Field observations on nestmate recruitment to millipedes in the chain-assembling ponerine ant *Leptogenys cyanicatena* (Formicidae: Ponerinae) in northern Thailand

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ABSTRACT. The foraging behavior of the millipede-specialized predatory ant *Leptogenys cyanicatena* was observed in Chiang Mai Province, northern Thailand. During the observations, millipede species from two families of the orders Polydesmida (family Paradoxosomatidae) and Spirostreptida (family Harpagophoridae) were hunted by *L. cyanicatena*. Lone foragers searched for millipedes on the forest floor. When a forager encountered a millipede, she touched the latter with antennae and mouthparts for up to 15 seconds, but never attacked the prey alone. Alternatively, she would then quickly return to the nest to recruit nestmates (scout behavior). Once the scout arrived at the nest entrance, a raiding party would be quickly formed upon stimulation. The scout would then lead the raiding party, consisting of 7–155 ants, and move towards the prey-discovery site in a single file. If the target millipede escaped from the original site, the ants of the raiding party would find the escapee by following its trail. Immobilized large millipedes were retrieved by "self-assembling chains" as reported in a previous study. Nestmate recruitment is necessary not only to retrieve, but also to immobilize prey, because the millipedes are too large to be successfully attacked by a single ant. Therefore, scouts recruit nestmates in advance of the attack.

Keywords	Prior recruitment, coordinated hunting, group attack, daisy chains, specialist predator
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INTRODUCTION

The genus *Leptogenys* consists of 314 species distributed globally in tropical and subtropical regions (Bolton 2021) and shows extreme diversity of life histories (e.g., Maschwitz et al. 1989, Ito 1997, Ito & Ohkawara 2000, Peeters & Ito 2001). Many species are specialist predators of specific prey, such as isopods, earwigs, earthworms, and termites (Wheeler 1936, Maschwitz & Mühlenberg 1975, Maschwitz & Schönegge 1983, Steghaus-Kovac & Maschwitz 1993, Duncan & Crewe 1994, Schmidt & Shattuck 2014). According to their prey preferences, their foraging behaviors are also very diverse with solitary foraging (*Leptogenys* spp., Dejean & Evraerts 1997), mass recruitment (*L. chinensis* (Mayr, 1870), Maschwitz & Schönegge 1983; *L. diminuta* (Smith, 1857)*,* Ito & Ohkawara 2000), and true group raiding (*L, processionalis distinguenda* (Emery, 1887), Maschwitz et al. 1989; *L. intermedia* (Emery, 1902) (= *L. nitida*), Duncan & Crewe 1994) having been documented in this genus.

Leptogenys cyanicatena Arimoto & Yamane, 2018 is distributed in Indochina and a specialist predator of large millipedes (Peeters & De Greef 2015). Millipedes protect themselves with hard exoskeletons, specialized setae, and/ or strong defensive secretions (Hopkin & Read 1992). Therefore, *Leptogenys* excluded, millipede predation is known to occur in only limited ant genera, such as *Gnamptogenys*, *Myopias*, *Plectroctena, Probolomyrmex* and *Thaumatomyrmex* (Bolton 1974, Willy & Brown 1983, Brandão et al. 1991, Brown 1992, Ito 1998, Dejean et al. 2001, Rabeling et al. 2012, Ito et al. 2020, Ito & Hosokawa 2020). Peeters and De Greef (2015) reported that *L. cyanicatena* preys on large millipedes in Cambodia and retrieves the millipedes by cooperative group transport with notable "self-assembling chains", but the entire process of foraging, including prey searching and nestmate recruitment is unknown. They further predicted that simultaneous attacks by a large number of ants would be necessary to immobilize the large millipedes, but the process of nestmate recruitment has not been clarified.

Colonies of the *Leptogenys chalybaea* species group (including *L. cyanicatena*) contain many virgin ergatoid queens (Arimoto & Yamane 2018). Their social structure is similar to that of the *Leptogenys diminuta* species group, a sister lineage of the *L. chalybaea* species group. In the *L. diminuta* group, some virgin ergatoid queens engage in foraging (Ito & Ohkawara 2000), but it has not been confirmed in *L. cyanicatena* (Peeters & De Greef 2015, Arimoto & Yamane 2018)*.*

In this paper, we report detailed field observations on prey searching and nestmate recruitment of *L. cyanicatena* in northern Thailand. We also investigate colony composition and raiding party participation of these ants, with focusing on the presence/absence of ergatoid queens. Furthermore, natural history information of *L. cyanicatena,* including the composition of a whole colony, is provided.

MATERIALS AND METHODS

Field observations were conducted in Omkoi National Forest, Chiang Mai Province, northern Thailand (alt. 950 to 1000 m). The study site is located in a dry dipterocarp forest, belonging to the *Aw* type of Köppen's climate classification, with distinct dry and rainy seasons (Mizuno et al. 2019). All observed ant colonies were identified morphologically as *L. cyanicatena*. Before the observations, one entire colony (RM's personal colony code RM-34) was excavated, and its composition was recorded. This colony was reared under laboratory conditions during a week in Kagawa Univ., Japan.

Observations were carried out from June to August 2017 and in July 2019 (Table 1). During the observations, 32 millipede individuals were collected as captured prey of *L. cyanicatena*. A large earthworm (ca. 15 cm long) was offered for foraging ants once. The prey millipedes' body length and weight were measured after identification at the family or genus level. Voucher specimens of ants were deposited in the Thailand Natural History Museum, while voucher specimens of millipedes were deposited in the collections of the Division of Plant Protection, Maejo University.

In advance of observation in the field, a total 56 ants in three *L. cyanicatena* colonies were marked and identified by using enamel paint markers (23 ants of colony 2017-2, 17 ants of 2017-3, and 16 ants of 2019-2). Observation at the nest entrance was carried out six times on four colo-

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Table 1 Date, colonies ID, type of observation, and duration of each observation episode. Abbreviations; NE, observation on nest entrance; RP, observation on raiding party; EX, experimental observation of recruitment and prey search.

Obs. ID	Date	Colony IDs	Type of observation	Duration
1	7 Jun. 2017	2017-1	NE	ca. 1h
2	27 Jun. 2017	2017-1	NE	2h8m
3	9 Jul. 2017	2017-2	NE	5h10m
4	10 Aug. 2017	2017-3	NE	ca. 0h30m
5	14 Aug. 2017	2017-3	NE	3h57m
6	13 Jul. 2019	2019-1	RP	1h1m
7	14 Jul. 2019	2019-1	RP	0h23m
8	15 Jul. 2019	2019-1	NE, RP	2h21m
9	15 Jul. 2019	2019-1	EX	0h15m
10	17 Jul. 2019	2019-1, 2, 3	EX	Total ca. 2h
11	17 Jul. 2019	2019-2	RP	ca. 1h
12	20 Jul. 2019	2019-2	EX	1h10m
13	20 Jul. 2019	2019-3	EX	0h30m
14	21 Jul. 2019	2019-2	EX	ca. 1h
15	21 Jul. 2019	2019-3	EX	ca. 2h

nies. The ants leaving and entering the nest were counted, and their behavior was observed. Foragers (= individuals searching for prey) leaving the nest alone were traced and observed. When nestmate recruitment occurred, the recruited raiding party was also traced (two raiding parties were observed). Hereafter, the individuals recruiting nestmates are referred to as "scouts". Large *Thyropygus* millipede juveniles (ca. 10 cm long each) were artificially offered to forager ants exiting a nest, to assess: (1) the behavior of foragers when encountering prey (n = 18), (2) the role of the scout in leading the raiding party (n = 2), (3) the attacking behavior of the raiding party against the prey (n = 18). The millipedes were placed two to five meters away from the nest entrances of the selected colonies.

Four simple experiments using large living *Thyropygus* millipedes were done to determine if ants actively traced the millipedes' trail: (1) A millipede was offered to a foraging ant. After contact with the forager, the route via which the millipede escaped was recorded (natural millipede trail experiment). (2) The offered millipede was immediately removed from the ground after the forager encountered it (removal experiment). (3)

Following the same method used in the removal experiment, an artificial millipede trail (50 cm) was drawn from the encounter site by dragging the millipede on the ground (artificial millipede trail experiment). (4) Using the same method as in the removal experiment, the removed millipede was offered again to an ant that had been recruited and was searching around the encounter site (re-encounter experiment).

As it is impossible to differentiate ergatoid queens from foraging workers in this species based on visual observation from a distance, since the two are morphologically similar. Thus, we collected two entire raiding parties (total 40 individuals) for closer examination and identification of ergatoid queens. In the Results section, we refer to observed individuals simply as "ants" without distinguishing between ergatoid queens and workers.

