

Behavior of the queen of *Leptanilla clypeata* Yamane et Ito collected in the Bogor Botanical Gardens, West Java, Indonesia (Hymenoptera; Formicidae), with a note on colony composition and a description of the ergatoid queen

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ABSTRACT. One colony of *Leptanilla clypeata* Yamane et Ito was collected in the type locality, the Bogor Botanical Gardens, West Java, Indonesia. The colony was composed of one ergatoid queen, 100 workers, and 120 almost uniformly sized larvae. Queen behavior was observed in the laboratory. As shown in *L. japonica* by Masuko (1989), the queen showed larval hemolymph feeding, and started to lay eggs after frequent feeding of larval and prepupal hemolymph. The morphology of the ergatoid queen is described for the first time.

Keywords caste, ergatoid queen, larval hemolymph feeding, prepupae

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INTRODUCTION

Recent phylogenetic studies of ants indicate that the extant ants comprise four phylogenetically distinctive groups: the poneroid and formicoid groups, and two early branching subfamilies, Leptanillinae and Martialinae (Brady et al. 2006; Rabeling et al. 2008; Kuck et al. 2011; Borowiec et al. 2019). Knowledge on the early branching subfamilies is indispensable for understanding the evolution and diversity of ants. However, solid information on behavior and ecology of Leptanillinae is only available for *Leptanilla japonica* Baroni Urbani, 1977, studied by Masuko (1989, 1990), because most members in this subfamily

are cryptic, underground species and rarely collected. One of the most interesting behavioral characteristics of *L. japonica* as shown by Masuko (1989, 1990) is larval hemolymph feeding (hereafter LHF) by ergatoid queens: larvae have a pair of novel larval hemolymph tap organs which secrete hemolymph when they are grasped by the queens. Larval hemolymph appears to be the only food ingested by queens, while workers consume other prey in addition to LHF. Beside *L. japonica*, larvae of other species of *Leptanilla* studied so far have similar structures to the hemolymph taps (Wheeler & Wheeler 1988; Barandica et al. 1994), therefore, the LHF behavior of queens may commonly occur in *Leptanilla* spe-

cies. Thus, to investigate the commonness of this behavior among *Leptanilla* species, additional studies are necessary.

We fortunately collected one colony of *L. clypeata* Yamane et Ito, 2001, in Bogor, West Java, and confirmed the occurrence of LHF. In this paper, colony composition and behavioral characteristics of a queen are reported. The morphology of the queen is described for the first time. Although this paper is based on fragmental observation of a single colony, we believe the report is worthwhile, because collection of *Leptanilla* colonies is very difficult and published data on their natural history are scarce. Furthermore, additional collection of this species seems to be impossible, because a drastic change of the ant fauna has recently occurred in the Bogor Botanical Gardens (Ito, pers. obs).

MATERIALS AND METHODS

Leptanilla clypeata was described based on worker specimens collected in the Bogor Botanical Gardens, West Java, Indonesia (Ito et al. 2001). An almost complete colony of *L. clypea-*

ta (personal colony code of F. Ito, FI01-7) was collected in the type locality, the Bogor Botanical Gardens (6° 36'06" S, 106° 47' 48" E), on 16 December, 2000, and kept in an artificial nest measuring 10 x 6.5 x 2.8 cm until the end of 2000 in the guest house in the botanical garden. The floor of the container was covered with plaster to keep the humidity high. During this period, small geophilomorph centipedes were given as prey. Queen behavior was observed for eight hours over three days under a binocular microscope in the guest house from 26 to 28 December 2000. During this period, the abdomen of the queen was slightly swollen and egg laying could be observed. The ergatoid queen and five workers were dissected for checking ovary condition under a binocular microscope. Maximum width of the head, thorax and abdomen of the queen and ten workers was measured under a microscope. Voucher specimens were deposited in the Bogor Zoological Museum.

