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Redescription of *Protomagalhaensia blaberae* Peregrine, 1970 (Apicomplexa: Eugregarinida: Blabericolidae) Parasitizing the Bolivian Cockroach, *Blaberus boliviensis* (Dictyoptera: Blaberidae)

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ABSTRACT: *Protomagalhaensia blaberae* Peregrine, 1970 is redescribed from the type host, the Bolivian cockroach *Blaberus boliviensis*. Complete morphometric data on all life cycle stages is presented, the taxon is stabilized with the deposition of new voucher specimen material, and *P. blaberae* is distinguished from all other species in the genus (*Protomagalhaensia cerastes*, *Protomagalhaensia granulosae*, and *Protomagalhaensia wolfi*). Species of *Protomagalhaensia* are distinguished by differences in relative metric ratios, morphology of oocysts, and by relative metric ratios of mature gamonts in association. The status of *Protomagalhaensia serpentula* is considered and the taxon is determined species inquirenda.

KEY WORDS: Apicomplexa, Blabericolidae, Gregarine, *Protomagalhaensia blaberae*, *Protomagalhaensia cerastes*, *Protomagalhaensia granulosae*, *Protomagalhaensia serpentula*, *Protomagalhaensia wolfi*.

The genus *Protomagalhaensia* (Apicomplexa: Eugregarinida: Blabericolidae) comprises 5 species of gregarines parasitic in the intestines of cockroaches. *Protomagalhaensia serpentula* (de Magalhaes 1900) Pinto 1918 (=*Gregarina serpentula* de Magalhaes 1900) parasitizing *Periplaneta americana* (Linnaeus, 1758) (=*Stylopyga americana* Fischer, 1846) (Dictyoptera: Blattaria: Blattidae: Blattinae) is the type of the genus and was described from material collected in Rio de Janeiro, Brazil (Pinto, 1918, 1922). *Protomagalhaensia granulosae* Peregrine, 1970, parasitizing *Blaberus discoidalis* Serville, 1839 (Dictyoptera: Blaberidae: Blaberinae), was described from cockroaches maintained in laboratory cultures in the Department of Zoology, University College Cardiff (Cardiff University), Cardiff, Wales, United Kingdom (Peregrine, 1970). Similarly, *Protomagalhaensia blaberae* Peregrine, 1970, parasitizing *Blaberus boliviensis* Princis, 1946 (Dictyoptera: Blattaria: Blaberidae: Blaberinae), was also described from cockroaches maintained in laboratory cultures in the Department of Zoology, University College Cardiff (Peregrine, 1970). *Protomagalhaensia wolfi* (Geus, 1969) Clopton and Hays, 2006 (=*Gregarina wolfi* Geus, 1969), parasitizing *Nauphoeta cinerea* (Olivier, 1789) (Dictyoptera: Blattaria: Blaberidae: Oxyhalinae: Nauphoetini), was described using material collected in Ndanda, Tanzania (Geus, 1969). *Protomagalhaensia cerastes* Clopton, 2010, parasitizing *Phoetalia pallida* (Brunner von Wattenwyl 1865) Princis, 1967 (=*Nauphoeta pallida* Brunner von Wattenwyl 1865) (Dictyoptera: Blattaria: Blaberidae: Blaberinae), was described from cockroaches maintained in laboratory colonies established using wild stock from Key West, Monroe County, Florida, U.S.A. (Clopton, 2010). *Protomagalhaensia serpentula* has not been reported since its original description and remains enigmatic, a problem compounded by confusion regarding the type host. Of the remaining 4 species in the genus, complete taxonomic data sets and voucher specimens exist for all but *P. blaberae* (see Clopton and Hays, 2006; Clopton, 2010, 2011;). Herein, we complete the stabilization of *Protomagalhaensia* by redescribing *P. blaberae* parasitizing *B. boliviensis*, differentiating it from existing species in the genus, discuss new voucher material deposited to stabilize the taxon, and reconsider the taxonomic status of *P. serpentula*.

MATERIALS AND METHODS

Blaberus boliviensis 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 & Balanced [Nestle Purina Pet Care Company, St. Louis, Missouri, U.S.A.]) and water were provided ad libidum. Adult or late-instar nymphal *B. boliviensis* individuals were examined for gregarine parasites as described by Clopton (2011). Likewise, preparation of permanent microscope slides, gametocyst collection and incubation protocols, techniques for studying oocysts in monolayer and free-rotational temporary mounts, and DNA extraction and storage protocols follow those detailed by Clopton (2011).

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 cellSens Dimension Desktop® v. 1.3 image

analysis software (Olympus Corp., Silver Spring, Maryland, U.S.A.). Photographic plates were processed and assembled using Adobe® PhotoShop® CS5 Extended software (Adobe Systems Inc., San Jose, California, U.S.A.).

The extended morphometric character set for *Protomagalhaensia* delineated by Clopton and Hays (2006) and the oocyst modifications described by Clopton (2011) are 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), polar dehiscence plate length (DPL), polar 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), total length of primite (PTL), width of protomerite at equatorial axis (PWE), maximum width of protomerite (PWM), oocyst residuum diameter (ResDia), and total length of satellite (STL).

Measurements are presented in micrometers as mean values followed parenthetically by range values, standard deviations, and sample sizes. Metric differences between species were identified using ANOVA protected Tukey's honestly significant difference (HSD) tests ($\alpha = 0.05$). Parasite ontogenetic and anatomical nomenclature largely follows that proposed by Levine (1971) but defers to Clopton (2009) in reference to association, syzygy, gametocyst development, and oocyst dehiscence. Nomenclature for the shapes of planes and solids follows Clopton (2004).

