults.

Type locality: Key West, Monroe County, Florida, U.S.A.

Symbiotype: Thirty-four symbiotype specimens are deposited in the Sam Houston State University Insect Collection (SHSUIC), Department of Biological Sciences, Sam Houston State University, Huntsville, Texas, U.S.A. Individual accession numbers are not assigned by SHSUIC. Symbiotype vouchers all bear 3 pin labels (locality, determination, synoptic identifier) and can be distinguished by the following 5-line synoptic identifier label: Line 1—"R.E. Clopton"; Line 2—"Synoptic Cockroach Collection"; Line 3—"Voucher"; Line 4—"♀" or "♂" (handwritten); Line 5—"Specimen #" followed by a handwritten number. The symbiotype series is composed of 34 specimens numbered as follows: 12 females, Synoptic Cockroach Collection numbers 26–30, 32, 34, 39, 44–47; 22 males, Synoptic Cockroach Collection numbers 2–4, 6, 8–10, 19–25, 63–68, 70–71.

Site of infection: Trophozoites were collected from ventricular cecae and intercecal region. Associations were collected from the ileum. Gametocysts were collected from host feces.

Prevalence: Prevalence in colony approaches 100%.

Records: Phoetalia pallida, nymphs and adults, research colonies, Peru State College, Peru, Nebraska, U.S.A.

Specimens deposited: The holotype slide is deposited in the Harold W. Manter Laboratory for Parasitology (HWML), Division of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska. The holotype slide HWML100031 (author's slide REC090006a) is a hapantotype containing multiple trophozoites, gamonts, and associations. It is one of a series of 65 hapantotype slides comprising the type series, which includes 66 paratype slides accessioned as HWML100032 (author's slides REC090002a–e, REC090003a–c, REC090004a–c, REC090005a–c, REC090006b–g, REC090008a–g, REC090009a–c, REC090010a–f, REC090011a–c, REC090012a–d, REC090013, REC090014a–n, and REC090015a–g).

Etymology: The specific epithet is taken from the Greek "kerastes" meaning "a horned serpent" and is given to note the general form of associations and the distinct "horned" nature of the primite–satellite junction in this species.

Remarks

The genus *Protomagalhaensia* consists of 5 named species, of which 4 are described from cockroaches: *P. wolfi* from *N. cinerea*; *P. serpentula*, (the type species), from *Blatta orientalis*; *Protomagalhaensia blaberae* Peregrine, 1970, from *Blaberus boliviensis* Princis, 1946; and *Protomagalhaensia granulosae*

Table 1. Comparative association morphometrics of *Protomagalhaensia cerastes* n. sp., *Protomagalhaensia serpentula*, *Protomagalhaensia granulosae*, *Protomagalhaensia blaberae*, and *Protomagalhaensia wolfi*.

	P. cerastes n. sp.	*P. serpentula**	*P. granulosae*†	*P. blaberae*†	*P. wolfi*‡
Primite					
PL	51.4	50.0	87.0	74.0	77.0
DL	278.3	350.0	563.0	481.0	460.0
PTL	323.1	400.0	650.0	555.0	533.0
PL:PTL	6.4	8.0	7.5	7.5	7.1
PWM:DWM	1.4	1.3	1.03	1.09	1.2
Satellite					
PL	34.4	50.0	62.0	55.0	62.0
DL	286.8	350.0	488.0	505.0	472.0
STL	317.9	400.0	550.0	560.0	535.0
PL:STL	9.3	8.0	8.85	10.2	9.1
PWM:DWM	1.4	1.3	1.02	1.04	1.3
PTL:STL	1.0	1.0	1.18	0.99	0.99
Oocyst					
OLM	7.3	7.5	7.0	8.0	9.2
OW	4.5	2.8	4.3	5.0	5.5
OLW:OW	1.6	2.6	1.6	1.6	1.7

* Data from de Magalhaes (1900) and Pinto (1918, 1922).
† Data from Peregrine (1970).
‡ Data from Clopton and Hays (2006).

Peregrine, 1970, from *Blaberus discoidalis* Serville, 1839. A fifth species, *Protomagalhaensia* (?)[sic] *attenuata* Setna & Bhatia, 1934, is described from Rainbow prawns, *Parapeneopsis sculptilis* (Heller, 1862), collected near Bombay, India. This fifth species is clearly not a member of *Protomagalhaensia* but probably represents a species of the genus *Cardiohabitans* Ball, 1959. The original placement in *Protomagalhaensia* was clearly provisional, and the doubt of the original authors was echoed by Bhatia (1938 pp. 104–105), "The form resembles *Protomagalhaensia* in as much as the sporonts are attenuate … until the spores are known, the form cannot definitely be placed." The multiple associations reported by Setna and Bhatia (1934) and Bhatia (1938), combined with the host association (Crustacea), most closely resemble members of the genus *Cardiohabitans*, and thus the species is transferred with the formation of *Cardiohabitans attenuata* (Stena and Bhatia, 1938) n. comb.

No type or permanent specimen of *P. serpentula* is known. The type series of *P. granulosae* (Register number: 1970.333 and 1970.334, British Museum of Natural History [BMNH], Cromwell Road, London SW7 5BD, United Kingdom) and *P. blaberae* (BMNH register number 1970.333) were examined and the neotype slide (hapantotype HWML 48314) of *P. wolfi* was reexamined.

The overall morphology of all 4 species is similarly elongate and serpentine (Table 1). Although Clopton and Hays (2006) remarked upon differences in epimerite structure among species of *Protomagalhaensia*, the epimerite morphology of *P. cerastes* is not clearly distinct from that of *P. wolfi*, and I believe that the ultimate utility of epimerite characters for species diagnosis within the genus cannot be evaluated until additional species are described and/or *P. blaberae*, *P. serpentula*, and *P. granulosae* are adequately redescribed.

Species of *Protomagalhaensia* are distinguished using relative oocyst and association morphometrics. All 4 species possess dolioform oocysts. Oocysts of *P. granulosae* and *P. serpentula* also possess apical corner spines or knobs (de Magalhaes, 1900; Pinto, 1918, 1922; Peregrine, 1970) that are absent in the oocysts of *P. wolfi*, *P. blaberae*, and *P. cerastes*. *Protomagalhaensia cerastes* and *P. wolfi* possess distinct polar plates, which are lacking in other members of the genus, but the oocysts of *P. cerastes* are notably smaller (OLM, 7.3; OW 4.5) than those of *P. wolfi* (OLM, 9.2; OW, 5.5 [Clopton and Hays, 2006]) or *P. blaberae* (OLM, 8.0; OW, 5.0 [Peregrine, 1970]). Associated gamonts of *P. cerastes* are also considerably smaller than those of all other known species of *Protomagalhaensia* (cf. Table 1).

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