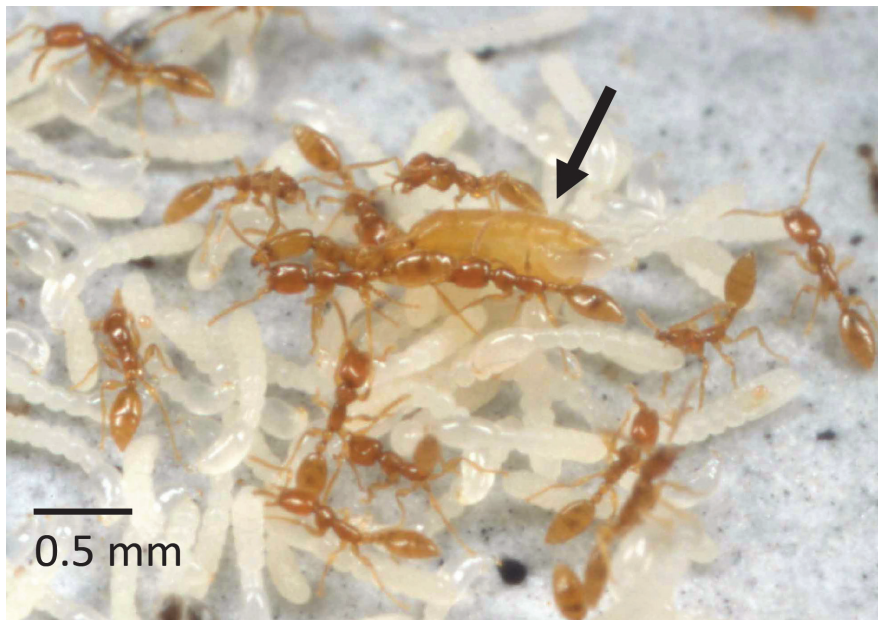


Fig. 1. Picture of a colony of *Leptanilla clypeata* collected in the Bogor Botanical Gardens (Kebun Raya Bogor). Arrow indicates the ergatoid queen. Abdomen of the ergatoid queen is slightly enlarged, and she just starts egg-laying. One prepupa is stacked to left side of the abdomen of the ergatoid queen.

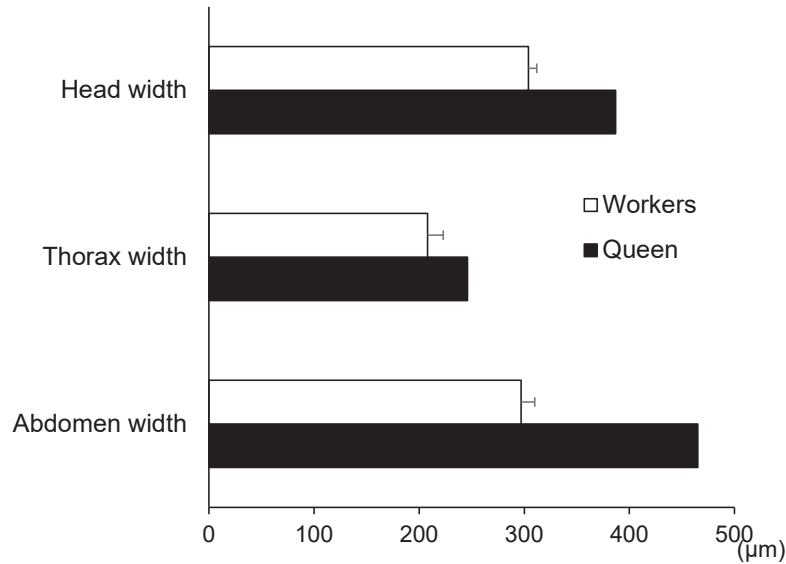


Fig. 2. Body size of the ergatoid queen and workers.

RESULTS

Colony composition

The colony was collected under a stone in a small forest in front of the guest house of the botanical garden. The colony contained one ergatoid queen, 100 workers, and 120 larvae. Larval body size was almost uniform (c.a. 2.0 mm in body length) without small larvae. Eggs and pupae were not present. These facts indicate that this species shows phasic egg production and synchronized brood development. The width of the head and thorax of the queen was slightly larger than that of workers while queen's abdomen was remarkably enlarged (Figs. 1 and 2). The thorax structure of the ergatoid queen was simplified as in workers. Dissection after observation indicated that workers had no ovaries, whereas the queen had a large spermatheca and well-developed ovaries. Unfortunately, the information on ovariole number of the queen was lost. Larvae of *L. clypeata* had a pair of "hemolymph feeding taps" on the IVth abdominal segment. Prepupae and pupae were naked without cocoons.

Queen behavior

When we started the behavioral observation at 14:00 on 26 Dec., some larvae had already become prepupae. The abdomen of the queen was slightly swollen. During the first three hours of observation before 20:00, the queen frequently walked around the nest, and was often groomed by workers. No licking behavior from the queen to the larvae was observed during this first period, but became frequently observed after 20:00 onward until the night on 27 Dec (five hours observation) (Fig. 3). Two types of licking behavior were observed: the first was that the queen just licked the body surface of larva or prepupa for a few seconds, and then walked away. The second type was that after a short time of licking, the queen grasped a larva or a prepupa with its mandibles and licked a certain point of the body surface for 20 to 90 seconds. We did not determine the precise site licked by the queen in the latter case, however, it was around the IVth abdominal segment. Workers also often showed LHF towards larvae and prepupae.

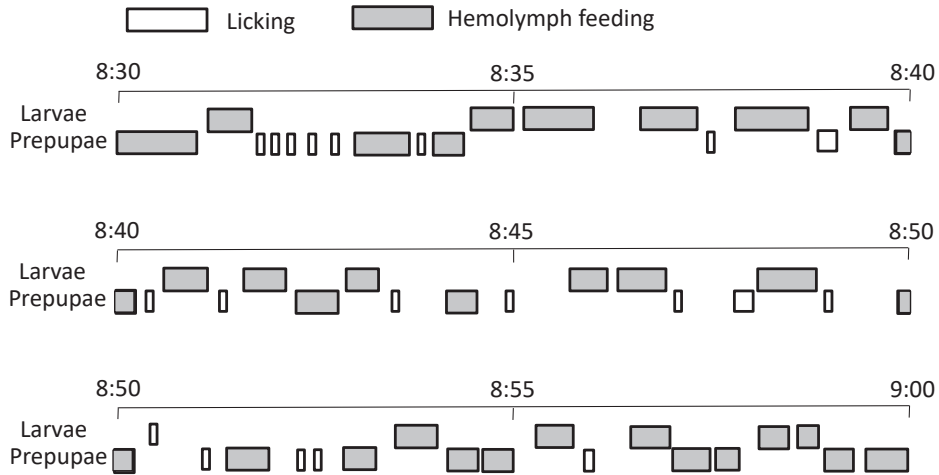


Fig. 3. Licking and hemolymph feeding behavior of the ergatoid queen of *L. clypeata* during 30 min. observed from 8:30 AM to 9:00 AM on 27 December 2000. Length of each bar indicates the duration of the behavior.

