The evidence of temporary social parasitism by *Polyrhachis lamellidens* (Hymenoptera, Formicidae) in a *Camponotus obscuripes* colony (Hymenoptera, Formicidae)

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Abstract

Polyrhachis lamellidens is a temporary socially parasitic ant whose new queen utilizes other ant species in the early stages of colony foundation. Field observations and rearing experiments suggest that *Camponotus japonicus* is a host species of *P. lamellidens*. It is presumed that *Camponotus obscuripes* is also a host of *P. lamellidens* by rearing experiments and field observations; however, there are no records of *P. lamellidens* workers or brood coexisting in *C. obscuripes* colonies in field observations, and there is no clear evidence that *C. obscuripes* is a natural host of *P. lamellidens*. We conducted detailed field observations, behavioral tests, and rearing experiments to show that *C. obscuripes* is a host of temporary social parasites. We found colonies with *P. lamellidens* queens, workers, and larvae intermixed with *C. obscuripes* workers in the field. Behavioral tests showed that workers of both species in mixed colonies did not attack each other and maintained nestmate recognition ability, which suggests a collaborative nestmate relationship. Furthermore, a rearing experiment confirmed social parasitism by *P. lamellidens* among *C. obscuripes* by producing a mixed brood-producing colony. These are the first field and laboratory records of temporary social parasitism involving *P. lamellidens* and *C. obscuripes*.

Keywords Social parasite · Host record · Rearing experiment · *Polyrhachis lamellidens*

Introduction

Polyrhachis lamellidens is a temporary social parasite that establishes new colonies with the assistance of a host species (Yano 1911; Kohriba 1963, 1966; Kubota 1974; Sakai 1996, 2000; Japanese Ant Database Group 2003; