ASIAN MYRMECOLOGY — Volume 15, e015009, 2022
ISSN 1985-1944 | eISSN: 2462-2362 DOI: 10.20362/am.015009

Seed-removal experiments indicate *Aphaenogaster* ants (Hymenoptera, Formicidae) as a potential keystone seed disperser of subtropical forest fragments and plantations in China

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ABSTRACT. Forest fragments and plantations have important values in ecological restoration under the global urbanization trend, which represent widely distributed however largely understudied ecosystems in China. Ants are biotic vectors mediating vegetation recovery via seed-dispersal. A keystone ant seed disperser exhibits high rate of seed-removal and provides consistent service directing seeds to microhabitats suitable for establishment. In this study, seed removal by ants was studied at three forested sites in Kunming, Yunnan, China during September - November 2021. An exclusion experiment using papaya seeds for ≤ 72 hours was performed to identify ant species contributing significantly to seed removal at each study site. Seed tracking was conducted at two of the sites, to understand the processes of transporting seeds from deposition spots to ant nest openings. Ants in the genus *Aphaenogaster* (*A. famelica*, *A. japonica, A. schurri*) removed the most seeds, followed by *Pheidole* (*P. fervens*, *P. nodus*) and *Nylanderia bourbonica*. Seeds carried into *Aphaenogaster* nest openings were at an average speed of 1.72cm per minute, with *Aphaenogaster* competing with *Pheidole* and *Ectomomyrmex* during seed transportation. The results showed a potential keystone role of the *Aphaenogaster* ants in seed dispersal, with important conservation implications for altered subtropical forest ecosystems in China.

Keywords Seed removal, forest fragments, forest plantations, China, *Aphaenogaster*

Citation Ran Dai (2022). Seed-removal experiments indicate *Aphaenogaster* ants (Hymenoptera, Formicidae) as a potential keystone seed disperser of subtropical forest fragments and plantations in China. Asian Myrmecology 15: e015009

Communicating Editor Omid Paknia

INTRODUCTION

Urbanization is a global driver for natural landscape fragmentation with biodiversity loss (Elvidge et al. 2012). Currently, there is an increasing amount of research driven by the call to restore ecological function of human-altered ecosystems (McConkey et al. 2012) to address the potential role of underrepresented soil organisms (Genes & Dirzo 2022). Ants are biotic vectors mediating vegetation recovery through the service of seed dispersal, considered primarily as a mutualistic interaction occurring along wide geographic gradients and under different vegetation types (Gómez & Espadaler 2013). Seed removal by ants from deposition site to ant nest opening triggers above-ground seed movements and contributes to effective dispersal distances (Anjos et al. 2020), which helps the plant to escape negative impacts of kin competition and increases chances of survival (Fernandes et al. 2020). Among various ants which interact with plant seeds, species vary in seed dispersal efficiency, with keystone species removing >75% of the seeds and consistently directing seeds to potentially suitable microhabitats for establishment (Ness et al. 2009, Warren & Giladi 2014). Examples of the keystone ant seed dispersers include *Aphaenogaster rudis* of North American (Ness et al. 2009), *Rhytidoponera violacea* of southwestern Australia (Lubertazzi et al. 2010) and *Pristomyrmex pungens* of central China (Zhu & Wang 2018).

Identification of ant species acting as potential keystone seed dispersers and quantify their relative importance are particularly important for altered environments. Changes in vegetation structure (Assis et al. 2018), soil and water processes (Debuse et al. 2007), and species relationships (Oliveras et al. 2005) affect ant assemblages, and hence the availability, magnitude and effectiveness of the interaction between the plant and its ant partner (Buono et al. 2022). Body size, diet and foraging habits (Silva & Brandão 2010), and the differences at individual and colony scales (Kaspari 1996) of ants greatly affect the type of seeds they could possibly interact with and influence seed dispersal distance (Kaspari 1996, Ness et al. 2004). Due to a diverse array of seeds that may be involved in mutualistic interactions with ants (Ness et al. 2009), both native and exotic plants may benefit from the efficiency of the keystone disperser (Ortiz et al. 2021), which possibly leads to negative implications of the conservation effectiveness of the ant's foraging behavior. Furthermore, ant communities are vulnerable to changes in the natural vegetation with human disturbances, which facilitate species invasion and alters the dominance regime of a local community. This may result in the displacement of the keystone species by an invader, however the invader may fail to perform equivalent seed-dispersal services (Warren et al. 2015).

