

The twists and turns of biological research: first record of an ergatoid queen in the ant genus *Pheidole* Westwood, 1839, and implications for dispersal and life history

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ABSTRACT. An ergatoid queen is reported for the first time in the ant genus *Pheidole* Westwood, 1839. Initially being investigated as a mermithergate, its true reproductive nature was finally proven after several years of searching for fresh specimens for dissection and imaging. Two possibilities remain unresolved regarding the reproductive strategy seen in the Philippine local populations of *P. aglae*: (1) that they are secondary reproductives; or (2) winged queens have been eliminated from the Philippine population due to isolation on oceanic islands. The exceptional reproductive strategy was found in the oceanic islands of Luzon and nearby Polillo. Further research is needed by taking account of the “island syndrome”. This study also highlights the unexpected directions that problems in biological research take the researcher.

Keywords dispersal, dependent colony foundation, ovary, Philippines, *Pheidole aglae*, Formicidae

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INTRODUCTION

Forel (1913) described the three female castes, major worker, minor worker, and dealate queen, of *Pheidole aglae* Forel, 1913. The dealate queen specimen, FoCol 1401, is housed at the Museum

für Naturkunde der Humboldt Universität, Berlin, Germany (ZMBH) and imaged on Antweb (2020) (Fig. 1). Eguchi (2001a) did not include this specimen when he revised the taxonomy of the major and minor workers of *P. aglae*. In his revision of the *Pheidole* of Borneo, Eguchi

(2001b) redescribed the worker and soldier of *P. aglae* but did not redescribe the reproductive caste although he collected worker-associated dealate queens from several nest series (Eguchi, pers. comm.).

In the Philippines, *Pheidole aglae* is one of the common species of the genus and is widely distributed from Luzon Island to Mindanao Island and several islands in between (General & Alpert 2011). In 2013, the first author of the present study, David Emmanuel M. General (DEMG), conducted a transect study in Polillo Island, off the eastern coast of Luzon Island (Fig. 2), and obtained nest series of ants from rotten twigs. Polillo Island is a small oceanic island dominated by low hills of karst. Each of two nest series of *P. aglae* contained a strange adult ant, although the other two nest series of *P. aglae* did not contain such strange ants. The strange ants possessed a smaller head and a larger gaster than the major worker, a mesosoma with a mesoscutum, and was intermediate in body size between the minor and major workers. These individuals were first considered to be mermithergates, since infection by a nematode is known to produce bizarre morphology in ant workers (Wheeler 1901, Wheeler 1928, Poinar 2012). Those specimens were transferred into ethanol in the field, and, consequently, those could not be examined further by dissecting them. According to a nematologist at University of the Philippines Los Baños (UPLB), Dr. Vachel Gay Paller (pers. comm.), the nematode needed to be recovered from a live mermithergate for taxonomic identification.

In 2014, DEMG again collected a nest series of *P. aglae* with a suspected mermithergate from a log in Mt. Bulusan, an active volcano in Sorsogon Province, Luzon Island (Fig. 2). This time, the nest fragment was kept alive until the mermithergate could be dissected. Unexpectedly, no nematodes were found in the abdomen, but instead developed ovaries were found. The suspected mermithergate turned out to be an ergatoid queen. However, unfortunately, no images of the ovaries and other reproductive organs were captured because DEMG did not know then that the reproductive organs would become unsuitable for dissection and imaging when stored in ethanol.

In 2019, a twig nest with an ergatoid queen was collected again from Mt. Isarog, a dormant volcano in Camarines Sur Province, Luzon Island (Fig. 2). The nest fragment was kept alive until the ergatoid queen could be successfully dissected and photographed.

The present study aims to report the first discovery of ergatoid queens in the genus *Pheidole* and make inferences about the patterns of colony foundation and growth.

MATERIALS AND METHODS

Specimen Depository

Specimens are deposited in the Philippine National Museum of Natural History, Manila, Philippines (PNM) and the University of the Philippines Los Baños, Museum of Natural History Entomological Collection, Los Baños, Laguna, Philippines (UPLB).

Measurements

All measurements are given in millimetres.

- HL Head length, maximum length of head capsule, excluding mandibles, measured in full-face view in a straight line from the midpoint of posterior head margin to anterior clypeal margin. If anterior clypeal margin and/or posterior margin of head are concave, the straight line is drawn between the midpoints of transverse lines spanning the anterior-most and/or posterior-most points of the head, respectively.
- HW Maximum head width, including eyes when they exceed the lateral margin of the head, measured in full face view.
- SL Scape length, maximum length of scape, excluding basal neck and condyle, measured at the appropriate angle such that the scape is positioned perpendicularly to the viewer.
- EL Maximum eye length, measured along the longest axis of eye.
- WL Weber's Length, mesosomal length measured from anterior edge of the pronotum (excluding the collar) to posterior edge of propodeal lobe.

- PnL Pronotal length, measured in lateral view, from the anterior edge of the pronotal collar to the pronotal-mesonotal suture.
- PnW Maximum width of pronotum, measured in dorsal view.
- GL Gaster length, measured in lateral view, from postpetiolar attachment to tip of gaster.
- GH Maximum height of gaster, measured in lateral view, maximum transverse distance between dorsal surface and ventral surface of gaster.
- GW Maximum width of gaster, measured in dorsal view.

Indices

- CI Cephalic index: $HW/HL \times 100$.
- SI Scape index: $SL/HW \times 100$.
- EI Eye index: $EL/HW \times 100$.

Specimens were examined with a Leica S6D stereomicroscope and measured using a Leica S8APO stereomicroscope with ocular micrometer. Ovaries were dissected from two specimens (UPLBMNH HYM-01776; UPLBMNH HYM-01777) but only the Mt. Isarog specimen (UPLBMNH HYM-01776) was imaged. Images were created using a Leica MC120HD digital camera attached to the Leica S8APO stereomicroscope. These images were stacked using Combine ZM (available from: <https://combinezm.en.lo4d.com/windows>). The stacked images were edited with Adobe Photoshop CS5. The map was created in QGIS 3.14 (available from: <https://www.qgis.org/en/site/>).

RESULTS

Description of Ergatoid Queen of *Pheidole aglae* (Figs. 3-6)

Measurements: see Table 1.

In full-face view, lateral margins of head convex; posterior margin of head concave; occipital corners of head rounded, not forming distinct lobes; occipital carina entire. Compound eyes located in front of midlength of head; median ocellus present. Frons with a shallow median depression anterior to median ocellus. Frontal carinae

subparallel and almost reaching posterior margin of head. Antennal scape long, when laid backwards extends beyond posterior margin of head by about 1/3 of scape length. Basal portion of antennal scape expanded laterally as a flange; antenna 12-segmented, with 3-segmented club. Antennal fossa large and deeply impressed. Frontal lobe reduced laterally, so that antennal condyle is partly visible, and terminally produced dorsally into a sharp flange. Anteromedian margin of clypeus convex, with a median emargination. Mandibles triangular. Mandibular dentition composed of apical and pre-apical tooth, followed by three denticles and basal tooth. Palp formula 2,2 (observed without dissection).

Dorsal margin of mesosoma in lateral view rising posterad to mesoscutum then angling downward, interrupted by mesoscutellar disc and metanotal groove, to base of propodeal spines. Pronotum broad. Anterior half of mesoscutum prominent. Flight sclerites absent; above the usual location of the wing scar, a shallow pit present just under mesoscutellar disc, unbounded posteriorly but bordered above and below by thick cuticular ridges. Mesopleuron divided by transverse suture. Propodeal spines short and triangular. Metapleural gland orifice subcircular, without guard hairs. Petiolar node in lateral view low, distinguished from peduncle by rounded obtuse angle. Postpetiole in lateral view low and dome-shaped. Gaster large, at least 3X volume of mesosoma.

Frons longitudinally densely carinate; antennal scrobe carinate; median portion of clypeus largely smooth, with a single median carina; lateral portion of clypeus carinate; malar area reticulate; mandibles smooth and shiny; pronotum transversely carinate dorsally, irregularly granular laterally; mesopleuron and lateral face of propodeum largely smooth; dorsum of mesoscutum transversely irregularly carinate, with occasional cross-hatches, over interstitial punctation; mesoscutellar disc shallowly reticulate; dorsum of propodeum, petiole and postpetiole dorsally transversely carinate with interstitial punctation; first gastral tergite finely granulate, overlain by weak reticulation; each of the remaining gastral tergites similar to the first, but with a smooth anterior half.

Table 1. Standard measurements of ergatoid queens and workers of *Pheidole aglae* (see also Figure 6).

Standard Measurements	Ergatoid queen (Isarog)	Ergatoid queen (Bulusan)	Major worker (Bulusan)	Minor worker (Bulusan)
	UPLBMNH HYM-01776	UPLBMNH HYM-01777	UPLBMNH HYM-01779	UPLBMNH HYM-01778
EW	0.20	0.20	0.16	0.13
HL	1.12	1.08	1.88	0.93
HW	1.16	1.04	1.60	0.63
SL	1.32	1.24	1.20	1.43
EL	0.28	0.24	0.20	0.18
WL	1.64	1.60	1.44	1.35
PnL	0.76	0.68	0.52	0.53
PnW	0.68	0.68	0.66	0.45
GL	damaged	1.44	1.40	0.55
GH	1.20	1.08	1.00	0.40
GW	1.44	1.40	1.00	0.30
CI	104	96	85	68
SI	114	119	75	228
EI	24	23	13	28



Fig. 1. Syntype dealate queen of *Pheidole aglae* Forel, 1913. Lateral view (Note: the image is flipped since this side presents a clearer view of the morphology of the dealate queen). Image created by Christiana Klingenberg. Images of FoCol #1401 were accessed from www.antweb.org, with express permission of Brian Fisher.

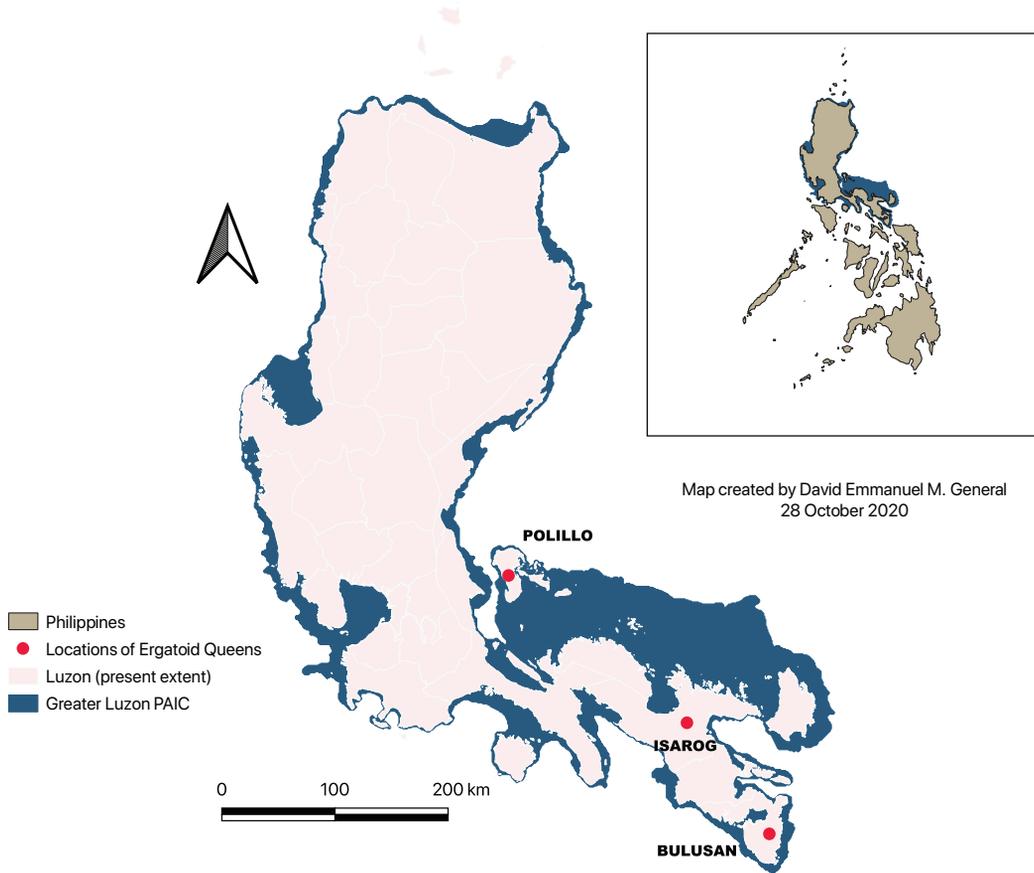


Fig. 2. Map of the known locations of the ergatoid queen of *P. aglae*. The current extent of Luzon Island and nearby Polillo Island (PhilGIS 2020) is super-imposed over the estimated extent of the Greater Luzon Pleistocene Aggregate Island Complex (PAIC) (modified from Heaney 1985 and Sánchez-González, et al. 2015). Inset map shows the entire Philippine archipelago.



Fig. 3. Ergatoid queen of *Pheidole aglae* Forel, 1913 from the Philippines (UPLBMNH HYM-01777). Full-face view.