RESULTS

Protomagalhaensia blaberae Peregrine, 1970 (Figs. 1–16)

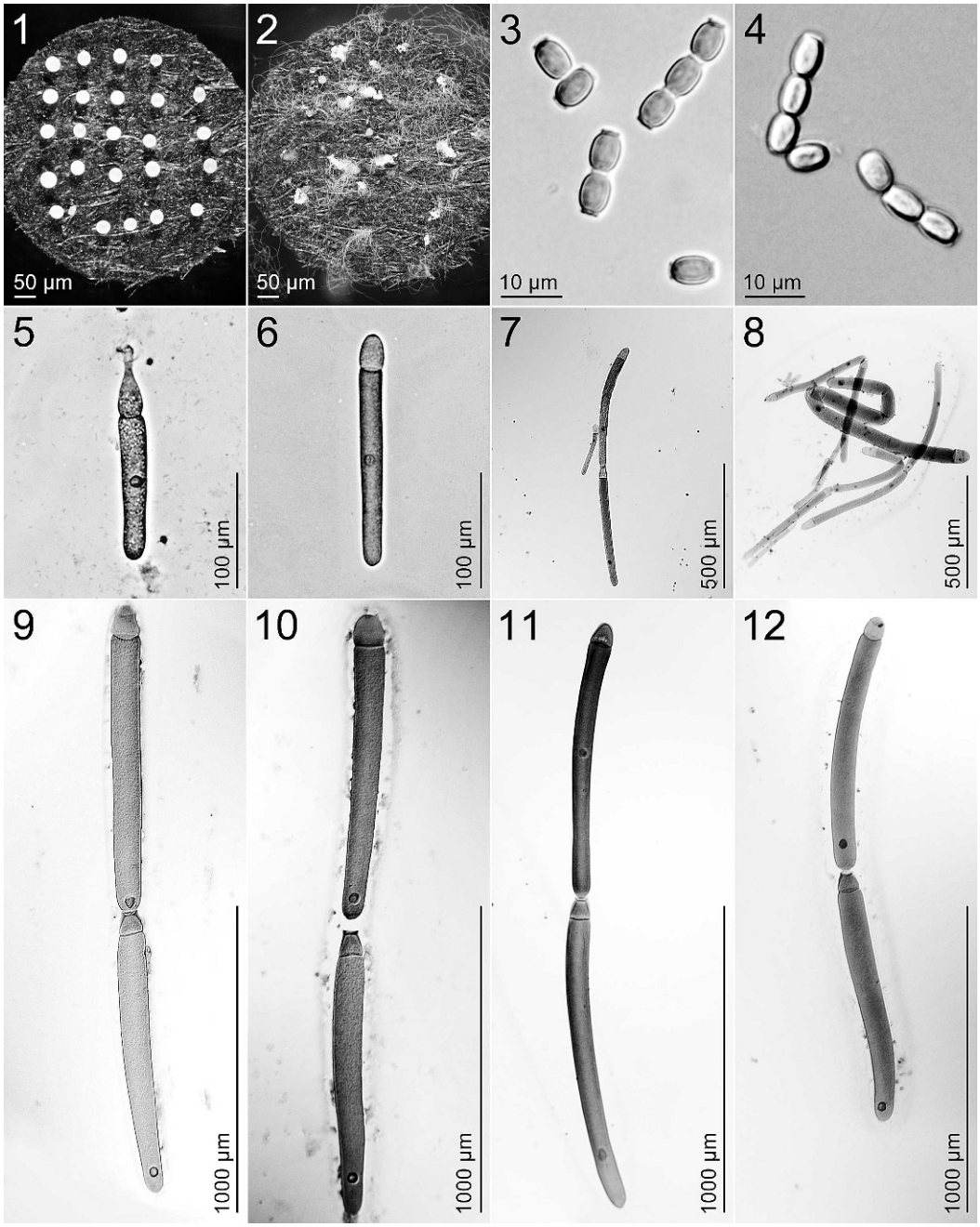
Generic diagnosis

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 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 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. 5, 6): 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. Epimerite very narrowly obpyriform, expanding in distal half (Fig. 5), absent in older trophozoites (Fig. 6). Protomerite broadly to shallowly ovoid in young trophozoites, becoming oblong in older solitary trophozoites, markedly constricted at protomerite–deutomerite septum (cf. Figs. 5, 6). Deutomerite obovoid in young solitary trophozoites, becoming very narrowly to linearly oblong in older solitary trophozoites (cf. Figs. 5, 6). Nucleus orbicular with 1 distinct, large, smooth-margined karyosome.

Association (Figs. 9–12): Presyzygial, gamontic; gamonts anisomorphic 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 (Figs. 9–12). Measurements taken from mature gamonts in association (cf. immature association of Figs. 7, 8 and mature associations of Figs. 9–12). Indices: PTL/STL 1.0 (0.9–1.2, ±0.07, 30), PPL/SPL 1.3 (1.0–2.6, ±0.31, 30), PPWM/SPWM 1.1 (0.9–1.4, ±0.14, 30), PDL/SDL 1.0 (0.9–1.2, ±0.08, 30), PDWM/SDWM 1.0 (0.8–1.3, ±0.14, 30), PDWE/SDWE 1.0 (0.8–1.3, ±0.12, 30).