RESULTS

Prey menu

During the observation period in 2017, small *Thyropygus* sp. (juveniles, 3.4–5.2 cm, n = 23) and millipedes of other unidentified genera (adults,

Enghoffosoma or *Anoplodesmus*, ca. 4 cm, n = 4) of the family Paradoxosomatidae were collected as prey captured by *L. cyanicatena*. We could only identify some prey to genus level because of their poor condition. During observations in 2019, larger *Thyropygus* sp. (also juveniles, 9.8–13.2 cm, n = 2) and unidentified species in Paradoxosomatidae (apparently same species as 2017, n = 3) were collected from the ants. Hereafter, these prey millipedes are referred to as "small *Thyropygus*", "Paradoxosomatid" and "large *Thyropygus*", respectively. The fresh weight of the small *Thyropygus* was 0.47–0.69 g (n = 7), that of large *Thyropygus* was 5.54–9.47 g (n = 6). Paradoxosomatids were not weighed but appeared lighter than the small *Thyropygus*. During observations, *L. cyanicatena* exclusively preyed on millipedes and never attacked other arthropods. A large earthworm (ca. 15 cm long) was artificially offered once but not attacked. The adult ants of captive colony RM-34 fed on offered Turkistan cockroaches (*Blatta lateralis* Walker, 1868) (n = 2).

The process of millipede raid

Almost all observed foragers left the nest and explored alone (n = 12 ants). One forager was traced: she explored at least 38.5 m from the nest (30 minutes after leaving the nest). Non-solitary exploration was observed only twice, in which cases two foragers traveled together in the same direction. If a single forager encountered a group of nestmate ants carrying prey to the nest, she never joined them and continued to explore alone (n = 2 foragers). When a lone forager encountered a millipede, she touched the millipede briefly (5–15 s) with her antennae and/or mouthparts (palpus?). After the forager encountered prey, she got frantic and quickly ran back to the nest excitedly (scout behavior). The movement of scouts is distinguishable from that before encountering the prey. During the first contact, foragers just touched the prey millipede briefly and did not exhibit any measuring behavior, such as antennation with walking along the prey's long axis. At this time, foragers never attacked the prey alone (n = 18).

Fig. 1. *Leptogenys cyanicatena* carrying a large *Thyropygus* millipede with long (11 ants) and short (four ants, and the fifth ant is trying to join) chains while the millipede is curling up.

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Fig. 2. *Leptogenys cyanicatena* carrying a Paradoxosomatid millipede with chain of two ants (shown by arrows).

At the nest entrance, 33 single ants were observed returning to the nest not carrying any prey and eight of them behaved as scouts. When the scouts returned to the nest, recruitment occurred: a "raiding party" consisting of 7–155 ants (21 raiding parties assessed) was quickly formed at the nest entrance (Video S1). Nestmate recruitment by marked scouts was observed twice (one example is provided in Video S1), the behavior of the scout and nestmates were constant: (1) When a scout approached the nest, the ants around the nest entrance reacted to the scout (even without any direct contact by antennae) and started to walk slowly in the direction of the scout's original route (started forming the raiding party). (2) About 30 seconds after a scout entered the nest, she appears from the nest entrance and walks to the head of the raiding party. (3) The scout walks on the head of the raiding party and leads them to the direction where the scout encountered the prey. A recruited raiding party always moved as a procession in single file to the prey-encounter site (n = 8, including

the observation with non-marked scouts). In the two raiding parties collected and examined (of 17 and 23 ants), only one queen was found (in the 17 ant party).

Two traced raiding parties succeeded in finding a prey at 25.3 m and about 30 m away from their nest, respectively. When a raiding party arrived at a possible prey site, they spread over an area up to 1 m × 1 m and searched on the ground with their antennae. One of the observed raiding parties first found a small *Thyropygus* under leaf litter. After that, the same raiding party also searched and found another small *Thyropygus* 1.5 m away from the first prey. Another raiding party found and retrieved a large *Thyropygus* in a chamber (possibly a molting chamber made by the millipede itself) under a rotting log.

When attacking, the ants attempted to sting the ventral side of a millipede, avoiding the hardest part of its exoskeleton. Ants seem to be unable to sting the dorsal side of millipedes. Attacked millipedes curled up to defend themselves.

Fig. 3. *Leptogenys cyanicatena* carrying a small *Thyropygus* millipede without chaining.

After multiple stinging by two to 13 workers for five to 10 minutes, a millipede relaxed and was linear. Most preyed millipedes were cooperatively transported to their nest (n = 32). Large *Thyropygus* millipedes were retrieved by chains of ants (up to 11 individuals, Fig. 1) as reported in the previous study (Peeters & De Greef 2015). Even for relatively small prey, the ants sometimes formed a short-chain according to the resistance to drag (e.g., friction between the prey and the ground surface, slope, or obstructive objects) (Fig. 2). When the millipede was insufficiently paralyzed, the ants carried it while it was curled up (Fig. 1), but at a slower speed. When the ants transported smaller prey (small *Thyropygus* or Paradoxosomatid), they mostly carried the prey without forming chains. Smaller Paradoxosomatid millipedes were observed to be transported solitarily (n = 3). However, seven to 20 ants usually walked together in this case, often helping each other with prey transport. The ants always grasped appendages of the millipede (antennae or legs) by their mandibles and held it under their body or just dragged it (Fig. 3).

Experimental observation of millipede tracing by ants

When the recruited raiding party arrived at the prey encounter site, the ants tapped the ground with their antennae, followed the trail by which the millipede escaped, and reached the escapee (Fig. 4C-1, natural millipede trail experiment, n = 4). At this time, the ants did not start to attack the prey until most of the raiding party members arrived. If the offered millipede was removed immediately after the scout's departure (removal experiment), the recruited raiding party broke up and searched around upon arrival at the prey-encounter site (Fig. 4C-2, n = 9). When the raiding party could not find the millipede after more than 10 minutes, the ants started to walk back to the nest. When an artificial millipede trail was drawn from the encountered site by dragging a millipede on the ground (artificial millipede trail experiment), the ants followed it and broke up and searched around at the ending point of the artificial millipede trail (Fig. 4C-3, n = 2). When the removed millipede was reintroduced to the area being searched (re-encounter

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experiment), the ant that first encountered the re-introduced millipede behaved like a scout (Fig. 5, n = 5). At this time, the ants around the scout would respond to the latter and start to walk in the direction the scout came from. This behavior was similar to the recruitment at the nest entrance. During the attack on the millipede, 1–3 additional raiding parties came from the nest and joined in the prey attack and transport (n = 2 times).

The entire process of the millipede raid of *L. cyanicatena* is shown in Fig. 6.

Natural history observations of *Leptogenys cyanicatena.*

The collected and observed colonies were nested in underground preexisting spaces in abandoned termite nests (n = 6) or under a standing decayed tree (n = 1). The exoskeletons of preyed millipedes and cocoon shells of *L. cyanicatena* were scattered near the nest entrance, as in former studies (Peeters & De Greef 2015; Arimoto & Yamane 2018). One whole excavated colony (RM's personal colony code RM-34) consisted of 454 workers, 62 ergatoid queens, nine males, 188 cocoons, many larvae, and eggs. Reproductive status of the ergatoid queens was not examined.

A. A forager encounters an offered millipede.

B. The forager runs back to the nest (as a scout), the millipede escapes.

C-1. Natural situation: if the millipede is not artificially removed (is escaping). Recruited ants follow the millipede trail and reach.

C-2. When the millipede is artificially removed at the site. Ants break up and search around the site.

C-3. When the millipede is removed after being dragged artificially on the ground. Ants follow the artificial millipede trail and break up at the ending point of it.

Artificial trail drawn by a millipede

Fig. 4. Schematic figure of millipede tracing.

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1. Discovered millipede is immediately removed and reintroduced for encountering a recruited ant (re-encountering).

Reintroduced
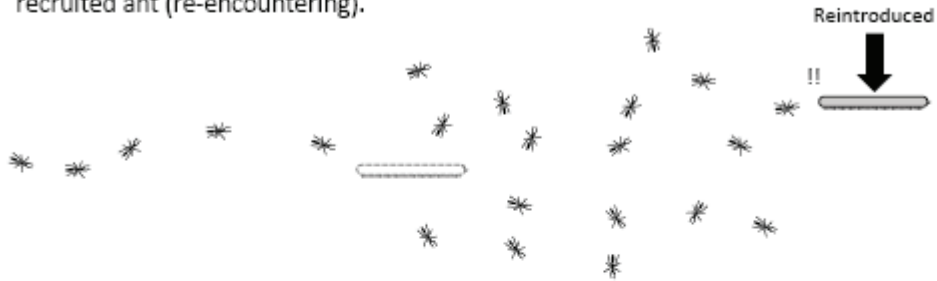
2. The encountered ant shows scout behavior (runs back to the nest). The ants around the encountered ant respond to her and walk in the direction where she came (to the millipede).

Fig. 5. Schematic figure of the results of millipede re-encountering experiment.

A forager search for a prey solitary

↓ Find a prey (not attack)

Runs back to the nest (as a scout)

↓ Nestmate recruitment

The scout leads a raiding party to the prey encountered site

↓ Arrive at the site

Search the millipede trail on the ground

↓ Follow it and find the prey

Attack and immobilize the prey collectively

↓

Carry the prey to the nest collectively
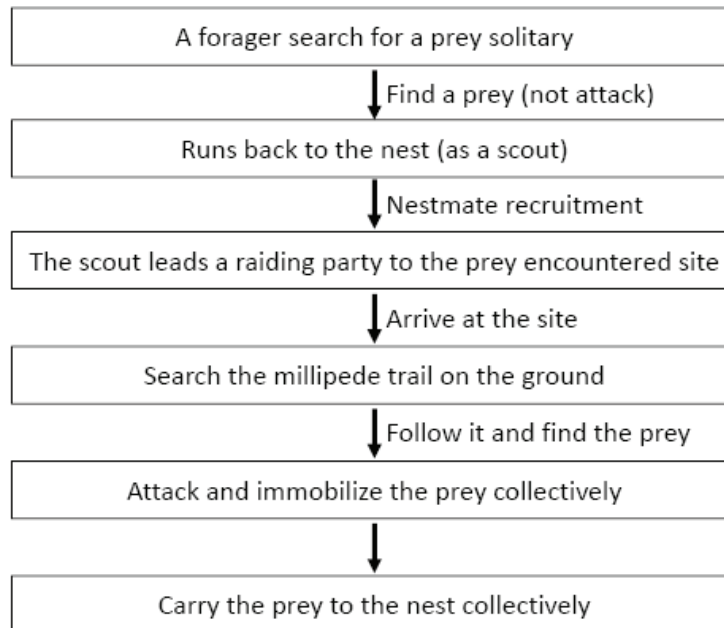
Fig. 6. Sequence of the millipede raid of *Leptogenys cyanicatena.*

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During the fieldwork, it was observed that *L. cyanicatena* piled up their cocoons at the nest entrance (Fig. S1, n > 10 times at five colonies). They seemed to heat or dry the cocoons. When it started to rain during observations, the ants all carried these cocoons back into the nest. Similar cocoon piling was also observed in *L.* cf. *diminuta* at the same locality (Mizuno unpubl.) and *L. nitida* in South Africa (Duncan & Crewe 1994).

It was observed that six individuals of small *Thyropygus* millipedes were piled together with cocoons under fallen leaves at the nest entrance of colony 2017-1 (Fig. S2, n = 1). *Gnamptogenys ingeborgae* leave their prey millipedes outside of the nest for 10 minutes to more than an hour, possibly for dissipation of defensive chemicals emitted by the millipede (Brown 1992). The millipede piling of *L. cyanicatena* also might be a similar behavior.

DISCUSSION

In this study, we identified the behavior of solitary scouts and of searching for escaped millipedes, which was not reported by Peeters and De Greef (2015). Additionally, as Peeters and De Greef (2015) predicted, our results confirm that the nestmate recruitment occurs prior to the attack against the prey. Specialized predation on millipedes, transportation of large millipedes with chaining behavior, and transportation of smaller millipedes without chaining was consistent with the previous study (Peeters & De Greef 2015). The prey millipedes obtained in this study belonged to the same family as in that of the previous studies (Peeters & De Greef 2015; Arimoto & Yamane 2018). However, the opportunistic predatory habits occasionally observed in the previous studies, such as predation on earthworms or snails with broken shells (Peeters & De Greef 2015, Arimoto & Yamane 2018), were not observed during the field observations in this study (though a captive colony accepted cockroaches and mealworms (*Tenebrio molitor* Linnaeus, 1758) (Jaitrong personal observation)). However, it is not clear whether similar prey are collected in field conditions.

In the excavated colony (RM-34), about 12% of the adult female ants were ergatoid queens and one ergatoid queen was found as a member of an examined raiding party. Ergatoid queens who can also work as colony laborers are called 'multi-purpose ergatoid queens' (Molet et al. 2009). In the case of *L. diminuta* group, a sister lineage of *L. cyanicatena*, only old virgin ergatoid queens engaged in foraging (Ito & Ohkawara 2000). It may be the same in *L. cyanicatena*, but the age of ergatoid queens was not evaluated in our study. The excess production of multi-purpose ergatoid queens is associated with opportunistic colony fission (Forder & Marsh 1989, Ito & Ohkawara 2000). Nest relocation is occasionally observed in *L. cyanicatena*, which nests in preexisting spaces (Peeters & De Greef 2015, Mizuno unpublished). In northern Thailand, heavy rain frequently falls during the rainy season, and it may disturb the nest site or interrupt nest relocation, resulting in unexpected colony fission. Therefore, as in *L. diminuta*, a notably high number of ergatoid queens in colonies of *L. cyanicatena* also may be associated with opportunistic colony fission.

