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SHORT COMMUNICATION

Colony composition and nesting habits of six species of *Aphaenogaster* in Thailand (Hymenoptera; Formicidae)

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INTRODUCTION

The myrmicine ant genus *Aphaenogaster* comprises 187 valid species (Bolton 2016) and is distributed in the temperate to tropical zones throughout the world. They are dominant ant species in many temperate forest ecosystems, and hold important positions as predators and dispersers of myrmecochorous plants (Bednar et al. 2013, Caut et al. 2016, Higashi et al. 1987, Lubertazzi 2012, Mizutani & Imamura 1980, Ohara & Higashi 1987, Richards 2009, Thomas et al. 2016). Until now, our knowledge on the biology of *Aphaenogaster* ants has been limited to species in temperate regions. In the tropics, the species diversity of *Aphaenogaster* is thought to be low (cf. Brühl et al. 1998; Malsch et al. 2003), though the nest density of *Aphaenogaster* in some tropical areas can be quite high (see Results), suggesting they may also have important functions in at least some tropical ecosystems. However, the biology and ecology of tropical *Aphaenogaster* species remains largely unknown. In this study, we investigated the biology of six species of *Aphaenogaster* in Thailand, and report on their nesting habits and colony composition.

MATERIALS AND METHODS

Colonies of six *Aphaenogaster* species were collected in northern (Chiang Mai Province), northeastern (Sakon Nakhon Province), central (Saraburi Province) and southern (Trang Province) Thailand (Fig. 1), from early June to the end of July in the rainy season of 2016. Because the taxonomy of *Aphaenogaster* ants in Southeast Asia is still inadequate, we gave a species code for each species (Table 1, Figs. S1-S18). *Aphaenogaster* sp. 2 is similar to *A. feae* described from Myanmar, however, we could not compare our samples to the type specimen. The habitus of the six species and a tentative key is presented in the Electronic Supplementary Material. Voucher specimens were deposited in the Thailand Natural History Museum and the Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University. The study sites in the north where sp.1 and sp. 2 were collected (a, b, c in Fig. 1) were located at high elevation (altitude 1277 m to 1367 m), while the three sites in southern and central Thailand, where sp. 4, sp. 5 and sp. 6 were found (f, g, h in Fig. 1), were lowland areas (altitude 66 m to 167 m). The el-

Table1. Colony composition of six *Aphaenogaster* species in Thailand (Eggs were not counted). **N:** Northern, **NE:** Northeast, **C:** Central, **S:** Southern, PS: possible satellite nests

Colony code	Numbers of individuals					
	Queens	Workers	Males	Females	Pupae	Larvae
Aphaenogaster sp.1						
N.31		277			293	
N.32		211			57	61
Mean±SD		244±46.7				
Aphaenogaster sp. 2						
N. 33	1	167			2	6
N. 34	1	122			10	25
N. 35	1	38			4	3
N. 36	1	57			4	12
N. 37	1	251				57
N. 38	1	177			3	26
N. 40	1	210			1	69
N.41	1					
Mean±SD		146±78.2				
Aphaenogaster sp. 3						
NE. 27	1	190			9	15
N. 39	1	14			2	1
Mean±SD		102±124.5				
Aphaenogaster sp. 4						
C.24	1	62	1			4
C.25	1	76				10
C.26	1	59	3		2	19
Mean±SD		65.7±9.1				
Aphaenogaster sp. 5						
C.23	1	79				8
C.28	1	48			1	5
C.29	1	81			1	3
C.30	1	70			5	8
Mean±SD		69±15.1				
Aphaenogaster sp. 6						
S.1	1	28				2
S.2	1	65	3		3	5
S.3	1	37			13	17
S.4	1	66	3		2	6
S.5		66			3	8
S.6		37				1
S.10	1	22			1	1
S.11	1	72	10	2	12	23
S.12		36			3	4
S.13	1	30				8
S.15	1	25		2		
S.16	1	34	2		2	2
S.17		23	2			6
S.18	1	11			1	
S.19	1	35	1			5