Primite: Observations and data taken from mature primites in association. Epimerite absent; protomerite finely deltoid to deltoid; PL 88.1 (63.4–111.0, ±11.71, 30), PWE 68.0 (50.5–94.5, ±12.04, 30), PWM 75.1 (54.8–99.7, ±11.40, 30), PLAM 58.4 (34.3–79.7, ±12.22, 30), PLPM 30.9 (7.8–52.6, ±10.81, 30), PDSW 70.1 (52.6–91.7, ±9.87, 30), PL/PWE 1.3 (1.0–1.7, ±0.20, 30), PL/PWM 1.2 (1.0–1.6, ±0.17, 30), PL/PDSW 1.3 (1.0–1.6, ±0.18, 30), PLAM/PL 0.7 (0.4–0.9, ±0.11, 30), PLAM/PLPM 2.3 (0.8–10.2, ±1.74, 30), PWM/PWE 1.1 (1.0–1.3, ±0.08, 30). Deutomerite elongated, very narrowly obovoid; DL 778.1 (685.0–846.0, ±48.32, 30), DWE 77.4 (52.7–99.9, ±12.32, 30), DWM 83.8 (63.5–116.2, ±12.13, 30), DLAM 151.9 (14.3–429.0, ±168.75, 30), DLPM 626.3 (348.0–825.0, ±171.94, 30), DL/DWE 10.3 (7.7–14.0, ±1.84, 30),



Figures 1–12. *Protomagalhaensia blaberae*. **1.** Gametocysts. **2.** Monete oocyst chains dehisced from mature gametocysts. **3.** Oocysts in agar monolayer; note apical "spines" created by polar plates. **4.** Oocysts freely rotating in thick water mount; note lateral depression and corpuscular appearance. **5.** Solitary trophozoite with epimerite. **6.** Older trophozoite without epimerite. **7, 8.** Immature associations. **9–12.** Variation in typical mature associations.

DL/DWM 9.5 (7.2–12.5, ±1.45, 30), DLAM/DL 0.2 (0.0–0.5, ±0.22, 30), DLAM/DLPM 0.4 (0.0–1.1, ±0.45, 30), DWM/DWE 1.1 (1.0–1.3, ±0.09, 30), PTL 861.1 (789.0–930.7, ±47.75, 30). Indices: PTL/PL 9.9 (7.2–13.0, ±1.31, 30), DL/PL 9.0 (6.2–12.1, ±1.32, 30), DWM/PWM 1.1 (1.0–1.3, ±0.10, 30), PTL/DL 1.1 (1.1–1.2, ±0.02, 30). Nucleus broadly elliptoid to very broadly elliptoid with a single, eccentric broadly elliptoid karyosome; NL 34.9 (28.1–51.1, ±4.70, 30), NW 31.1 (25.3–40.1, ±3.96, 30), NDS 559.2 (223.0–756.0, ±157.15, 30), KD1 15.7 (8.6–21.7, ±4.00, 30), NL/NW 1.1 (0.8–1.6, ±0.17, 30), NDS/NL 16.3 (6.4–23.4, ±4.80, 30), DL/NDS 1.6 (1.1–3.7, ±0.66, 30), NL/KD1 2.4 (1.5–4.3, ±0.70, 30).

Satellite: Observations and data taken from mature satellites in association. Protomerite very shallowly to deeply deltoid, anterior membranes forming a cup-shaped acetabulum (Figs. 10, 12) PL 68.4 (38.5–100.0, ±12.42, 30), AcW 43.7 (31.9–57.5, ±5.69, 30), AcD 11.3 (6.9–16.2, ±2.39, 30), PWE 61.8 (42.1–87.1, ±10.45, 30), PWM 67.5 (42.2–86.9, ±10.98, 30), PLAM 48.5 (32.0–70.7, ±7.94, 30), PLPM 20.2 (9.0–38.0, ±8.04, 30), PDSW 64.7 (42.3–76.0, ±8.49, 30), AcW/AcD 4.0 (2.4–6.2, ±1.00, 30), AcW/PWM 0.7 (0.5–0.8, ±0.07, 30), AcD/PL 0.2 (0.1–0.4, ±0.05, 30), PL/PWE 1.2 (0.5–1.9, ±0.31, 30), PL/PWM 1.1 (0.5–1.9, ±0.29, 30), PL/PDSW 1.1 (0.5–1.8, ±0.27, 30), PLAM/PL 0.7 (0.6–0.9, ±0.09, 30), PLAM/PLPM 2.8 (1.2–5.3, ±1.20, 30), PWM/PWE 1.1 (1.0–1.2, ±0.06, 30). Deutomerite elongated, very narrowly obovoid; DL 763.3 (693.0–877.2, ±40.35, 30), DWE 76.2 (44.9–92.0, ±12.64, 30), DWM 84.0 (57.8–110.1, ±14.03, 30), DLAM 182.6 (20.9–425.0, ±138.82, 30), DLPM 571.9 (381.0–767.0, ±127.15, 30), DL/DWE 10.4 (7.5–17.1, ±2.24, 30), DL/DWM 9.4 (6.7–13.2, ±1.86, 30), DLAM/DL 0.2 (0.0–0.5, ±0.18, 30), DLAM/DLPM 0.4 (0.0–1.1, ±0.36, 30), DWM/DWE 1.1 (1.0–1.4, ±0.09, 30), STL 830.9 (758.8–935.4, ±38.50, 30). Indices: STL/PL 12.6 (7.9–19.8, ±2.46, 30), DL/PL 11.6 (6.9–18.2, ±2.35, 30), DWM/PWM 1.3 (1.1–1.5, ±0.11, 30), STL/DL 1.1 (1.1–1.2, ±0.01, 30). Nucleus broadly elliptoid to very broadly elliptoid with a single, eccentric, broadly elliptoid karyosome; NL 31.3 (25.5–40.4, ±3.82, 30), NW 29.0 (23.0–39.8, ±3.98, 30), NDS 634.9 (363.0–764.0, ±85.36, 30), KD 14.0 (8.2–23.0, ±3.48, 30), NL/NW 1.1 (0.8–1.5, ±0.17, 30), NDS/NL 20.5 (9.0–24.7, ±3.13, 30), DL/NDS 1.2 (1.1–2.1, ±0.20, 30), NL/KD 2.4 (1.6–4.1, ±0.56, 30).