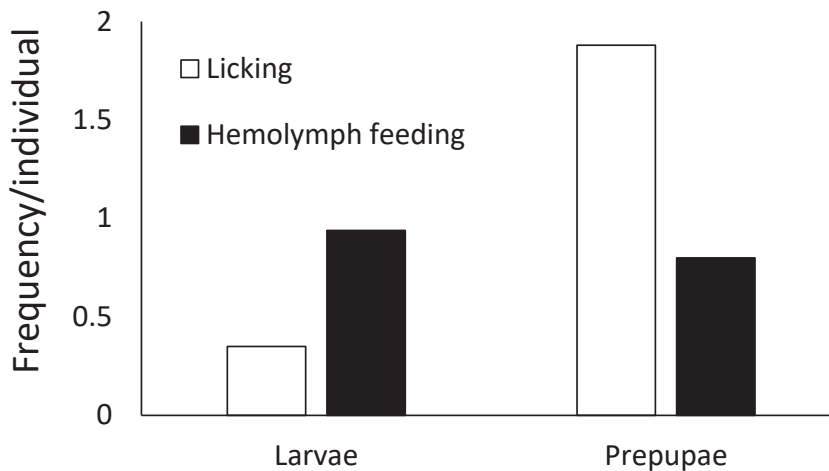


Fig. 4. Relative frequency of licking and hemolymph feeding towards larvae and prepupae during a 4 hour observation on 27 Dec.

During a four hours observation period on 27 Dec, with 37 larvae and 44 prepupae present, licking brood and feeding hemolymph behaviors by the queen were observed in total 166 times. The frequency (no. observed episodes/no. individuals) was calculated separately for larvae and prepupae (Fig. 4). The behavior of the queen apparently differed toward larvae or prepupae:

she licked prepupae more frequently than larvae (Fisher test, $P < 0.0001$) while the frequency of feeding hemolymph was similar. The duration of hemolymph feeding per single feeding episode was not different between larvae (31.4 ± 11.4 sec., $n = 27$) and prepupae (31.1 ± 12.0 sec., $n = 34$) (Welch two sample t-test, $t = -0.238$, $df = 54.8$, $p = 0.813$).

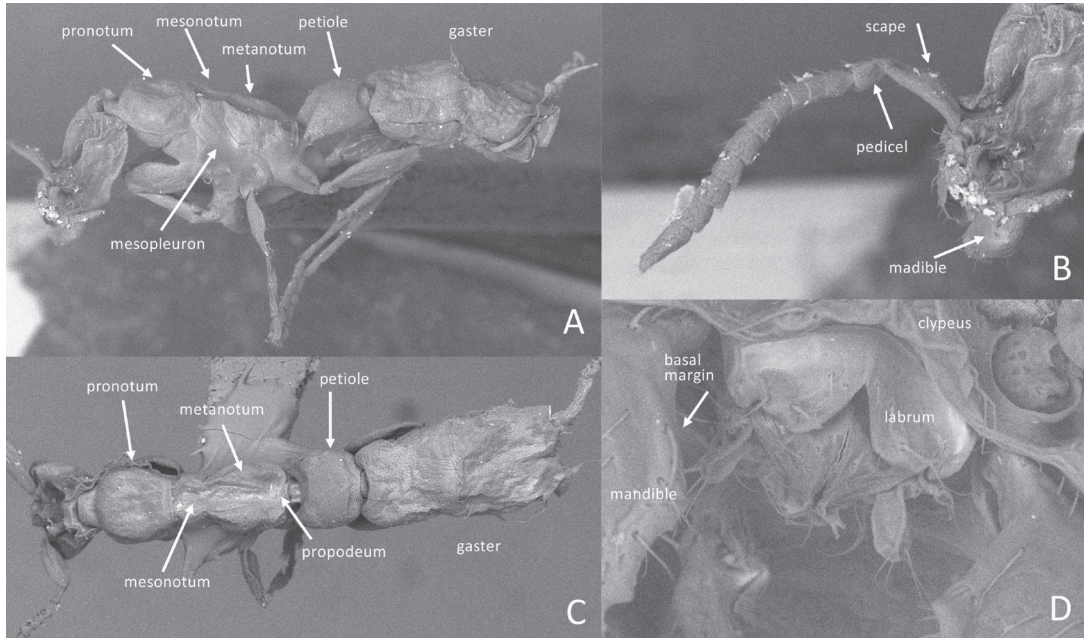


Fig. 5. Ergatoid queen of *Leptanilla clypeata*. A: Body in lateral view. B: Head in obliquely lateral view, showing right antenna and mandibles. C: Body in dorsal view. D: Mouth parts. The color of specimens kept in ethanol for 20 years turned white, probably because the quality of ethanol had some problems. When the specimen was picked up from the ethanol and dried up, it immediately deformed.