S.20	1	17
S.22	1	34
Mean±SD		37.5±18.5
PS.7		11
PS.8		5
PS.9		8
PS.14		17
PS.21		10

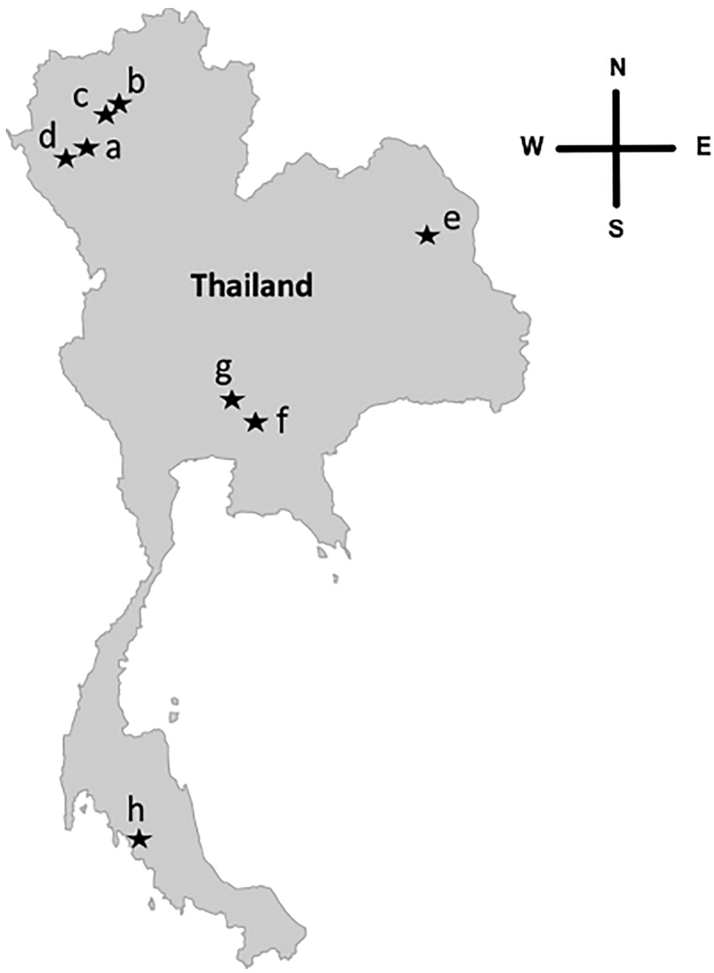

Fig. 1. The location for collecting the colony in Thailand. a, b, c and d in Chiang Mai Provoince, e in Sakon Nakhon Province, f and g in Saraburi Prov., h in Trang Prov. Altitude, forest types, and species collected were as follow. **a)** Omkoi, 1277 m, Hill evergreen forest, sp. 1, **b)** Khun Chang Khian Highland Research Station, 1314 m, Hill evergreen forest, sp. 2, **c)** Forest camp of Faculty of Forestry, 1367 m, Hill evergreen forest, sp. 2, **d)** Chomthong, 618 m, Dry dipterocarp forest, sp. 3, **e)** Phu Phan, 363 m, Hill evergreen forest, sp.3, **f)** Klokeidok, 167 m, Evergreen forest, sp. 4, **g)** Pukae, 78 m, Evergreen forest, sp. 5, **h)** Khao Chong Botanical garden, Trang Prov. 66 m, evergreen forest, sp. 6.

evations of the two sites in northeastern Thailand where sp. 3 was collected (d and e in Fig. 1) were 363 m. and 618 m. *Aphaenogaster* nests are usually hidden under the litter or underground so we located the nests by following foraging workers. First, we tried to find foragers and then we offered them live or dead termites. If the foragers retrieved items to their nests, we followed them to find their nest. We recorded the nest location and habitat condition. All nests were carefully excavated by shovel and put in a plastic bag with soil, and then brought back to the laboratory. We separated the ants from the soil and counted the numbers of each caste and brood stage. Queens (N = 1 to 5 queens/species for 5 species) and workers (N = 10 to 142/species) were dissected under a dissecting microscope to check the number of ovarioles and reproductive condition. Mean colony size between southern and northern Thai species, and between temperate and tropical species were compared by Mann-Whitney U test packaged in R ver. 3.4.2 (R Development Core Team 2017).

RESULTS

Nesting habit

We found in total 36 nests of six species of *Aphaenogaster*, the majority of which were found in a slope nearby a forest path. In most cases, the nest opening appeared as a small chimney made of sand debris and/or leaf litter (Fig. 2). A single tunnel connected the entrance to the underground nest chamber. In all cases of underground nests (N = 33), there was only one nest chamber. The depth of nests was shallow, about 20-30 cm from the soil surface. In addition to such underground nests, we collected one nest of *Aphaenogaster* sp. 3 under a stone, one nest of *Aphaenogaster* sp. 4 under leaf litter, and one nest of *Aphaenogaster* sp. 4 in rotten wood fallen on the ground. It appeared that nests of *Aphaenogaster* sp.2 (N = 5) and sp. 6 (N = 10) were aggregated.