Gametocysts (Figs. 1–2): Opaque, broadly elliptoid to orbicular in outline, length (GL) 318.8 (266.5–361.0, ±23.35, 41), width (GW) 302.5 (207.0–335.6, ±23.58, 41), GL/GW 1.1 (1.0–1.3, ±0.06, 41). Of 43 gametocysts collected and stored under moist conditions, most dehisced within 60–72 hr, releasing oocysts in monete chains by extrusion (cf. Fig. 1, 2).

Oocysts (Figs. 3–4): Dolioform in outline, terminal dehiscence plate present, oocysts dolioform in outline without dehiscence plate; presenting as dolioform in outline with apical corner knobs with dehiscence plate (Figs. 3, 4); sagittally flattened, corpuscular with dorsal surface depression (Fig. 4); OLM 7.4 (7.1–7.8, ±0.19, 30), OLI 6.4 (6.0–6.8, ±0.26, 30), OW 5.1 (5.0–5.2, ±0.07, 30), DPW 2.8 (2.5–3.2, ±0.18, 30), DPL 0.6 (0.4–0.9, ±0.11, 30), ResDia 0.8 (0.6–1.0, ±0.11, 30), OLM/OLI 1.2 (1.1–1.2, ±0.03, 30), OLM/OW 1.5 (1.4–1.5, ±0.04, 30), OLI/OW 1.3 (1.2–1.4, ±0.05, 30), DPW/DPL 5.2 (3.6–7.0, ±0.89, 30), OW/DPW 1.8 (1.6–2.1, ±0.12, 30), OLM/DPL 13.7 (8.8–19.4, ±2.60, 30), OLM/ResDia 9.2 (7.2–12.0, ±1.29, 30), OW/ResDia 6.4 (4.9–8.5, ±0.94, 30).

Taxonomic summary

Type host: Blaberus boliviensis Princis, 1946 (Dictyoptera: Blattaria: Blaberidae: Blaberinae), nymphs and adults.

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

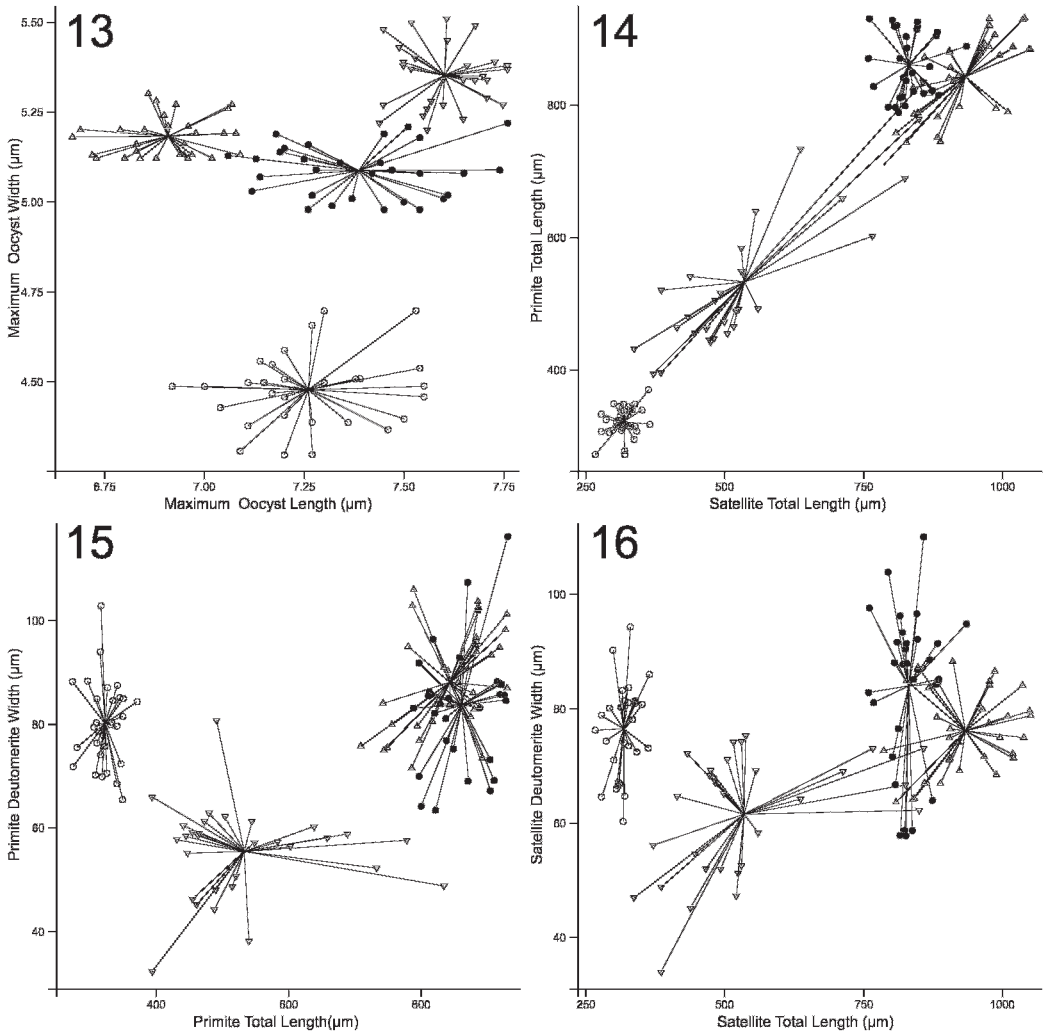
Types examined: Syntype series, hapantotype slide containing multiple trophozoites, gamonts, and associations, registration numbers 1970:3:3:5, The Natural History Museum, London, England, United Kingdom.