Fig. 4. Ergatoid queen of *Pheidole aglae* Forel, 1913 from the Philippines (UPLBMNH HYM-01777). Lateral view.

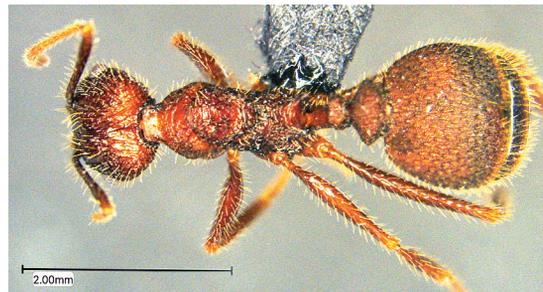


Fig. 5. Ergatoid queen of *Pheidole aglae* Forel, 1913 from the Philippines (UPLBMNH HYM-01777). Dorsal view.

Antennal scape with evenly spaced suberect hairs; dorsum of head with sparse erect hairs; mesoscutum and mesoscutellar disc with abundant erect hairs; pronotum, propodeum, petiole, postpetiole, and gaster with less abundant erect hairs with intervals about the length of an individual hair.

Body dark reddish brown, with darker mesoscutum, mesoscutellar disc and gaster.

Reproductive organ (two ergatoid queens were dissected): A queen from Mt. Bulusan (UPLBMNH HYM-01777) having ovaries in which eggs are present. A queen from Mt. Isarog (UPLBMNH HYM-01776) having 4+4 ovarioles in which several developed eggs and yellow bodies are present. The spermatheca was not identified among the other organs, thus was not examined for the presence of sperm (Fig. 7).



Fig. 6. *Pheidole aglae* Forel, 1913 from the Philippines. Lateral view. Ergatoid queen (UPLBMNH HYM-01777) (top); major worker (UPLBMNH HYM-01779) (middle); minor worker (UPLBMNH HYM-01778) (bottom). The Ergatoid queen and the minor worker are from one nest series while the major worker is from another nest series. These nest series were collected from Mt. Bulusan at the same time. (Note: the three specimens were temporarily arranged on a single pin to allow visual comparison of the relative sizes of the head, mesosoma, and gaster among the 3 subcastes).

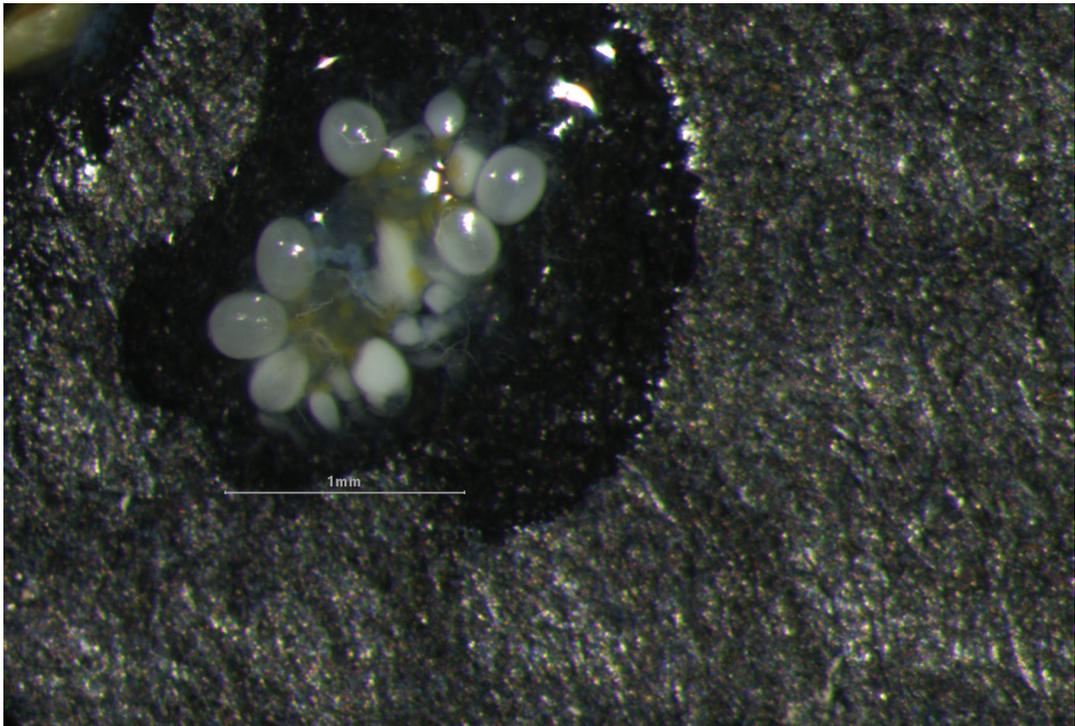


Fig. 7. Ovaries of ergatoid queen of *Pheidole aglae* Forel, 1913 from the Philippines. The specimen was collected from Mt. Isarog. Each ovary has four active ovarioles and yellow bodies are present.