In China, the ecology of seed dispersal has been studied using various animal models including mammals (e.g. primates [Bai 2020, Li et al. 2019, Li et al. 2021], rodents [Zhang et al. 2016, Chang et al. 2012], bats [Tang et al. 2005], foxes [Sun 2013]), birds (Wang 2020, Lu 2020, Li et al. 2014, Yang et al. 2017, Jin et al. 2021) and invertebrates (e.g. hornets, ants [Chen et al. 2021], beetles [Cao 2021]). Many studies on ant seed dispersal focused on the pairwise relationships between ants and plant species producing disposable diaspores, for example *Broussonetia papyrifera* (Moraceae) - *Camponotus parius* and *Pheidole sp.* (Wang 2013), *Flaveria bidentis* (Asteraceae) - *Tetramorium caespitum* (Zhang 2013), *Taxus chinensis* (Taxaceae) - *Aenictus bobaiensis* and *Odontomachus monticola* (Chen 2009). However, the universal role of myrmecochory in vegetation recovery within the human-disturbed ecosystems, and the comparative importance of ant species co-existing in an area remained poorly understood. On the other hand, deforestation in China has a long history, with widespread forest fragmentations being a major ecological consequence of human land-use transformation and economic booms (Liu et al 2019). In the southern and inland provinces, which are economically less developed, geographically diverse and forest rich (Démurger et al. 2002), forest fragments are found in areas inconvenient for human land exploitation (Liu et al. 2019). As a possible measurement, afforestation via introducing artificial forests to previously deforested areas has been widely adopted, which successfully boosted national forest coverage since initiation (Zhang & Song 2006). However, the theory and practice of afforestation initiatives have been criticized for ignoring the complex nature of ecological processes underlying forest recovery (Li et al. 2021), whereas localized plant-animal relationships potentially determine the long-term well-being of the afforested vegetations (Staples et al. 2020). In this study, seed-removal experiments were conducted in the subtropical forests of Kunming, capital city of Yunnan Province (China). Yunnan is traditionally a biodiversity hotspot and is particularly rich in myrmefauna (Fontanilla et al. 2019), however the ecology of myrmecochory has only been studied or mentioned in the tropics and/or less-disturbed environments (e.g. Zhang 2006, Chen et al. 2017), where species composition and diversity of both ants and plants vary significantly from those of the subtropical forests influenced by humans. I hypothesized that ant species living in the subtropical forest fragments and plantations contributed differently to seed removal, with only one or few ants removing most of the seeds. The objectives were to identify the potential "keystone" ants and quantify seed removal, and to understand the process of above-ground seed transportation mediated by ants.

MATERIALS AND METHODS

Study sites

The natural vegetation in Kunming is classified as subtropical montane broadleaved evergreen forest, where diverse floral compositions are found under various combinations of altitudinal, topographic and soil-water conditions (Tang 2010). In September - November 2021, fieldwork was carried out at three urban/suburban forests (S1, S2 and S3, S for Site; Fig. 1.). S1 was in a secondary-growth forest and tree plantation dominated by *Acacia dealbata* (Fabaceae) in Changchongshan ("Snake Mountain"). *Acacia dealbata* (and *A. mearnsii* in other areas of Changchongshan) are currently widespread and had been introduced decades ago possibly for greening purposes. Other tree species in the genera *Cupressus* (Cupressaceae), *Eucalyptus* (Myrtaceae), *Juniper* (Cupressaceae), *Pinus* (Pinaceae) were found in small stands. S2 was in a secondary shrub forest composed of *Bauhinia* (Fabaceae), *Pistacia* (Anacardiaceae), *Osteomeles* (Rosaceae), and *Thalictrum* (Ranunculaceae) among others, with the uppermost areas comprising corn fields (*Zea Mays*, Poaceae) in Yingongshan ("Silver Mercury Mountain"). In recent years, the natural vegetation of upper Yingongshan was further fragmented by the construction of a tunnel road (Sun 2021). S3 was situated in Mianshan ("Sleeping Mountain"), a large, singular forest patch (~28 ha) located ~ 6km from downtown Kunming, where a radio-transmission tower (Xu et al. 2019) had been built near the hilltop, with several old factories and human residents on the lower part of the hillside. Trees from the genera *Castanea* (Castanea), *Celtis* (Cannabaceae), *Cupressus*, *Eucalyptus*, *Pinus*, *Prunus* (Rosaceae), and *Quercus* (Fagaceae) dominated the plantation canopy, with several shrubs in the genera *Myrsine* (Primulaceae), *Viburnum* (Adoxaceae), and *Rhododendron* (Ericaceae) sparsely distributed along the forest interiors.