Host vouchers: Nine 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.

Site of infection: Trophozoites were collected from ventricular caecae and postintercaecal 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.; laboratory



Figures 13–16. Centroid cluster separation of *Protomagalhaensia blaberae* (●), *Protomagalhaensia cerastes* (○), *Protomagalhaensia granulosae* (□), and *Protomagalhaensia wolfi* (□) based on differences in relative morphometric size and shape of 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 drawn from population sample members within 2 standard deviations of the mean centroid). **13.** Ratio of maximum oocyst length and maximum oocyst width. **14.** Ratio of primite total length and satellite total length. **15.** Ratio of primite total length and primite deutomerite width. **16.** Ratio of satellite total length and satellite deutomerite width.

cultures, Department of Zoology, University College Cardiff (Cardiff University), Cardiff, Wales, United Kingdom.

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 57 hapantotype slides containing multiple trophozoites, gamonts, and associations accessioned as HWML100049 (57 slides, author's slide numbers REC090086A–G; REC090088A–J; REC090089A–E, H–N; REC090090A, D–G; REC090091D–E; REC090092A–G; REC090093A–N).

Remarks

The hapantotype of *P. blaberae* (Registration numbers 1970:3:3:5, The Natural History Museum,

Table 1. Comparative oocysts metrics* of *Protomagalhaensia blaberae*, *Protomagalhaensia cerastes*, *Protomagalhaensia granulosae*, and *Protomagalhaensia wolfi*. (Mean followed parenthetically by range and standard deviation; $n = 30$ for each taxon.)

Metric	*P. blaberae*	*P. cerastes*	*P. granulosae*	*P. wolfi*
OLM	6.91 (6.67–7.09 ± 0.12)	7.26 (6.92–7.55 ± 0.17)	7.60 (7.44–7.76 ± 0.10)	7.39 (7.06–7.76 ± 0.19)
OLI	6.13 (5.77–6.44 ± 0.19)	6.63 (6.28–6.97 ± 0.18)	6.69 (6.24–7.09 ± 0.19)	6.40 (6.03–6.78 ± 0.25)
OW	5.18 (5.12–5.30 ± 0.06)	4.48 (4.30–4.70 ± 0.10)	5.35 (5.20–5.51 ± 0.08)	5.09 (4.98–5.22 ± 0.07)
DPW	2.81 (2.51–3.11 ± 0.16)	2.10 (1.82–2.40 ± 0.15)	2.61 (2.11–3.02 ± 0.23)	2.83 (2.45–3.16 ± 0.18)
DPL	0.44 (0.26–0.64 ± 0.10)	0.35 (0.16–0.57 ± 0.09)	0.51 (0.32–0.81 ± 0.12)	0.56 (0.38–0.86 ± 0.11)
ResDia	0.79 (0.51–1.02 ± 0.13)	0.69 (0.54–0.93 ± 0.10)	0.92 (0.75–1.15 ± 0.10)	0.82 (0.60–1.02 ± 0.11)

* OLM, maximum exterior length; OLI, interior length; OW, width; DPW, polar dehiscence plate width; DPL, polar dehiscence plate length; ResDia, residuum diameter.

London, England, United Kingdom) was 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. Data presented in the redescription herein is consistent with that of Peregrine (1970) and the hapantotype sample but reflects the larger, mature end of the gamontic range.

DISCUSSION

Status of *Protomagalhaensia serpentula*

Of the 5 species comprising *Protomagalhaensia*, all but the type species, *P. serpentula*, have been fully described or redescribed, allowing robust morphometric comparison among all species in the genus except the type species. *Protomagalhaensia serpentula* is a problematic taxon. Although it appears to be a member of *Protomagalhaensia*, available morphometric data is limited to a few sketches and individual length measurements. No type specimen is known and additional taxonomic samples are unlikely because the host taxon is unclear.

Protomagalhaensia serpentula was originally described as *Gregarina serpentula* de Magalhaes 1900 infecting *Periplaneta americana* Fabricius. In the same manuscript, de Magalhaes (1900) also reported gregarines from "*Blatta* (*Periplaneta orientalis*)" without ascribing authorship to the host name. He indicated that the same or a similar gregarine infected *B. orientalis* but provided neither a description nor a differential diagnosis.

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 *Stylopyga americana* Fischer, 1846 in Rio de Janeiro, Brazil (Pinto, 1918, 1922). This material was typotypic but may or may not have come from the same host species.

Periplaneta americana (Linnaeus, 1758) (=*Blatta americana* Linnaeus, 1758) was not described by Fabricius as indicated by de Magalhaes (1900). Fabricius did describe a related cosmopolitan pest species, *Periplaneta australasiae* (Fabricius, 1775) (=*Blatta australasiae* Fabricius, 1775) that is very

Table 2. Homogenous groups formed by ANOVA protected Tukey's honestly significant difference tests ($\alpha = 0.05$) using oocysts metrics* of *Protomagalhaensia blaberae*, *Protomagalhaensia cerastes*, *Protomagalhaensia granulosae*, and *Protomagalhaensia wolfi*. Shared letters indicate homogenous groups, letters indicate relative size for each metric (A > B > C > D).

Metric	*P. blaberae*	*P. cerastes*	*P. granulosae*	*P. wolfi*
OLM†	B	C	D	A
OLI	B	A	C	A
OW†	C	D	B	A
DPW‡	A	C	A	B
DPL	A	C	B	A
ResDia‡	B	C	B	A

* OLM, maximum exterior length; OLI, interior length; OW, width; DPW, polar dehiscence plate width; DPL, polar dehiscence plate length; ResDia, residuum diameter.

