



Notes on the biology of *Protanilla* sp. (Hymenoptera, Formicidae) collected in Ulu Gombak, Peninsular Malaysia

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Abstract

A colony of *Protanilla* sp. was collected from the floor of a selectively logged forest in Ulu Gombak, Peninsular Malaysia, in March 2011. The colony contained 17 ergatoid queens and 25 workers. The ergatoid queens had 6–10 ovarioles per individual with a sperm-filled spermatheca while workers had two ovarioles and a vestigial spermatheca. In the laboratory, foraging workers responded to *Occasjapyx* sp. (Japygidae) only; larvae, workers, and ergatoid queens fed on this prey. Ergatoid queens frequently showed larval and egg care, and transported larvae when the nest was disturbed. Compared to *Leptanilla*, morphological and behavioral specialization of both castes in *Protanilla* sp. does not seem developed.

Keywords Behavioral characteristics · Caste specialization · Ergatoid queen · Japygidae

Introduction

Recent phylogenetic studies of ants indicate that the subfamily Leptanillinae is an early-branching subfamily with Martialinae (Brady et al. 2006; Kück et al. 2011; Branstetter et al. 2017; Borowiec et al. 2019). Although the phylogenetic position is still controversial, knowledge on their biology and ecology is very important for understanding the evolution and diversity of ants. Rigid biological information based on sufficient data, however, is only available from *Leptanilla japonica* Baroni Urbani, 1977 (Masuko 1989, 1990), because most members in this subfamily are cryptic and live in the underground, which makes it difficult to collect entire colonies. In addition to *L. japonica*, some biological information such as colony composition, queen behavior and/or prey preference has been reported for *Leptanilla clypeata* Yamane and Ito, 2001 (Ito and Yamane, 2020), *Protanilla jongi* Hsu et al., 2017 (Hsu et al. 2017), and *P. lini* Terayama, 2009 (Katayama and Tsuji 2011; Yamamuro 2018).

The colony life of *Protanilla* appears very different from *Leptanilla*. Dealate queens are reported for all five *Protanilla* species for which queens are known (Baroni Urbani and De Andrade, 2006; Hsu et al. 2017; Man et al. 2017; Terayama 2013; Yamamuro 2018), while in all nine species of *Leptanilla* so far studied queens are dichthadiiform and therefore permanently wingless (ergatoid queen) (Emery 1870; Wheeler 1932; Kutter 1948; Brandica et al. 1994; Ogata et al. 1995; Xu 2002; Terayama and Kinomura 2015; Ito and Yamane 2020). We managed to collect one colony of *Protanilla* in Ulu Gombak, peninsular Malaysia. Unexpectedly ergatoid queens reproduced in this species. Here we reported our preliminary observations on the colony composition and their behavioral characteristics.

Materials and methods

A colony of *Protanilla* sp. was collected from a small dead twig (ca. 3 cm diameter, 15 cm length) under fallen leaves in the Ulu Gombak forest reserve on 17 March 2011 (Colony code, FI11-96). The colony composition was recorded just after collection. The colony was kept in the laboratory in a polystyrene box (148 × 84 × 32 mm). The bottom of the box was covered with Plaster of Paris for keeping the humidity high. Inside the box, a smaller polystyrene box (68 × 39 × 15 mm) with an entrance hole served as a nest

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chamber. The bottom layer of the chamber was also covered with Plaster of Paris.

Protanilla sp. in this paper was identified as *P. wallacei* by Dr. Bob Taylor (pers. comm.). This scientific name already appeared in *The Ants* (Hölldobler and Wilson, 1990, p 592, Figures 16–18), however, the species is still not formally described. We, therefore, refer to this “*P. wallacei*” as *Protanilla* sp. in this paper. We already reported the novel exocrine system of this species (Billen et al. 2013).