All the sites were at least 8km from one another, selected based on the relatively mild topography allowing for establishment of transect lines and fixation of multiple cages on the ground (see below). Despite the presence of agriculture and sporadic tourism encountered during the study, all the sites showed clear evidence of regular and intensive human activity, and the forests were at their early successional stages as evidenced by a lack of native tree species in the canopy layers and relatively complex structures for the herbaceous and shrub layers (Swanson et al. 2011).

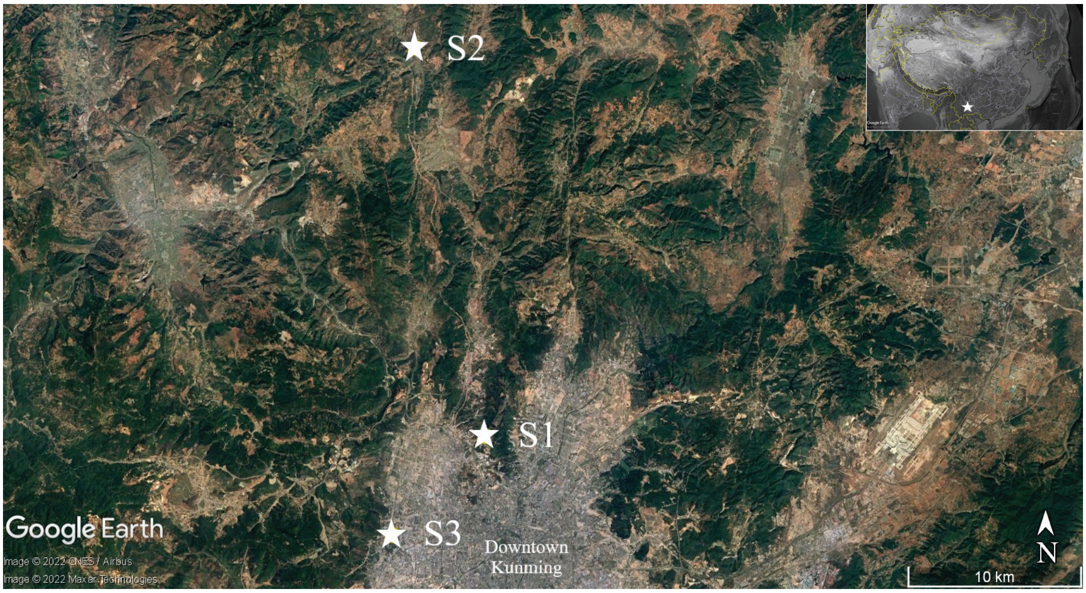

Fig. 1. Geographic locations of the study sites (S1, S2, S3) in Kunming, Yunnan Province, southwest China. S1 was located near the urban-rural boundary, where small and large forest patches were fragmented by human land uses. S2 was in a rural hilly forest, with agriculture and villages occupying the lower bodies of the hill. S3 was a forest patch located near the highly urbanized downtown.

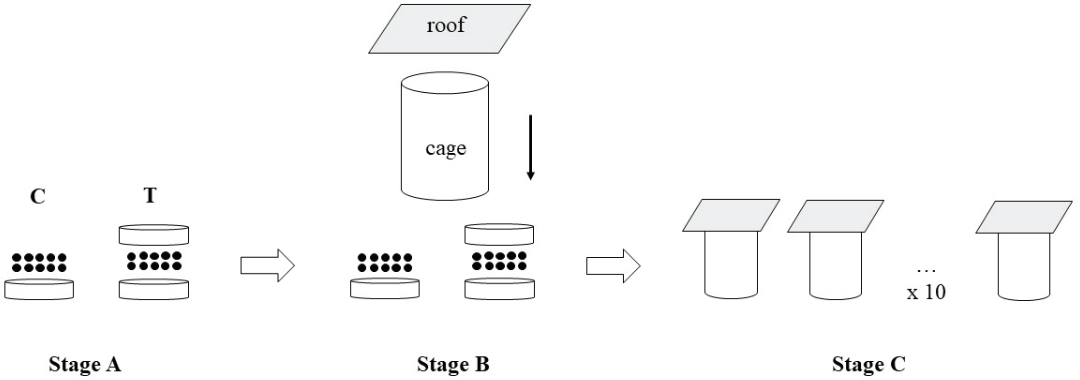

Fig. 2. Experimental set-up and flow for testing seed removal by ants in the field. Stage A: establish paired control (open petri-dish allowing ant-access) and treatment (sealed petri-dish excluding ant access), each containing 10 papaya seeds. Stage B: protect the C & T pairs from larger animals and rainfall using an iron cage with roof. Stage C: assign 10 pairs at each transect line (totally three lines for each site), with 2-m interval between neighboring pairs. Image composited using Microsoft PowerPoint 2019 (ver. 2210, Microsoft Co Lit.).

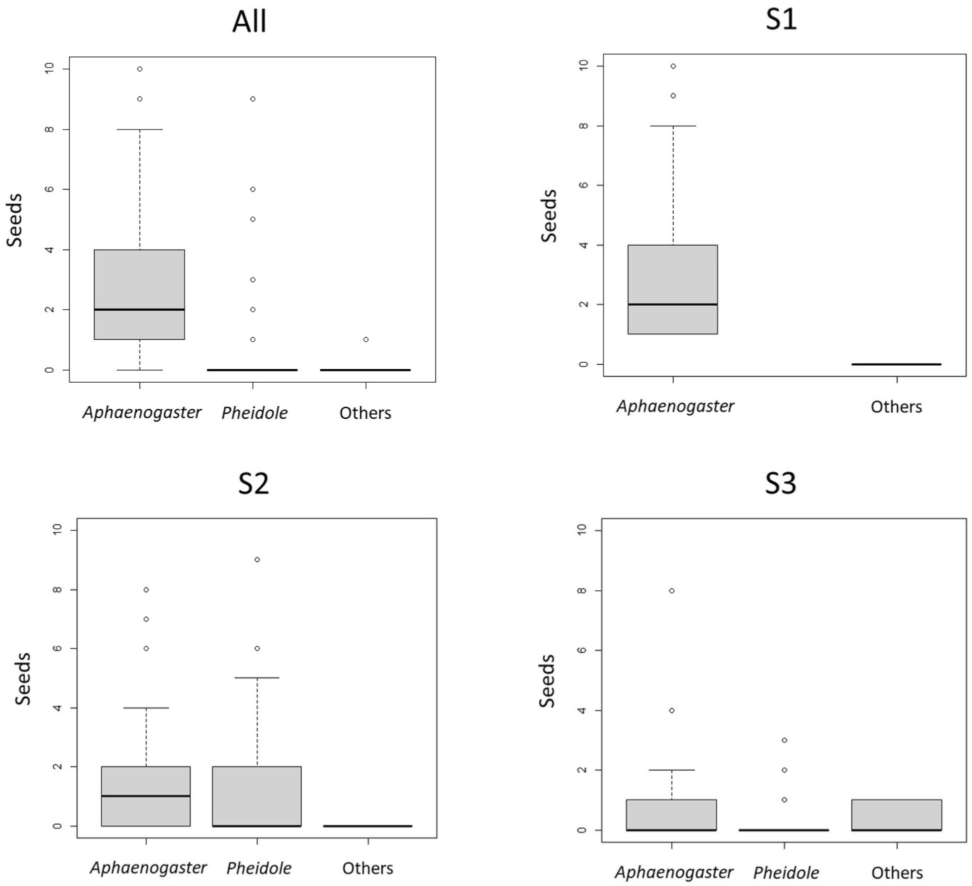

Fig. 3. Boxplots showing seed removal (number of seeds removed per two hours of observation) by ant categories (*Aphaenogaster*, *Pheidole*, Others) for each study site (S1, S2, S3) and for all sites combined. In S1, no *Pheidole* ants were removing seeds from the experimental trails. More *Pheidole* ants were recorded removing seeds in S3 compared to S2. In all cases, the *Aphaenogaster* ants were mainly responsible for seed removals. The boxplots were generated using R (version 4.1.2, R Core Team 2021).

Fig. 4. (Left) *Aphaenogaster famelica* workers pulling two papaya seeds from an open petri-dish (control) at S1. (Right) a threaded seed moved by *Aphaenogaster* workers to a nest opening positioned on the surface of a mushroom. After finding out the seed was too big to fit the opening, an additional worker emerged from a nearby nest opening on the ground with larger diameter, and directed the seed to be transported into the later nest opening.

The subtropical climate of Kunming is characterized by dry/wet seasonality and relatively mild temperature variations year-round. The wet season with the most precipitation was from June to October, and the dry season was from November to May. In September - November 2021, mean air temperature ranged between 4°C - 27°C, and accumulated rainfall was 85.2mm. The temperature values were within the normal range of the ten-year (2012 - 2021) averages (3°C - 27.9°C), whereas rainfall was less than the ten-year average (842.8mm). (Qweather 2022).