† Metrics for which each species is significantly different from all other species in the analysis.

‡ Metrics for which *P. blaberae* and *P. granulosae* form a homogenous subgroup.

Table 3. Comparative gamont metrics* of *Protomagalhaensia blaberae*, *Protomagalhaensia cerastes*, *Protomagalhaensia granulosae*, and *Protomagalhaensia wolfi*. (Mean is followed parenthetically by range and standard deviation.)

Metric	*P. blaberae*†	*P. cerastes*†	*P. granulosae*‡	*P. wolfi*†
PriTL	861.1 (789.0–930.7 ± 47.7)	323.1 (274.0–371.0 ± 23.0)	843.0 (708.9–929.8 ± 61.4)	533.3 (394.6–835.6 ± 111.8)
PriPL	88.0 (63.4–111.0 ± 11.7)	51.4 (36.0–64.9 ± 7.9)	84.5 (61.4–109.5 ± 9.4)	77.1 (53.0–101.0 ± 11.6)
PriPWE	68.0 (50.5–94.5 ± 12.0)	49.4 (41.2–62.1 ± 5.2)	55.0 (37.1–71.6 ± 7.2)	41.8 (29.1–56.5 ± 6.5)
PriPWM	75.1 (54.8–99.7 ± 11.4)	56.2 (46.7–67.6 ± 5.2)	69.7 (55.0–84.3 ± 7.5)	46.8 (33.1–61.7 ± 6.8)
PriPLAM	58.4 (34.3–79.7 ± 12.2)	36.7 (22.9–44.7 ± 5.4)	71.8 (58.3–85.8 ± 7.1)	57.0 (40.1–82.1 ± 11.6)
PriPLPM	30.9 (7.8–52.6 ± 10.8)	14.9 (1.3–38.6 ± 6.5)	14.4 (3.8–23.5 ± 5.1)	20.3 (10.0–32.1 ± 5.9)
PriPDSW	70.1 (52.6–91.7 ± 9.9)	55.8 (47.2–63.9 ± 4.3)	65.5 (51.9–76.7 ± 7.0)	43.6 (29.0–65.4 ± 8.2)
PriDL	778.1 (685.0–846.0 ± 48.3)	278.3 (224.0–312.0 ± 20.9)	763.8 (635.5–846.2 ± 57.7)	459.4 (303.8–756.7 ± 112.5)
PriDWE	77.4 (52.7–99.9 ± 12.3)	76.7 (58.8–94.4 ± 8.2)	67.8 (55.3–85.8 ± 7.6)	45.9 (21.3–60.0 ± 8.2)
PriDWM	83.8 (63.5–116.2 ± 12.1)	80.2 (65.6–103.0 ± 8.2)	88.2 (71.5–106.0 ± 9.7)	55.5 (32.2–80.8 ± 9.1)
PriDLAM	151.9 (14.3–429.0 ± 168.8)	117.3 (55.1–189.0 ± 32.6)	87.6 (18.9–221.0 ± 42.2)	70.0 (20.7–219.6 ± 50.5)
PriDLPM	626.3 (348.0–825.0 ± 171.9)	160.5 (94.6–223.0 ± 31.8)	678.1 (474.3–789.1 ± 68.9)	390.4 (242.5–614.3 ± 99.8)
PriNL	34.9 (28.1–51.1 ± 4.7)	28.0 (21.1–33.7 ± 2.7)	37.0 (25.9–50.7 ± 5.6)	25.0 (15.3–32.1 ± 4.3)
PriNW	31.1 (25.3–40.1 ± 4.0)	25.7 (19.8–35.3 ± 3.3)	33.3 (19.6–47.3 ± 6.1)	19.9 (12.6–25.3 ± 3.5)
PriNDS	559.2 (223.0–756.0 ± 157.1)	31.2 (3.6–154.0 ± 31.6)	148.7 (13.2–407.9 ± 92.3)	211.4 (29.6–575.6 ± 126.5)
PriKD1	15.7 (8.6–21.7 ± 4.0)	8.0 (5.7–10.8 ± 1.3)	10.3 (6.4–16.2 ± 2.0)	9.4 (6.0–13.6 ± 1.7)
AcW	43.7 (31.9–57.5 ± 5.7)	45.5 (29.3–59.2 ± 5.8)	41.6 (33.1–57.1 ± 5.4)	35.1 (20.6–48.3 ± 6.8)
AcD	11.3 (6.9–16.2 ± 2.4)	7.5 (4.0–10.9 ± 1.7)	13.0 (7.7–19.2 ± 2.9)	10.3 (6.4–14.2 ± 2.1)
SatTL	830.9 (758.8–935.4 ± 38.5)	317.9 (267.0–365.0 ± 23.5)	932.8 (785.9–1,050.2 ± 74.2)	535.0 (338.5–859.3 ± 138.8)
SatPL	68.4 (38.5–100.0 ± 12.4)	34.4 (27.8–42.7 ± 4.0)	74.4 (57.8–92.8 ± 8.2)	61.5 (35.3–86.2 ± 13.1)
SatPWE	61.8 (42.1–87.1 ± 10.5)	51.5 (41.5–63.2 ± 5.8)	50.4 (33.8–63.3 ± 7.7)	42.6 (26.2–62.2 ± 8.3)
SatPWM	67.5 (42.2–86.9 ± 11.0)	56.8 (45.0–67.6 ± 5.8)	57.4 (44.1–69.3 ± 6.8)	47.1 (30.0–67.2 ± 8.6)
SatPLAM	48.5 (32.0–70.7 ± 7.9)	22.7 (17.3–30.4 ± 3.0)	61.4 (48.8–75.5 ± 7.2)	41.7 (24.2–63.8 ± 9.6)
SatPLPM	20.2 (9.0–38.0 ± 8.0)	11.4 (5.3–21.1 ± 3.7)	14.1 (9.0–20.0 ± 2.9)	19.5 (1.3–34.4 ± 7.4)
SatPDSW	64.7 (42.3–76.0 ± 8.5)	56.3 (46.3–66.4 ± 5.9)	53.2 (39.0–62.9 ± 5.3)	43.0 (23.4–59.3 ± 8.9)
SatDL	763.3 (693.0–877.2 ± 40.4)	286.8 (244.0–330.0 ± 22.7)	862.6 (728.0–979.0 ± 71.5)	471.8 (288.0–792.1 ± 138.7)
SatDWE	76.2 (44.9–92.0 ± 12.6)	71.0 (54.0–94.2 ± 9.1)	65.6 (50.7–86.7 ± 7.7)	54.4 (32.7–69.2 ± 9.0)
SatDWM	84.0 (57.8–110.1 ± 14.0)	76.6 (60.5–94.4 ± 8.1)	76.1 (63.6–88.3 ± 7.2)	61.5 (34.0–75.3 ± 10.8)
SatDLAM	182.6 (20.9–425.0 ± 138.8)	99.0 (40.8–225.0 ± 33.1)	162.9 (63.3–263.9 ± 55.1)	117.7 (33.9–359.2 ± 81.4)
SatDLPM	571.9 (381.0–767.0 ± 127.2)	189.3 (88.6–252.0 ± 33.4)	689.9 (312.9–854.1 ± 100.0)	346.5 (83.8–603.5 ± 103.0)
SatNL	31.3 (25.5–40.4 ± 3.8)	28.3 (22.8–37.1 ± 3.7)	37.3 (25.4–53.7 ± 5.7)	26.5 (16.5–39.7 ± 5.9)
SatNW	29.0 (23.0–39.8 ± 4.0)	24.1 (16.1–31.8 ± 4.0)	31.1 (20.6–44.1 ± 5.3)	20.1 (13.1–26.7 ± 3.6)
SatNDS	634.9 (363.0–764.0 ± 85.4)	67.1 (5.1–239.0 ± 80.0)	289.9 (29.5–592.3 ± 140.6)	249.7 (61.6–697.8 ± 166.0)
SatKD1	14.0 (8.2–23.0 ± 3.5)	7.0 (5.3–9.7 ± 1.1)	11.0 (7.4–15.0 ± 1.9)	10.2 (6.0–14.3 ± 1.9)