During the observation periods spread across three days, the queen was never observed feeding on the geophilomorph prey offered. During 90 min. observation on 27 Dec, egg-laying by the ergatoid queen was observed five times. In all cases, when an egg emerged from the abdominal tip of the queen, a worker picked it up and brought it to an egg pile. Larvae and prepupae were often observed physically attached (through unknown means) to the queen abdomen (Fig 1.).

DISCUSSION

Out of 47 described species of *Leptanilla*, the queen caste has been described for only eight species (Emery 1870; Wheeler 1932; Kutter 1948; Brandica et al. 1994; Ogata et al. 1995; Xu 2002; Terayama & Kinomura 2015). *Leptanilla* queens are ergatoid, and queen-worker body size difference is apparent. Knowledge on colony composition is still scarce, however, with the available information suggesting that they show monogyny

with moderate colony size (100~200 workers), phasic egg production and synchronized brood development (Masuko 1990; Ogata et al. 1995; Terayama & Kinomura 2015). The present observation confirms that these biological characteristics applies to other species within the *Leptanilla* genus as shown in *L. clypeata*.

As known in some other species of *Leptanilla* (e.g. Wheeler & Wheeler 1988), larvae of *L. clypeata* had a pair of “hemolymph feeding taps”. The ergatoid queen of *L. clypeata* shows larval hemolymph feeding as previously demonstrated in *Leptanilla japonica* (Masuko 1989), with this behavior directed not only towards larvae but also towards prepupae. Masuko (1989, 1990) did not mention such queen behavior in *L. japonica* towards prepupae, however, the reason might be that there were only few prepupae available during his observation period (Masuko, pers. com). It can thus not be excluded that *L. japonica* queens also show this behavior towards prepupae. In *L. clypeata*, prepupae were more frequently licked by the queen, while the frequency

of hemolymph feeding was similar for larvae and prepupae. This may indicate that some prepupae were not suitable for hemolymph feeding because of their progress of metamorphosis. The queen is possibly able to recognize the brood condition by licking the body surface. In summary, biological characteristics of *L. clypeata* studied in this research are very similar to those of *L. japonica*.