Colony composition

Among the 36 colonies, 30 colonies were queenright and six colonies were queenless (Table 1). These queenless colonies might be the result of failure to capture queens during nest excavation. In addition, we found five small nests of *Aphaenogaster* sp. 6 near the main colonies, lacking a queen and brood, and with only a few workers. These small nests might be satellite nests (Table 1.). One colony of sp. 2 was likely a newly founded colony, with one dealate queen and small egg pile. All queenright colonies were monogynous with one dealate queen. All dealate queens dissected (N = 11 for 5 species) were inseminated, had eight ovarioles (4-4), and dense yellow bodies and mature oocytes in their ovarioles. Workers of all six species had two ovarioles (1-1). Several

Fig. 2. The obvious entrance of nest of *Aphaenogaster* sp. 2 was built by debris of sand or leaf litter.

workers of all six species had developing oocytes which were likely to be trophic eggs as in *A. japonica* (Iwanishi et al. 2003). Alate queens were found in two colonies of sp. 6 and males were found in six colonies of sp. 6 and two colonies of sp. 2. The mean number of workers in the species collected in northern and northeastern sites (sp. 1- 3) was larger than in species in the central and southern sites (sp. 4-6) (Mann-Whitney U test, Z = -1.96, $N_1 = 3$, $N_2 = 3$, $p = 0.049$). In addition to these colonies, three single dealate queens of sp. 2 were found alone on the ground, which may have been founding queens looking for new nest sites.

DISCUSSION

All but one *Aphaenogaster* species so far reported from the temperate zone are monogynous (N = 7 species, Table 2), having only one mated queen per colony (Chéron et al 2009; Cronin & Monnin 2009; Frumhoff & Ward 1992; Mizutani & Imamaura 1980; Talbot 1954; Boulay et al. 2007). Frequent occurrence of multiple-queen colonies was observed only in *A. rudis* (Headley 1949; Talbot 1951), however, even in this species, there was only one mated egg-laying queen (Lubertazzi 2012). Five of the six species of *Aphaenogaster* collected in Thailand also showed monogyny. Nonetheless, the limited available data indicate differences in biological characteristics between temperate and tropical *Aphaenogaster* species in terms of nest structure and colony size (Table 2). Both tropical and temperate species make their nests in soil. However, whereas nests of temperate species have several small chambers (Mizutani & Imamura1980; Talbot 1951, 1954; Headley 1949; Tschinkel 2011), all nests of the six species studied in this paper had only one chamber. The number of nest entrances in Thai species is only one, while at least three temperate species (*A. fluva*, *A. longiceps*, *A. treatae*) had multiple entrances (Headley 1949, Richards 2009, Talbot 1954). The existence of the entrance chimney in our Thai species is also unique.

Kaspari and Vargo (1995) proposed that "Bergmann's rule" could explain the decreasing colony size observed in ants with decreasing latitude. They compared colony size of three different taxonomic categories (subfamily, genus and species) between temperate and tropical ants (< 23.3 latitude), and found that mean colony size of both species and genus in the tropics was smaller than in the temperate zone. They further examined colony size of 19 ant genera that are distributed both in temperate and tropical regions and found that, 17 had smaller average colony sizes in the tropics. In *Aphaenogaster*, they used only one tropical species and two temperate species. We compared our data and data of three tropical and nine temperate species found in the literature and our unpublished information (Fig. 3). When colony size was compared between tropical and temperate species according to the analysis by

Table 2. A comparison of the biological features of *Aphaenogaster* species between Thailand and temperate zone

	Thailand	Temperate zone
No. of nest entrances	1 (six species)	2.3 (*A. fluva*, Headley 1949) 1-5 (*A. treatae*, Talbot 1954) 1 to "multiple" (*A. longiceps,* Richards 2009)
No. of nest chambers	1 (six species)	5.81 (*A. floridana*, Tschinkel 2011) 4.2 (*A. ashmeadi*, Tschinkel 2011) 7 (*A. japonica*, Mizutani and Imamura 1980) 8.8 (*A. treatae*, Talbot 1954) 6.5 (*A. fluva*, Headley 1949)
No. of queens	1 (five species)	1 (*A. treatae*, Talbot 1954; *A. japonica*, Mizutani and Imamura 1980; *A. senilis*, Boulay et al 2007; *A. rudis*, Lubertazzi 2012; *A. albisetosus, A. subterranea*, Frumhoff and Ward 1992) 1-2 (*A. fluva*, Headley 1949)