* Pri, primite; Sat, satellite; AcD, satellite acetabulum depth; AcW, satellite acetabulum width; DL, length of deutomerite; DLAM, distance from protomerite–deutomerite septum to deutomerite axis of maximum width; DLPM, distance from posterior end of deutomerite to deutomerite axis of maximum width; DWE, width of deutomerite at equatorial axis; DWM, maximum width of deutomerite; KD1, diameter of major karyosome; NDS, distance from nucleus to protomerite–deutomerite septum; NL, length of nucleus; NW, width of nucleus; PDSW, width of protomerite–deutomerite septum; PL, length of protomerite; PLAM, distance from anterior end of protomerite to protomerite axis of maximum width; PLPM, distance from protomerite–deutomerite septum to protomerite axis of maximum width; PWE, width of protomerite at equatorial axis; PWM, maximum width of protomerite; TL, total length of gamont.

† $n = 30$.

‡ $n = 34$.

similar in appearance and habit. Pinto's reports of the gregarine from *Stylopyga americana* Fischer, 1846 are consistent with a single host taxon hypothesis, as this is a junior synonym of *Periplaneta americana* (Linnaeus, 1758). The report of a similar gregarine from "*Blatta* (*Periplaneta orientalis*)" clouds the issue: *Periplaneta americana* (Linnaeus, 1758) is the senior synonym of *Blatta orientalis* Sulzer, 1776 but not of *Blatta orientalis* Linnaeus, 1758. (For details of cockroach nomenclature, see Princis, 1966.) There has been no new report of *Protomagalhaensia serpentula* since Pinto (1922). The type host for this gregarine is probably *Periplaneta americana* (Linnaeus, 1758) but could easily be *Periplaneta australasiae* (Fabricius, 1775), *Blatta orientalis* Linnaeus, 1758, or another domiciliary species of *Periplaneta* that was either undescribed or unknown to de Magalhaes and Pinto. No specimen of the type species is known and existing descriptions are insufficient to clearly differentiate *P. serpentula* from

Table 4. Homogenous groups formed by ANOVA protected Tukey's honestly significant difference tests ($\alpha = 0.05$) using gamont metrics* of *Protomagalhaensia blaberae*, *Protomagalhaensia cerastes*, *Protomagalhaensia granulosae*, and *Protomagalhaensia wolfi*. Shared letters indicate homogenous groups, letters indicate relative size for each metric (A > B > C > D).