The foraging behavior of *L. cyanicatena* is classified as group raids (Lanan 2014). Many *Leptogenys* species in Southeast Asia show relatively small colony size (less than 50 adult ants, Ito 1997), except for handful species (e.g. *L. processionalis distinguenda*, more than 50000 workers, Maschwitz et al. 1989; *Leptogenys myops* (Emery, 1887), 1540 workers, Ito 1997; *L. diminuta* group, up to 700 adult ants, Ito & Ohkawara 2000). Similar to their sister lineage *L. diminuta* group, the colony size of *L. cyanicatena* (about 400–1000 adult ants, Arimoto & Yamane 2018) is relatively large and might be related to their foraging style. Recruitment of nestmates always occurred prior to the attack (hereafter prior recruitment). This nestmate recruitment might be a specialized behavior required for the group attack to immobilize huge millipedes. Prior recruitment is thus associated with the need for a large number of ants to sting the prey simultaneously. The reduced efficiency of transportation of incompletely paralyzed prey may further support the importance of complete paralysis by simultaneous attacks with multiple ants. Prior recruitment behaviour has been observed in other species. The scouts of *Leptogenys diminuta* recruit nestmates before the attack when the prey is too large to be immobilized by a single forager, but small prey is hunted by a single forager (Witte et al. 2010). The millipede predatory ponerine ant *Plectroctena minor* usually attacks prey solitarily;

the solitary forager recruits nestmates only when the prey is difficult to immobilize alone (Dejean et al. 2001). Prior recruitment has also been observed in termite predators, such as *Leptogenys chinensis* (Mayr, 1870) (Maschwitz & Schönegge 1983) and *Megaponera analis* (Latreille, 1802) (= *M. foetens*) (Longhurst & Howse 1979), and a doryline ant predator, *Lioponera* cf. *turneri* (Hölldobler 1982). For termite and ant predators, prior recruitment might be an adaptation for effective retrieval of groups of small prey. Prior recruitment behavior appears to have evolved and adapted to varied prey types and sizes across different ant lineages.

Based on the behavior of the recruited ants at nest entrances, it seems that the scouts lay a chemical trail and stimulate their nestmates with stridulation and/or volatile pheromones. The number of recruited individuals in a raiding party varied greatly from a dozen to more than a hundred. This may suggest 'graded recruitment' – a mechanism in place that adjusts the number of recruited individuals according to the size and condition of the food source, is occurring. Graded recruitment has been reported in other genera, (e.g., *Paraponera clavata* (Fabricius, 1775), Breed et al. 1987; *Gnamptogenys sulcata* (Smith, 1858), Daly-Schveitzer et al. 2007). Further experiments are needed to verify this behavior in *L. cyanicatena*. The number of ants required to immobilize and transport a small to a large millipede (the heaviest millipede weighed about 20 times as much as the lightest one) may differ remarkably. According to Witte et al. (2010), a *L. diminuta* scout determines in a few seconds whether to kill the prey alone or to recruit nestmates (as well as the number of nestmates to recruit) depending on the characteristics of the prey (type, size, and speed of movement). Such graded recruitment also might be possible for *L. cyanicatena* in hunting of various sizes of millipedes.

The recruited raiding party dispersed at the site where the scout encountered the prey and followed trails of an escaping millipedes. Thus, *L. cyanicatena* may recognize chemical substances left on the ground, such as 'kairomones' – a chemical cue used by predators or parasites to mark prey or hosts. Because of prior recruitment behavior of *L. cyanicatena*, it is likely that the encountered millipede may escape from the encountered site while the scout returns to the nest to recruit nest-mates. Therefore, prey tracing ability is important for the hunting success of *L. cyanicatena*. We have no evidence that *L. cyanicatena* recognizes and traces the defensive chemicals of millipedes, but the defensive chemicals might leave strong vestiges of millipede existence. Parasitic flies use the strong defensive substances of millipedes as signals of their host (Hash et al. 2017). Using prey scent for hunting is known in some ants. For example, *Plectroctena minor* Emery, 1892 recognizes chemicals in millipede molting chambers' walls and destroys the chamber to prey on the millipedes inside (Dejean et al. 2001). Also, Brown (1992) suggests that *Gnamptogenys ingeborgae* Brown, 1993 uses the millipedes' defensive chemicals as a cue for prey recognition. In *M. analis*, workers recognize chemicals emitted from the soil sheeting laid down by the termites as kairomone for prey search (Longhurst & Howse 1978). However, the prey millipedes used by *L. cyanicatena* consisted of two unrelated taxa (genus *Thyropygus* belongs to family Harpagophoridae of order Spirostreptida, family Paradoxosomatidae belongs to order Polydesmida). These millipedes use different defensive substances (reviewed in Shear 2015). Since all the experiments in this study were conducted with large *Thyropygus*, it is not clear whether the hunting style of *L. cyanicatena* also differs among prey of different orders or whether other prey can be traced. Hunting behavior against different millipedes and analysis of their chemical recognition is an interesting topic for further study.