Kaspari and Vargo (1995), the tropical species of *Aphaenogaster* had significantly smaller colonies (Mann-Whitney U test, $Z = -3.13$, $N_1 = 9$, $N_2 = 9$, $p = 0.002$). Among the six Thai species, colony size of three southern and central species was also smaller than that of three species collected in northern sites, even the number of colonies examined was small for most species (Table 1). Thus, the variation of colony size in *Aphaenogaster* seems to fit well to "Bergmann's rule", though the mechanism underlying "Bergmann's rule" is still controversial, and may vary among animal groups (Blackburn et al. 1999). Larger numbers of workers may help colonies survive during winter (Kaspari & Vargo 1995). The difference in nest structure between temperate and tropical *Aphaenogaster* may partly be explained by colony size. Detailed investigation of ecology and behavior of tropical and temperate *Aphaenogaster* may provide important insights into the mechanism underlying "Bergmann's rule" in ant colony size.

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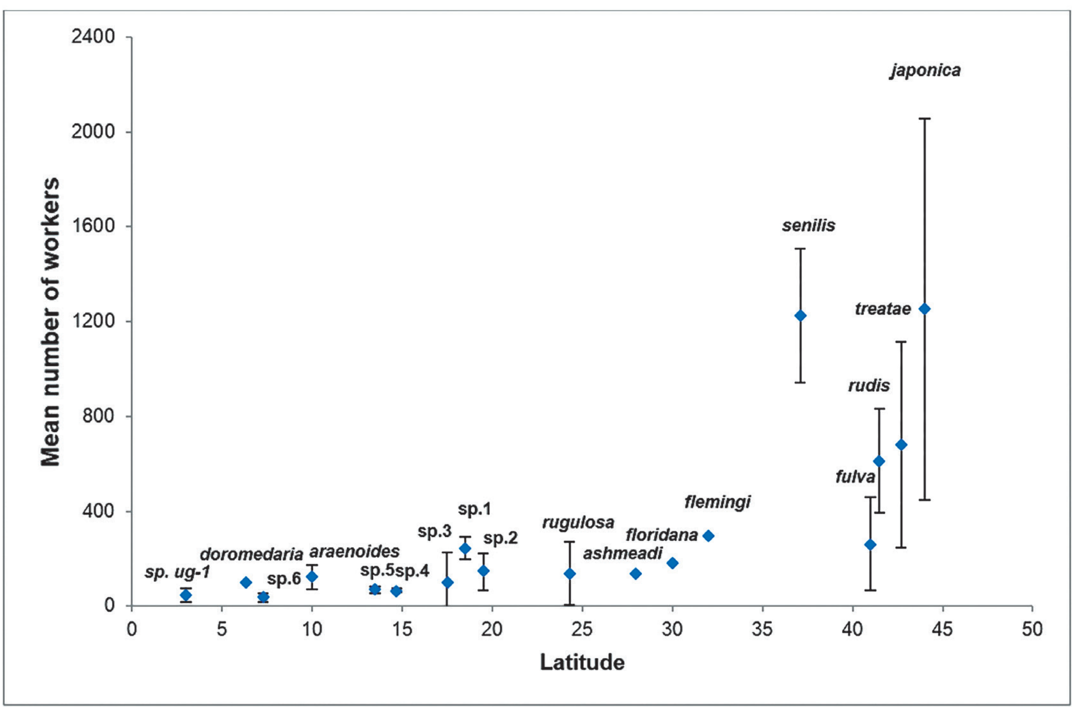

Fig. 3. The relationship between colony size of *Aphaenogaster* species (mean ± SD) and latitude. Colony size of 12 species studied outside Thailand were calculated based on Avargues-Weber and Monnin (2009), Boulay et al. (2007), Cronin and Monnin (2009) for *A. senilis* (N = 243), Headley (1949) for *A. fluva* (N = 46), Ito (unpublished) for *A.* sp. ug-1 (N = 8), Ito et al. (unpublished) for *A. rugulosa* (N = 3), King and Porter (2007) for *A. flemingi* (N = not mentioned), Lubertazzi (2012) for *A. rudis* (N = 17), McGlnn et al. (2002) for *A. araenoides* (N = 16), Mizutani and Imamura (1980) for *A. japonica* (N = 13), Talbot (1954) for *A. treatae* (N = 30), Tschinkel (2011) for *A. floridana* (N = 20) and *A. ashmeadi* (N = 7), Wilson (1959) for *A. doromedaria* (N=1)

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