Metric	*P. blaberae*†	*P. cerastes*†	*P. granulosae*‡	*P. wolfi*†
PriTL§	A	C	A	B
PriPL§	A	C	A	B
PriPWE|	A	C	B	D
PriPWM|	A	C	B	D
PriPLAM	B	C	A	B
PriPLPM	A	C	C	B
PriPDSW|	A	C	B	D
PriDL§	A	C	A	B
PriDWE	A	A	B	C
PriDWM§	A, B	B	A	C
PriDLAM	A	A, B	B, C	C
PriDLPM§	A	C	A	B
PriNL§	A	B	A	C
PriNW§	A	B	A	C
PriNDS|	A	D	C	B
PriKD1	A	C	B	B
AcW§	B	D	A	C
AcD	A, B	A	B	C
SatTL|	B	C	A	B
SatPL|	B	D	A	C
SatPWE	A	B	B	C
SatPWM	A	B	B	C
SatPLAM|	B	D	A	C
SatPLPM	A	B	B	A
SatPDSW	A	B	B	C
SatDL|	B	D	A	C
SatDWE|	A	B	C	D
SatDWM	A	B	B	C
SatDLAM§	A	B	A	B
SatDLPM|	B	D	A	C
SatNL	B	C	A	C
SatNW§	A	B	A	C
SatNDS	A	C	B	B
SatKD1	A	C	B	B

* Pri, primite; Sat, satellite; AcD, satellite acetabulum depth; AcW, satellite acetabulum width; DL, length of deutomerite; DLAM, distance from protomerite–deutomerite septum to deutomerite axis of maximum width; DLPM, distance from posterior end of deutomerite to deutomerite axis of maximum width; DWE, width of deutomerite at equatorial axis; DWM, maximum width of deutomerite; KD1, diameter of major karyosome; NDS, distance from nucleus to protomerite–deutomerite septum; NL, length of nucleus; NW, width of nucleus; PDSW, width of protomerite–deutomerite septum; PL, length of protomerite; PLAM, distance from anterior end of protomerite to protomerite axis of maximum width; PLPM, distance from protomerite–deutomerite septum to protomerite axis of maximum width; PWE, width of protomerite at equatorial axis; PWM, maximum width of protomerite; TL, total length of gamont.

† $n = 30$.

‡ $n = 34$.

§ Metrics for which *P. blaberae* and *P. granulosae* form a homogenous subgroup.

| Metrics for which each species is significantly different from all other species in the analysis.

other members of the genus. Until the type host is determined, new collections are made, and the species is redescribed, *P. serpentula* is considered species inquirenda (sensu Clopton et al., [2007]), although it remains the type species of *Protomagalhaensia* under Article 70.1 of the International Code of Zoological Nomenclature. *Protomagalhaensia serpentula* adequately establishes the genus but is simply too poorly known to be readily differentiated from other species within the genus.

Species differentiation within *Protomagalhaensia*

The four known species of *Protomagalhaensia*, *P. granulosae*, *P. blaberae*, *P. wolfi*, and *P. cerastes* are

recognized primarily by separation of morphometric centroids and morphology of oocysts and gamonts in association. Their epimerites are simple gladiate to deltoid holdfasts that lack a diamerite, serving as a shared character uniting the genus but with little taxonomic value in species differentiation.

Across the genus, oocysts are dolioform to broadly dolioform in form but differ in size, morphometric ratio, surface ornamentation, and the shape of the polar dehiscence plate. Oocysts of *P. blaberae*, *P. granulosae*, and *P. wolfi* are distinctly dolioform with distinct polar dehiscence plates that form apical corner knobs or spines. These spines are relatively pronounced on the oocysts of *P. blaberae* (Fig. 3) and *P. granulosae* but are small and abaxial in the oocysts of *P. wolfi* oocysts. In contrast, the oocysts of *P. cerastes* possess distinct polar dehiscence plates that do not form apical corner spines. When observed in a thick, temporary water mount, oocysts of *P. blaberae* (Fig. 4), *P. granulosae*, and *P. wolfi* exhibit distinct sagittal compression with a dorsal surface depression, making the oocysts distinctly corpuscular in appearance. Neither sagittal compression nor corpuscular appearance is present in the oocysts of *P. cerastes*. Summary descriptive statistics by taxon and grouping results (Tukey's HSD) for oocyst metrics are presented in Tables 1 and 2, respectively. Among species of *Protomagalhaensia*, oocysts differ significantly across all species in both maximum length and width (Fig. 13; Tables 1, 2). In all other oocyst metrics, those of *P. blaberae* significantly overlap only *P. granulosae* (DPW, ResDia), and *P. wolfi* (DPL) (Tables 1, 2).

Summary descriptive statistics by taxon and grouping results (Tukey's HSD) for gamont metrics are presented in Tables 3 and 4, respectively. Separation of differential morphometric centroids for gamonts of *Protomagalhaensia* are displayed in Figures 14–16. The figures illustrate the population variation and central morphometric tendency of each species, and clearly distinguish *P. blaberae*, *P. cerastes*, *P. granulosae*, and *P. wolfi*, although gamont metrics do not separate taxa as distinctly as do oocyst metrics; this is especially true for *P. blaberae* and *P. granulosae* (cf. Figs. 13, 14–16). Among species of *Protomagalhaensia*, gamonts differ significantly across all species in the following metrics: PriPWE, PriPWM, PriPDSW, PriNDS, SatTL, SatPL, SatPLAM, SatDL, SatDWE, and SatDLPM (Figs. 14–16; Tables 3, 4). Morphometrically, gamonts of *P. blaberae* significantly overlap with those of *P. granulosae* (PriTL, PriPL, PriDL, PriDWM, PriDLPM, PriNL, PriNW, AcW, SatDLAM, and SatNW). Some morphometric overlap exists with *P. wolfi* (PriPLAM, AcD, and SatPLPM) and *P. cerastes* (PriDWE, PriDWM, and AcW), but these metrics do not correlate in a coordinated way to the overall shape and morphology of protomagalhaensid gamonts. Of known species in the genus, gamonts of *P. blaberae* are most similar to those of *P. granulosae*. In general, gamonts of *P. blaberae* and *P. granulosae* are both broader and longer than those of *P. cerastes* and *P. wolfi*. Primites of *P. blaberae* tend to be wider than those of *P. granulosae* while the satellites of *P. blaberae* are generally narrower and shorter than those of *P. granulosae*.

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