To determine the prey preferences of *Protanilla* sp., several kinds of arthropod animals including geophilomorph centipedes ($N=7$), termites ($N=5$), small cricket nymphs ($N=6$), Campodeoidea (Diplura) ($N=4$), and *Occasjapyx* sp. (Jyapygidae, Diplura) ($N=5$) were given to the ants. The behavior of workers towards these prey items was observed.

The behavior of queens and workers was recorded by raster scan visual sampling (77 times over 2 weeks) under a binocular microscope. In each scan session, we recorded the number of ergatoid queens and workers performing the following behaviors: foraging, caring for larvae, caring for eggs, grooming of ergatoid queens, grooming of workers, and feeding on prey. The cumulative data, including the number of individuals performing non-specific behavior such as resting and wandering was analyzed by a X^2 test with subsequent residual analysis to compare the frequency of each behavior and the expected value. To observe nest emigration, the cover of the nest chamber was removed five times, and the caste of individuals transporting brood was recorded. After observation, 27 individuals were dissected to check their reproductive condition.

To observe morphological characteristics of ergatoid queens and workers, a few individuals of each caste were photographed under a scanning electron microscope (JCM-7000, JEOL). The width of head and metasoma of workers ($N=17$) and queens ($N=15$) was measured under a binocular microscope (SZX7, Olympus).

Results

Colony composition

The colony contained 42 wingless females and 29 larvae (Fig. 1). The wingless females were divided into two types according to the presence or absence of compound eyes consisting of 4–10 ill-defined ommatidia, and vestigial ocelli (Fig. 2E, F, G). After finishing the observations, dissections revealed that the individuals with compound eyes and ocelli had a spermatheca filled with sperms (Fig. 3) and 6–10 ovarioles per individual (Fig. 4, average 7.7 ± 1.0 SD); the blind individuals had a very small empty spermatheca (Fig. 3) with two ovarioles per individual (Fig. 4), indicating that the former type are ergatoid queens and the



Fig. 1 Colony of *Protanilla* sp. Some ergatoid queens are indicated by black arrows

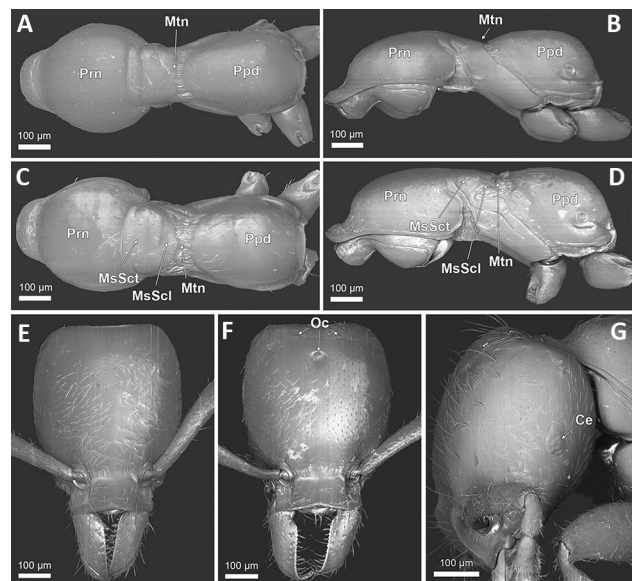


Fig. 2 Dorsal view of mesosoma and head of workers (A, C, E) and ergatoid queen (B, D, F), and lateral view of head of ergatoid queen (G). Oc ocelli, Ce compound eye, MsScl mesoscutellum, MsSet mesoscutum, Mtn metanotum, Oc ocelli, Ppd propodeum, Prn pronotum

latter are workers. The worker spermathecae could not be found under a dissecting microscope during dissection of individuals, but the occurrence of a spermatheca in workers was confirmed by observation of semi-thin sections of the metasoma ($N=3$, Fig. 3 inset). In total, 17 ergatoid queens and 25 workers occurred in the colony. Mesosomal morphology of ergatoid queens is similar to workers (Fig. 2A–D), but differs from the latter in the following characters: (1) mesonotum without distinct scuto-scutellar sulcus and scuto-scutellar suture, only weakly divided into mesoscutum and mesoscutellum, the latter being distinctly more narrow posteriorly (Fig. 2C, D) (scutum and