Seed-removal experiment

To quantify seed removal and identify important ant species, an exclusion experiment which supplied the ants with seeds of commercially available papaya (*Papaya arica*) (size = 4.79mm x 3.44mm [length x width], weight = 0.12 [SE = ± 0.14]g; n = 30 seeds) was performed at all study site (S1, S2 and S3) during 07 September - 26 October 2022. Ripe papaya seeds were covered by a semi-translucent layer of sweet-tasting aril attractive to the foraging ants, and have been used for studies of ant seed removal and seed dispersal elsewhere (e.g. Almeida et al. 2013, Zelikova et al. 2008). These seeds were also larger (and heavier) than the wild seeds (e.g. *Acacia dealbata* [see Discussion] and unknown grass [similar to *Glyceria striata*]) observed being carried by the ants at the study area. It was therefore expected that seed species that are smaller/lighter than the papaya could also be impacted by the ants, based on the ant-seed size hypothesis (Ness et al. 2004). As papaya is a non-native plant, under natural settings the papaya seedlings could not survive the low winter temperatures in Kunming (Liu & Li 2003), thus excluding the possibility of introducing alien species to the study area.

At each study site, three transect lines were set up, each measuring 20m in length and separated from neighboring lines by 20m - 80m. One line at S2 was in the corn field adjacent to two forest patches (where the two other lines were located). Due to the small sample size, data from the agricultural land was pooled with data from the forest. A feeding station was established every 2m at each transect line (ten stations in total), which contained a treatment (ten seeds put in a petri-dish [3.5cm in diameter and 1.1cm tall], sealed with clear tapes to exclude ant access while allowing for counting of the seeds within) and a control (ten seeds in an open petri-dish allowing for ant access). The paired control and treatment were protected by a cylinder cage (15cm in diameter and 30cm tall; made of iron mesh wire [mesh size = 1.5cm x 1.5cm, diameter = 0.7cm]) fixed into the ground, to protect seeds from larger animals (e.g. birds and mammals), and covered by a plastic roof (25 x 25cm) to reduce the impact of the rain (Fig. 2.).

The seeds were then left in the field for up to three days (72 hours). Each day I counted the number of seeds remaining in each control and treatment dish at two-hour intervals between 10:00-18:00. During the seed check, I also recorded ant species in and near a feeding station, which either interacted or not with the experimental seeds. Decreases in the number of seeds were assumed to be the result of ant species present between two seed checks (seed reductions occurring beyond the observational period, e.g. at nights, were not attributed to any ant species). When zero seeds remained in a petri-dish, ten new papaya seeds were added. Voucher ants (up to 32 individuals in each species) were collected for identification using available online databases, including AntWiki (http://antwiki.org) and Ants China (or "mayiwang", http://ants-China.com), while minimizing the impacts to seed transportation process (e.g. scouts and seed-carrying individuals were avoided during collection). For *Pheidole nodus* (see Results), both minor and major workers were collected for accurate identification. The specimens were then preserved in 75% alcohol as part of the author's personal collection. Due to logistic issues, observations were not performed in the evenings and at nights.

Ant species were categorized into three different groups based on the contribution to seed removal: species removing >75% of the experimental seeds were classified as "major facilitator", species removing the second-most seeds following the major facilitator were classified as "minor facilitator", and species removing the least seeds were classified as "others".

Tracking seed movements

To understand the transportation process from the seed depot to ant nest opening, direct observation was performed on tagged seeds at four randomly-selected spots along the transect lines of S1 and S3 (two places per site, separated from each other by > 20 m) during 19 October - 18 November 2021. Due to the dense shrub which made observations of ant behavior difficult, this part of the study was not carried out at S2. In one experimental trial (lasting from 10:00 to 18:00), ten papaya seeds (in total 40 seeds) each tied to a light weight and brightly colored silk thread [e.g. light yellow; for tracking of seed movements across the dimly-lit forest floor; Fig. 4 - B.] measuring 15cm, and marked (using a marker pen) with a unique number of 1 - 10 bars to enable identification and tracking of individual seeds) were placed on the ground. Following seed allocation, I recorded the time at which the seeds were first discovered and interacted with the scout(s) (usually one to two individual ants) and noted ant species, when a seed was moved and by how many ants at the same time, and calculated the duration of time(min) from the onset of seed movement up until the seed was seen disappearing within an ant nest opening. Distance(cm) between the seed depot spot to an ant nest opening was measured using a standard tape, and diameter of the nest opening was measured using a vernier calliper.