DISCUSSION

A key to the ecological success of ants is the innovation of wingless workers that perform maintenance and foraging, while retaining flying queens that disperse and initiate new colonies (Keller et al. 2014; Peeters & Molet 2010). The winglessness of ant workers allowed the evolution of an extreme divergence relative to winged queens in many species, unlike in social wasps and bees (Peeters & Ito 2015). In most formicoid ants (89% of extant species), the enlarged thorax of the queen houses hyperdeveloped wing muscles that are broken down after the nuptial flight in order to feed the first generation of workers during independent colony foundation (Wheeler & Buck 1996). A big gaster houses enlarged ovaries that underlie higher fecundity (Peeters & Ito 2015). Nonetheless, there is a striking trend towards the loss of aerial dispersal by females across all ant lineages (Peeters 2012). Ergatoid queens are primarily wingless reproductives with a simplified (worker-like) thorax and are known to have evolved sporadically in 64 genera from 11 subfamilies (compiled by CP in AntWiki 2020a). Moreover, short-winged brachypterous queens occur in 18 genera from 7 subfamilies (compiled by CP in AntWiki 2020b). Both these flightless reproductives are associated with a shift from independent to dependent colony foundation (Cronin et al. 2013). Colonies of *Acanthomyrmex careoscrobis* Moffett, 1986 can produce both winged and ergatoid queens, and the latter may function as cheaper secondary reproductive (Yamada et al. 2018). Ergatoid queens have the same number of ovarioles as winged queens and show variable intermediate external morphology between soldiers and winged queens (Yamada et al. 2018).

However, surprisingly, wingless reproductives have not been recorded from highly speciose formicoid genera such as *Pheidole* Westwood, 1839, *Camponotus* Mayr, 1861, *Crematogaster* Lund, 1831, and *Tetramorium* Mayr, 1855. *Pheidole* is the most diverse of all ant genera and is distributed on all continents except the frigid Poles. Globally, there are 1,095 valid species and 132 valid subspecies (Bolton 2020).

Eguchi (2001) revised *Pheidole* from Borneo and recognized 52 species, including 23 newly described. Wilson (2003) revised the genus in the New World, describing more than 300 new species. However, while specialized breeding strategies such as social parasitism are known in *Pheidole* (Wilson 1984; Fischer et al. 2020), there was no evidence of the existence of the ergatoid queen until the present study.

Two winged queens of *P. aglae* from West Java (Bogor) had 18 and 20 ovarioles, respectively. They were collected from nests in rotten logs on forest floor of the botanical gardens, and the colonies had only a few hundred workers (F. Ito, personal communication).

The presence of developed eggs and yellow bodies in ovarioles (Fig. 7) is unequivocal evidence that this ergatoid queen reproduced in its colony. In *Pheidole*, both workers and soldiers lack ovaries (Fletcher & Ross 1985; Frumhoff & Ward 1992), which makes our observation unambiguous. Therefore, this is the first record of an ergatoid queen in the highly speciose genus *Pheidole* that have been overlooked so far.

Two possibilities remain unresolved regarding the reproductive strategy of the Philippine populations of *P. aglae*: (1) ergatoid queens coexist with winged queens, with the former functioning as secondary reproductives, to extend the lifespan of colonies that were founded by a single dealate queen; (2) independent colony foundation where full-winged queens have been eliminated completely from the local populations, and dependent colony foundation is obligate. Both Polillo and Luzon Islands are oceanic islands, never connected to the Asian continent (Heaney 1985, Heaney et al. 2016). Such geographical factor may be associated with the exceptional reproductive strategy seen in the Philippine populations. Further research is needed by taking account of the “island syndrome”.

This study also illustrates the roundabout way that research sometimes proceeds, complete with wrong turns and missed opportunities, until the biological truth is revealed by evidence. What was first being investigated as a mermithergate turned out to be a major discovery of a novel reproductive strategy in the genus *Pheidole*.

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