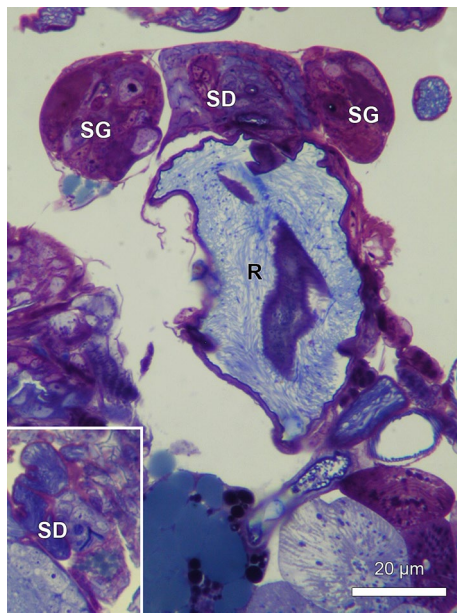


Fig. 3 Semithin section of the queen spermatheca. *R* reservoir, *SD* spermatheca duct, *SG* spermatheca gland. The inset shows the vestigial worker spermatheca, both images shown at same magnification. Inset image modified after Billen et al. (2013)

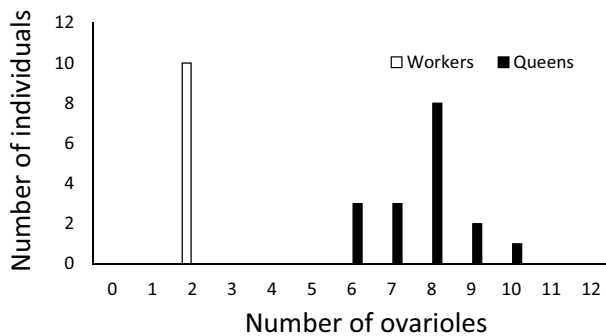


Fig. 4 Ovariole numbers per individuals in ergatoid queens and workers

scutellum not differentiated, with almost straight posterior margin in the worker (Fig. 2A, B), (2) metanotum well defined both anteriorly and posteriorly with sulci, forming a transverse belt that is coarsely sculptured (Fig. 2C) [metanotum a simple transverse groove that is not margined with anterior and posterior sulci, and longitudinally ruglose in the worker (Fig. 2A)]. 3) axilla and axillula not differentiated, but area between mesoscutellum and upper sector of mesopleuron irregularly sculptured (Fig. 2D) [such sculptured area is absent in the worker (Fig. 2B)]. The body size of ergatoid queens was larger than that of workers, although variation was high: head width of some ergatoid queens was similar to that in workers (Fig. 5A),

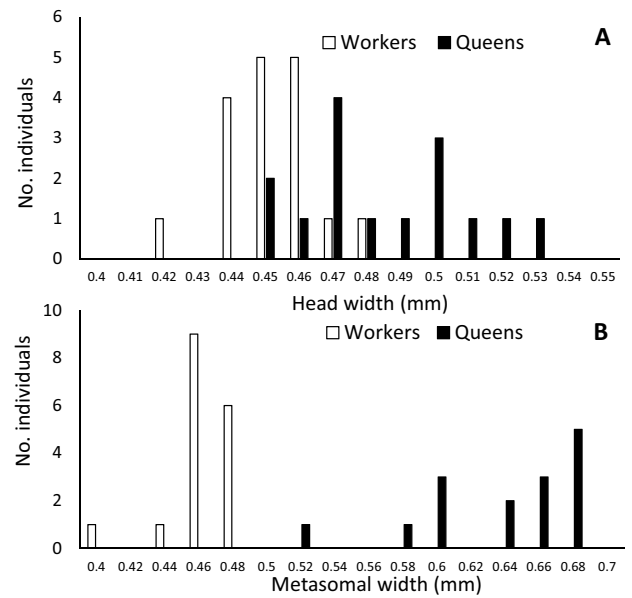


Fig. 5 Distribution of head width (A) and metasomal width (B) of ergatoid queens and workers