RESULTS

Fourteen ant species belonging to nine genera including *Aphaenogaster* (three species), *Pheidole* (two species), *Nylanderia* (three species), *Camponotus* (one species), *Dendrolasius* (one species), *Formica* (one species), *Tetramorium* (one species), *Ectomomyrmex* (one species) and *Crematogaster* (one species) were present during the observational period, with *Aphaenogaster*, *Pheidole, Nylanderia* and *Ectomomyrmex* ants (in seed-tracking experiment) observed moving seeds and/or feeding on the seed aril, and the rest species not found actively interacting with the seeds, with decrease in the number of seeds also not recorded following their presence (Table 1). At all study sites, 1,061 out of 1,610 seeds assigned to the control were removed (seed-removal rate = 65.9%), after the exposure in the field for up to 72 hours, with the remaining 549 (or 34.1%) seeds in the feeding stations un-removed. No seed was removed from the sealed treatment (total number of seeds assigned = 900). Of all the removed seeds, 439 (or 41.38%) were with identified ant species, and the rest 622 (58.62 %) were with unknown species (Table 1). Whereas some other ant species (e.g. *Solenopsis invicta*, *Lasius spp*., *Kartidris nyos*, *K. sparsipila*, *Monomorium sp*., *Odontoponera sp*., *Ponera sp*.) have also been found near the study area, they were not observed during experiments, and also appeared less common (except *S. invicta*, which was observed in great abundance at the lower altitudes near S1 and S2).

During the experimental period, ants in the genus *Aphaenogaster* (*A. famelica* [Fig. 4 - A], *A. japonica* and *A. schurri*) removed a total of 352 - 364 (or 80.18% - 82.92%) seeds with identified ant species, therefore they were classified as "major facilitator". Ants in the genus *Pheidole* (*P. fervens* and *P. nodus*; only the minors were observed carrying seeds during the observation period, with the majors present however not directly involved in seed transportations) removed 45 - 54 (or 10.25% - 12.3%) of the seeds, and were classified as “minor facilitator”. Other ant species (*Nylanderia bourbonica*) removed 15 - 17 (3.42% - 3.87%) of the seeds, classified as “others” (Fig. 3.). Twelve seeds were with more than one ant species/genera, including one seed at S1 removed by *Aphaenogaster famelica* and/or *Nylanderia bourbonica*, nine seeds at S2 by *A. schurri* and/or *Pheidole nodus*, and two seeds at S3 by *Aphaenogaster sp.* and/or *N. bourbonica.*

Table 1. Details of the study sites including geographical locations, dates studied and of seed-removal experiment, including numbers of seeds assigned (to controls and treatments) and removed (total vs. with identified ants [identified]) from the controls with seed-removal rates, ant species and number of seeds removed (pooled for *Aphaenogaster* and *Pheidole*, if more than one species present at one site might be responsible for seed removal).

Study site	Geo-location; a.s.l.	Date	Seeds assigned (control + treatment)	Total removal (rate%)	Identified (rate%)	Ant species	Seed removal	Rate (%)
S1 - Changchongshan	N 25°06.09’ E102°41.29’; 2,041 m	07 - 10 Sep 2021	1120 (820 + 300)	651 (79.39)	277 (42.55)	*Aphaenogaster famelica*	276 - 277	99.64 - 100
						Nylanderia bourbonica	0 - 1	0 - 0.36
S2 - Yingongshan	N 25°18.01’ E102°39.07’; 1,959 m	22 - 25 Sep 2021	750 (450 + 300)	244 (54.22)	107 (43.85)	*Aphaenogaster schurri*	57 - 66	53.27 - 61.68
						Pheidole nodus	37 - 46	34.58 - 42.99
						Nylanderia bourbonica	0	0
						Nylanderia flavipes, Nylanderia sp.*, Camponotus tonkinus, Dendrolasius* sp., *Formica* sp., *Tetramorium* sp.	0	0
S3 - Mianshan	N 25°02.96’ E102°38.27’; 1,950 m	23 - 26 Oct 2021	640 (340 + 300)	166 (48.82)	55 (33.13)	*Aphaenogaster schurri*	19 - 21	34.55 - 38.18
						Aphaenogaster japonica		
						Pheidole nodus	8	14.55
						Pheidole fervens		
						Nylanderia bourbonica	15 - 17	27.27 - 30.91
						Ectomomyrmex astutus	0	0