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REFERENCES

Arimoto K and Yamane Sk, 2018. Taxonomy of the *Leptogenys chalybaea* species group (Hymenoptera, Formicidae, Ponerinae) from Southeast Asia. Asian Myrmecology 10, e010008: 1 – 31. https://doi.org/10.20362/am.010008

Bolton B, 1974. A Revision of the Ponerine Ant Genus *Plectroctena* F. Smith (Hymenoptera, Formicidae). Bulletin of the British Museum (Natural History) Entomology 30(6): 310 – 338.

Bolton B, 2021. An online catalog of the ants of the world. Available from https://antcat.org. (accessed 16 Apr. 2021).

Brandão CRF, Diniz JLM and Tomotake EM, 1991. *Thaumatomyrmex* strips millipedes for prey: a novel predatory behaviour in ants, and the first case of sympatry in the genus (Hymenoptera: Formicidae). Insectes Sociaux 38(4): 335 – 344. https://doi.org/10.1007/BF01241869

Breed MD, Fewell JH and Moore AJ, 1987. Graded recruitment in a ponerine ant. Behavioral Ecology and Sociobiology 20(6), 407 – 411. https://doi.org/10.1007/BF00302983

Brown WL, 1992. Two new species of *Gnamptogenys*, and an account of millipede predation by one of them. Psyche 99(4): 275 – 289. https://doi.org/10.1155/1992/59431

Daly-Schveitzer S, Beugnon G and Lachaud JP, 2007. Prey weight and overwhelming difficulty impact the choice of retrieval strategy in the Neotropical ant *Gnamptogenys sulcata* (F. Smith). Insectes Sociaux 54(4) 319–328. https://doi.org/10.1007/s00040-007-0949-2

Dejean A and Evraerts C, 1997. Predatory behavior in the genus *Leptogenys*: A comparative study. Journal of Insect Behavior 10(2): 177 – 191. https://doi.org/10.1007/BF02765551

Dejean A, Suzzoni JP and Schatz B, 2001. Behavioral adaptations of an African Ponerine ant in the capture of millipedes. Behaviour 138(8): 981 – 996.

Duncan FD and Crewe RM, 1994. Group hunting in a ponerine ant, *Leptogenys nitida* Smith. Oecologia, 97(1): 118 – 123. https://doi.org/10.1007/BF00317915

Forder JC and Marsh AC, 1989. Social organization and reproduction in *Ocymyrmex foreli* (Formicidae: Myrmicinae). Insectes Sociaux. 36(2): 106 – 115. https://doi.org/10.1007/BF02225906

Hash JM, Millar JG, Heraty JM, Harwood JF and Brown BV, 2017. Millipede defensive compounds are a double-edged sword: natural history of the millipede-parasitic genus Myriophora Brown (Diptera: Phoridae). Journal of chemical ecology, 43(2), 198 – 206. https://doi.org/10.1007/s10886-016-0815-7

Hopkin SP and Read JH, 1992. The Biology of Millipedes. Oxford University Press, New York, 233 pp.

Hölldobler B, 1982. Communication, raiding behavior, and prey storage in *Cerapachys* (Hymenoptera: Formicidae). Psyche 89(1 – 2), 3 – 23. https://doi.org/10.1155/1982/28390

Ito F, 1997. Colony composition and morphological caste differentiation between ergatoid queens and workers in the ponerine ant genus *Leptogenys* in the Oriental tropics. Ethology Ecology & Evolution 9(4): 335 – 343. https://doi.org/10.1080/08927014.1997.9522876

Ito F, 1998. Colony composition and specialized predation on millipedes in the enigmatic ponerine ant genus *Probolomyrmex* (Hymenoptera, Formicidae). Insectes Sociaux 45(1): 79–83. https://doi.org/10.1007/s000400050070

Ito F and Hosokawa H, 2020. Biological notes of *Probolomyrmex okinawaensis* Terayama & Ogata collected in Yonagunijima Island, and five species of *Probolomyrmex* collected in Japan and Southeast Asia. Asian Myrmecology 12: e011003 (1 – 6). https://doi.org/10.20362/am.012003

Ito F and Ohkawara K, 2000. Production and behavior of ergatoid queens in two species of the Indonesian ponerine ant genus *Leptogenys* (*diminuta*-group)(Hymenoptera: Formicidae). Annals of the Entomological Society of America 93(4): 869 – 873. https://doi.org/10.1603/0013-8746(2000)093[0869:PABOEQ]2.0.CO;2

Ito F, Nguyen DA, Eguchi K, Toyota A and Billen J, 2020. Colony composition, queen behavior, specialized predation on millipedes, and exocrine glands in the ponerine ant *Myopias conicara* Xu, 1998 (Hymenoptera: Formicidae). Entomological Science 23(2): 142 – 151. https://doi.org/10.1111/ens.12406

Lanan M, 2014. Spatiotemporal resource distribution and foraging strategies of ants (Hymenoptera: Formicidae). Myrmecological news 20: 53 – 70.