Fig. 6 A worker attacks *Occasjapyx* sp.

however, metasomal width of ergatoid queens was distinctly larger than that of workers (Fig. 5B).

Prey

Among the prey animals we offered in the laboratory, foraging workers responded to *Occasjapyx* sp. (Japygidae) only ($N=5$). Workers in the foraging arena aggressively attacked the prey (Fig. 6): the typical behavioral sequence was as follows: when a worker encountered *Occasjapyx* sp. in the foraging arena, the worker grasped the prey with its appendages (legs or antennae) and stung it in its dorsal part of the

prey. After paralyzing the prey, the worker carried it into the nest chamber, where larvae were put on the prey, and they directly fed on it. Ergatoid queens and workers also ate the prey. However, all prey items ($N=5$) were not completely consumed. The prey remains were left in a corner of the nest chamber.

Behavior of queens and workers

During our observations, no pupae occurred but ca 20 larvae and up to 15 eggs were counted. Eggs were found during the observation period when larvae occurred. Egg size was relatively large ($0.2\text{ mm} \times 0.44 \sim 0.5\text{ mm}$, $N=4$): the length was almost equivalent to the head width of ergatoid queens. Figure 7 shows the frequency per individual of each behavior performed by ergatoid queens and workers. Ergatoid queens never showed foraging activity outside the nest chamber. Inside the chamber, they frequently performed brood care. The frequency of each behavior was significantly different between the two castes ($\chi^2 = 199.0$, $P < 0.01$, Cramer's $V = 0.248$). The residual analysis indicated that the frequency of caring for larvae and eggs by ergatoid queens was significantly higher than that observed for workers, and the frequency of grooming toward workers and foraging was significantly smaller than that by workers. When we disturbed nests, adult individuals grasped the larvae and carried them to the outside (Billen et al. 2013). In the five repetitions, 1–13 ergatoid queens transported the larvae, while the number of workers showing this behavior was 1–9 (Table 1). The ratio of individuals transporting larvae was not different between the two castes in four out of five trials. In the second trial, a significantly larger ratio of ergatoid queens transported larvae. As already mentioned, both queens and

Table 1 Number of workers and ergatoid queens that engaged in larval transport after nest disturbance

Caste	No. individuals transporting larvae				
	A	B	C	ID	E
Workers	4	9	1	6	1
Ergatoid queens	3	13	3	2	1
Fisher test	NS	$P < 0.05$	NS	NS	NS

Repetition was five times (A to E)

workers fed on *Occasjapyx* sp., and we did not observe larval hemolymph feeding nor trophic egg feeding.