During seed-tracking experiment, 34 out of 40 tagged seeds were moved by ants from seed depot spots, of which 11 were transported into *Aphaenogaster* nest openings during the observational period. The seeds were first discovered by one to two *Aphaenogaster* or one *Pheidole* scouts within 5min - 20min following seed deposition. And seed transportation was mediated by 5 - 20 *Aphaenogaster* workers (or more, possibly present however unseen beneath the soil litter) that arrived 3min - 30min after the discovery by the scout(s). Those ant(s) gripped at the seed coat, and carried the seed away from its deposition spot. Up to five *Aphaenogaster*, four *Pheidole* or one *Ectomomyrmex* workers were observed carrying individual seeds at the same time. In the majority of the cases, a single *Aphaenogaster* worker was found carrying a seed alone, with additional worker(s) joining the transportation only when the seed was approaching a nest opening. The *Aphaenogaster* nest openings (N = 7) in which the seeds were transported were mostly single holes (mean diameter = 10.92mm, range = 4.86mm - 14.44mm) situated on the forest floor or the surface of a mushroom (*Calvatia sp.*, Fig. 4 - B), located 34cm - 259cm from the seed depot spots. Time spent for carrying individual seeds (totally N = 11 seeds) into an *Aphaenogaster* nest opening ranged from 57min to 4h and 41min, with the average speed being 1.72cm per minute.

Competitive relationships were noted between *Aphaengoaster* and *Pheidole* and between *Aphaenogaster* and *Ectomomyrmex* at S3. In one case, seed transportation by an *Aphaenogaster* worker was interrupted by four *Pheidole* workers which gripped at the seed without physically aggressive behavior involved during the encounter with the *Aphaenogaster* worker; the final seed fate was however unknown. In another case, an *Aphaenogaster* worker carrying a seed was attacked (bit at the thorax) by an *Ectomomyrmex* worker, after the later had attempted to grab the seed from the former, but failed. After fighting with each other for < 10 seconds, the *Ectomomyrmex* worker was driven away, and the *Aphaenogaster* worker continued to transport the seed into the nest.

DISCUSSION

In this study, seed removal and seed tracking were studied at three subtropical forest fragments and plantations in China. The results showed an important role of *Aphaenogaster* as potential keystone ant seed disperser. This conclusion is based on the high overall seed-removal rate, quick seed discovery, low worker recruitment (c.f. *Pheidole* workers), and consistency of seed dispersal services provided by *Aphaenogaster* ants (Warren & Giladi 2014). Though this study did not examine the underground seed-handling processes by the ants and the possibility of re-dispersal, seeds transported into *Aphaenogaster* nests are known to thereafter be either stored within the nest chambers or discarded near the nest openings, where seed germination might occur in the fertile soil of the ant mound (Andersen 1988). Since *Aphaenogaster* ants are central-place foragers (Campos et al. 2014), where workers regularly carry food and other items in and out of the same nest openings, which function as centers of information exchange (Campos et al. 2014), the ant-plant interaction may foster symbiotic relationships which ultimately lead to co-evolution (Gove et al. 2007, Ness et al. 2009). Though nocturnal activities of the ants were not studied here, *Aphaenogaster* might act as seed disperser both during the day and at night (Johnson & Rutowsk 2021), and *A. schurri* near S2 was also observed foraging around mid-nights (Dai personal observation), hence responsible for seed reductions occurring beyond the observational period.

In comparison with *Aphaenogaster*, *Pheidole* appeared more numerically abundant in the study sites and was able to disrupt seed transportation by *Aphaenogaster*. It was likely that quick seed discovery and removal contribute to a major advantage of *Aphaenogaster* over *Pheidole*, as the longer time a seed was left in the field, the more likely it might be monopolized by large numbers of recruited *Pheidole* workers. Both *Aphaenogaster* (with behavioral advantage) and *Pheidole* (with numeric advantage) might serve as efficient seed dispersers, and act as major competitor to each other at the study system. Similar to *Aphaengoaster*, *Ectomomyrmex* ants also foraged individually and competed with *Aphaenogaster* for seeds. Although the *Ectomomyrmex* worker was larger in size and appeared more aggressive, the *Aphaenogaster* worker was able to defend its food from this seed competitor. Also, the *Ectomomyrmex* ants at the study sites appeared more of an opportunistic forager, with workers often found alone wandering in search for food (Dai personal observation), which might turn out to be a disadvantage when compared with the foraging pattern exhibited by *Aphaenogaster*, that included both single searching and group collaboration during seed-finding and transportation. At S2, a “passive” mode of seed transportation was observed in *Dendrolasius*, in which numerous workers formed a steady foraging trail running cross the corn field to the shrub forest, where the nests might be located. As the workers matched along on the forest floor by adhering to the trail-following activity driven by pheromone (Kern et al. 1997), active foraging for papaya seeds outside the trail was not observed in individual workers, and only seeds dropped on the trail were carried along. Seed transportation by *Dendrolasius* thus appeared opportunistic in comparison with seed-seeking activities by other ants (e.g. *Aphaenogaster* and *Pheidole*) in the area.