Longhurst C and Howse PE, 1978. The use of kairomones by *Megaponera foetens* (Fab.) (Hymenoptera: Formicidae) in the detection of its termite prey. Animal Behaviour 26(4): 1213–1218. https://doi.org/10.1016/0003-3472(78)90111-2

Longhurst C and Howse PE, 1979. Foraging, recruitment and emigration in *Megaponera foetens* (Fab.)(Hymenoptera: Formicidae) from the Nigerian Guinea savanna. Insectes Sociaux 26(3): 204 – 215. https://doi.org/10.1007/BF02223798

Maschwitz U and Mühlenberg M, 1975. Zur jagdstrategie einiger orientalicher *Leptogenys*-arten (Formicidae: Ponerinae). Oecologia 20(1): 65 – 83. https://doi.org/10.1007/BF00364322

Maschwitz U and Schönegge P, 1983. Forage communication, nest moving recruitment, and prey specialization in the oriental ponerine *Leptogenys chinensis*. Oecologia 57(1): 175 – 182. https://doi.org/10.1007/BF00379578

Maschwitz U, Steghaus-Kovac S, Gaube R and Hänel H, 1989. A South East Asian ponerine ant of the genus *Leptogenys* (Hym., Form.) with army ant life habits. Behavioral Ecology and Sociobiology 24(5): 305 – 316. https://doi.org/10.1007/BF00290907

Mizuno R, Suttiprapan P, Jaitrong W and Ito F, 2019. Daily and Seasonal Foraging Activity of the Oriental Non-army Ant Doryline *Cerapachys sulcinodis* Species Complex (Hymenoptera: Formicidae). Sociobiology, 66(2): 239 – 246. https://doi.org/ 10.13102/sociobiology.v66i2.3775

Molet M, Fisher BL, Ito F and Peeters C, 2009. Shift from independent to dependent colony foundation and evolution of 'multi-purpose' ergatoid queens in *Mystrium* ants (subfamily Amblyoponinae). Biological Journal of the Linnean Society, 98(1): 198 – 207. https://doi.org/10.1111/j.1095-8312.2009.01257.x

Peeters C and De Greef S, 2015. Predation on large millipedes and self-assembling chains in *Leptogenys* ants from Cambodia. Insectes Sociaux, 62(4): 471 – 477. https://doi.org/10.1007/s00040-015-0426-2

Peeters C and Ito F, 2001. Colony dispersal and the evolution of queen morphology in social Hymenoptera. Annual review of entomology 46(1): 601 – 630. https://doi.org/10.1146/annurev.ento.46.1.601

Rabeling C, Verhaagh M, Garcia MVB, 2012. Observations on the specialized predatory behavior of the pitchfork-mandibled ponerine ant *Thaumatomyrmex paludis* (Hymenoptera: Formicidae). Breviora (533):1 – 8.

Schmidt CA and Shattuck SO, 2014. The higher classification of the ant subfamily Ponerinae (Hymenoptera: Formicidae), with a review of ponerine ecology and behavior. Zootaxa, 3817(1), 1 – 242. https://doi.org/10.11646/zootaxa.3817.1.1

Shear WA, 2015. The chemical defenses of millipedes (Diplopoda): biochemistry, physiology and ecology. Biochemical Systematics and Ecology 61: 78 – 117. https://doi.org/10.1016/j.bse.2015.04.033

Steghaus-Kovac S and Maschwitz U, 1993. Predation on earwigs: a novel diet specialization within the genus *Leptogenys* (Formicidae: Ponerinae). Insectes Sociaux 40(3): 337 – 340. https://doi.org/10.1007/BF01242370

Wheeler WM, 1936. Ecological relations of ponerine and other ants to termites. Proceedings of the American Academy of Arts and Sciences 71:159 – 243.

Willey RB and Brown WL, 1983. New species of the ant genus *Myopias* (Hymenoptera: Formicidae: Ponerinae). Psyche 90(3) 249 – 285. https://doi.org/10.1155/1983/79524

Witte V, Schliessmann D and Hashim R, 2010. Attack or call for help? Rapid individual decisions in a group-hunting ant. Behavioral Ecology 21(5): 1040 – 1047. https://doi.org/10.1093/beheco/arq100