Discussion

So far only alate queens have been described in *Protanilla* (Baroni Urbani and De Andrade 2006; Hsu et al. 2017; Man et al. 2017; Terayama 2013; Yamamuro 2018). *Protanilla* sp. in this paper is the only species in this genus that reproduces through ergatoid queens. Comparing to alate queens of congeneric species described so far, remarkable morphological characteristics in ergatoid queens of *Protanilla* sp. are the reduced compound eyes, vestigial ocelli and a simplified mesosoma. These body parts are more simplified and/or absent in dichthadiiform queens of *Leptanilla*: queen's compound eyes consist of one or two ommatidia without ocelli, and a metanotal groove is absent (Ogata et al. 1995; Terayama and Kinomura 2015). Unlike dichthadiiform queens of the genus *Leptanilla*, morphological and behavioral specialization of queens is not conspicuous in *Protanilla* sp. In *Leptanilla*, queens rarely showed social behaviors such as brood care and grooming of nestmates, and fed exclusively on a larval hemolymph diet (Masuko 1989; Ito and Yamane 2020). The queen body size of *Leptanilla* is large in comparison to workers. *Leptanilla* queens are apparently specialized for reproduction. In contrast, body size of ergatoid queens in *Protanilla* sp. is larger than that of their workers but the difference is not conspicuous. There is a remarkable body size variation among ergatoid queens: the head width of the smallest ergatoid queen was similar to that of workers. The queens frequently showed larval and egg care, and fed on prey animals directly. Furthermore, queens as well as workers transported larvae during nest emigration under laboratory conditions. Thus, the ergatoid queens of this species are “multi-purpose” ergatoid queens in the sense of Molet et al. (2007). Morphological specialization of workers is also weak as compared to *Leptanilla*: workers of *Leptanilla* lack both ovaries and a spermatheca (Masuko 1990; Ito and Yamane 2020), whereas workers of *Protanilla* sp. retain ovaries as well as a small spermatheca, even though

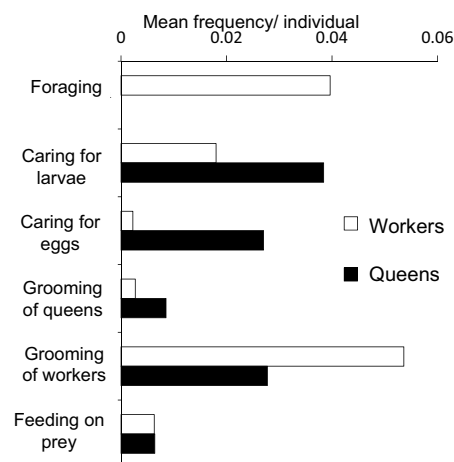


Fig. 7 Frequency of each behavior performed by ergatoid queens and workers

it is unknown if worker ovaries and spermathecae retain any functional potential. In sum, caste specialization is comparatively weak in *Protanilla* sp.

In *Leptanilla*, all species so far studied exhibit monogyny: only one dichthadiiform queen exists in a colony (Masuko 1990; Ogata et al. 1995; Terayama and Kinomura 2015; Ito and Yamane 2020) while we here report on the coexistence of 17 ergatoid queens in a colony of *Protanilla* sp. In *P. lini*, the colony is monogynous with one dealate queen (Yamamuro 2018). Thus, *Protanilla* shows greater variation in social organization than *Leptanilla*.

In the laboratory, *Protanilla* sp. produced eggs coincident with the presence of larvae. In cases of ant species showing phasic reproduction, eggs are only produced when larvae become pupae (e.g. Ito et al. 2018). Egg production under the presence of larvae in *Protanilla* sp. therefore indicates this is not a phasic species. When the colony was collected, however, brood composition was made up of larvae only, but as larval development was not easy in the laboratory, it remains unclear whether this species shows phasic reproduction like *Leptanilla*.

Leptanilla are specialized predators on geophilomorph centipedes (Masuko 1989). Katayama and Tsuji (2011) and Yamamuro (2018) reported that the Japanese species *P. lini* accepts several different kinds of small arthropods as prey, including geophilomorph, lithobiomorph, and scolopendromorph centipedes, cockroaches (*Margattea satsumana* [Blattellidae] and *Periplaneta australasiae* [Blattidae]) and Isopoda. *Protanilla jongi* feed on geophilomorph centipedes (Hsu et al. 2017). In contrast to these species, *Protanilla* sp. observed here accepted only *Occasjapyx* sp. (Jaypigiidae, Diplura). However, we could keep this colony only for 6 weeks, and the ants never fed on the prey in its entirety. We, therefore, do not know whether this remarkable difference reflects a species specific prey preference, or was the result of laboratory conditions. Further research is necessary to understand prey specialization in this genus.

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Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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