Among seed-harvesting ants, *Aphaenogaster* and *Ectomomyrmex* appeared to be less active in areas with intensive human disturbance, whereas *Pheidole* and *Nylanderia* showed adaptations to disturbance. For example, nests of *Nylanderia* at S1 were sometimes found in polluted soil mixed with construction wastes (the same as *Solenopsis*), where hardly any other ants inhabited. And some *Pheidole* species also occurred close to human residence. Other human activities, such as the use of herbicides (e.g. Glyphosate ammonium, 2-methyl-4-chlorophenoxyacetic acid [MCPA] and mesotrione [used for corn and maize], of which used packages were recovered from S2) on crops planted near the forest edges can have negative influence on ant abundance (e.g. Glyphosate-ammonium [Guiseppe 2016]) and ant food source (e.g. mesotrione pollution on honeydew, Calvo-Agudo et al. 2022). Future research is needed to better understand the distribution and density of the keystone as well as less important ant dispersers in relation to the physical environments and human interference.

As to the natural interactions between ants and plant species in and around the study area, *A. famelica* workers at S2 were as frequently observed carrying seeds of *A. dealbata* with elaiosomes and grass seeds (Poaceae) during the annual fruiting seasons. Piles of *Acacia* seeds derived of the elaiosomes (consumed by the ants possibly inside their nest chambers) were found nearby ant nest openings. It was also possible that the mass-fruiting/seeding *Acacia* trees (Correia et al. 2016) ultimately affect the density and distribution of *Aphaenogaster* nests. While multiple seed-dispersal agents co-existed at the study area, the ants might provide plants with more available, stable and predictable services. For instance, seed dispersal by gravity (e.g. *Quercus glauca*, Fagaceae), wind (e.g. *Senecio spp*., Asteraceae), water and soil movements (e.g. *Eupatorium adenophorum*, Asteraceae) deliver plant seeds into various spots, however the functionality of which may largely depend on the physical conditions such as topography, vegetation density, and weather, although the natural factors are also known to affect ant activities (for example, continuous rainfall might have contributed to the low seed-removal rates at S3). Several bird species such as sooty-headed bulbul (*Pycnonotus aurigaster*) swallow seeds enclosed by juicy pulps (e.g. *Pistacia weinmannifolia,* Anacardiaceae) and disperse them via defecation (Dai personal observation). Domestic animals (e.g. goats, dogs) as well as humans might serve as agents for epizoochorous dispersal (e.g. for *Bidens pilosa*, Asteraceae), passing seeds often along the forest trails walked. However, both the animals and human as seed dispersers are not as abundant as the ants, with their dispersal destinations also less predictable. In comparison, the “directed dispersal” provided by ants affects plant demography in a positive way (Hanzawa et al. 1988), although this could strictly be limited to small-seeded plant species. Currently, researches on the importance of ant seed dispersal in forest regeneration for the study system are lacking, and it is of interest to further the understanding of different dispersal mechanisms and compare seed dispersal efficiencies.

In conclusion, this is the first study assessing the relative importance of several seed-removing ants in the disturbed subtropical ecosystem of southern China. The *Aphaenogaster* ants at the study area exhibited high seed-removal rate, with aspects of seed transportation mediated by individual workers conforming to the descriptions of a “keystone” seed disperser. The finding suggests a potential role of *Aphaenogaster* in conserving and restoring vegetations of forest fragments and plantations in China.

ACKNOLEDGEMENTS

I would like to thank R An for assisting the preliminary ant survey, N Dai for assisting field data collection; SK An, ZQ Dai, HX Yuan and TX Yuan for helping with the logistics; ZX Lu for advising on ant identification during the ant survey; and editors of Asian Myrmecology and two anonymous reviewers for their detailed comments on the manuscript.

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