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REFERENCES

- Barandica JM, López F, Martínez MD, Ortuño VM, 1994. The larvae of *Leptanilla charonea* and *Leptanilla zaballosi* (Hymenoptera, Formicidae). *Deutsche Entomologische Zeitschrift* 41: 147 – 153.
- Borowiec ML, Rabeling C, Brady SG, Fisher BL, Schultz TR, Ward PS, 2019. Compositional heterogeneity and outgroup choice influence the internal phylogeny of the ants. *Molecular Phylogenetics and Evolution* 134: 111 – 121.
- Brady SG, Schultz TR, Fisher BL, Ward PS, 2006. Evaluating alternative hypotheses for the early evolution and diversification of ants. *Proceedings of the National Academy of Sciences of the United States of America* 103: 18172 – 18177.
- Emery C, 1870. Studi mirmecologici. *Bull. Soc. Entomol. Ital.* 2: 193-201 (page 196, *Leptanilla* in Dorylinae [Dorylidae])
- Ito F, Yamane Sk, Eguchi K, Noerdjito WA, Sih Kahono, Tsuji K, Ohkawara K, Yamauchi K, Nishida T, Nakamura K, 2001. Ant species diversity in the Bogor Botanic Garden, West Java, Indonesia, with descriptions of two new species of the genus *Leptanilla* (Hymenoptera, Formicidae). *Tropics* 10: 379 – 404.
- Kuck P, Hita Garcia F, Misof B, Meusemann K, 2011. Improved phylogenetic analyses corroborate a plausible position of *Martialis heureka* in the ant tree of life. *Plos one* 6(6): e21031.
- Kutter H, 1948. Beitrag zur Kenntnis der Leptanillinae (Hym. Formicidae), Eine neue Ameisengattung aus Sud-Indien. *Mitteilungen der Schweizer Entomologischen Gesellschaft* 21: 286 – 295.
- Masuko K, 1989. Larval hemolymph feeding in the ant *Leptanilla japonica* by use of a specialized duct organ, the “larval hemolymph tap” (Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology* 24: 127 – 132.
- Masuko K, 1990. Behavior and ecology of the enigmatic ant *Leptanilla japonica* Baroni Urbani (Hymenoptera: Formicidae: Leptanillinae). *Insectes Sociaux* 37: 31 – 57.
- Ogata K, Terayama M, Masuko K, 1995. The ant genus *Leptanilla*: discovery of the worker-associated male of *L. japonica*, and a description of a new species from Taiwan (Hymenoptera: Formicidae: Leptanillinae). *Systematic Entomology* 20:27 – 34
- Rabeling C, Brown JM, Verhaagh M, 2008. Newly discovered sister lineage sheds light on early ant evolution. *Proceeding of the National Academy of Science of the United States of America* 105:14913 – 14917.
- Terayama M, Kinomura K, 2015. Rediscovery of *Leptanilla kubotai* Baroni Urbani (Hymenoptera: Formicidae) from Kochi Prefecture, Japan, with a description of queen. *Ari-Journal of the Myrmecological Society of Japan* (37):17 – 22.
- Wheeler GC, Wheeler J, 1988. The larva of *Leptanilla japonica*, with notes on the genus (Hymenoptera: Formicidae: Leptanillinae). *Psyche* 95:185 – 190.
- Wheeler WM, 1932. An Australian *Leptanilla*. *Psyche* 39:53 – 58.
- Xu Z, 2002. A systematic study on the ant subfamily Leptanillinae of China. *Acta Entomologica Sinica* 45: 115 – 120.

APPENDIX

Description of queen

One mounted founding queen specimen is available, but is heavily damaged due to shrinking after getting dried (Fig. 5A, C). Dichthadiiform. According to a photo taken when it was alive (Fig. 1) body measuring around twice as long as worker in total body length (estimated to be ca. 3.1 mm). Head in full-face view more elongate than worker. Mandible broad, short and thick (Fig. 5B); masticatory margin with sharp apical tooth and 3 (or more) low, blunt teeth; basal margin edentate (Fig. 5D). Anterior margin of clypeus nearly straight (or shallowly emarginate); labrum anteriorly with deep triangular incision (Fig. 5 D). Eye apparently absent (but not sure). Antenna 12-segmented; scape relatively long, as long as pedicel and flagellar segments (FLs) 1–5; pedicel as long as FL 1; FLs 2–4 broader than long, 5–8 nearly as long as broad, 9 longer than broad, 10 (apical) more than twice as long as broad and tapered apicad (Fig. 5B). With thorax in dorsal view (Fig. 5C) pronotum excluding anterior protrusion (neck) as

long as broad, laterally roundly convex; mesonotum narrower than pronotum and metanotum+propodeum; pro-mesonotal suture distinct, but meso-metanotal suture weaker. Mesopleuron not divided into upper and lower areas; separation of metapleuron from propodeal side indistinct. Propodeum slightly inflated (in live specimen), with posterodorsal corner not angulate; spiracle round with slightly elongate orifice, located anteriorly on lateral face of propodeum. Petiole large and dorsally shallowly convex, in dorsal view broader than long with deeply concave anterior margin (Fig. 5A, C). Gastral tergite 1 in dorsal view anteriorly broadly emarginated to form roundly produced lateral corners (Fig. 5C).

Entire body superficially sculptured to nearly smooth, shiny (in live specimen). Dorsa of head including mandible, and mesosoma with many appressed (?) to standing hairs (short, fine and inconspicuous); short obliquely standing hairs present at apices of antennal segments; legs only with appressed short hairs, and spines at apices of femora, tibiae and basitarsi. Body (in live specimen) entirely light brown as in the worker.

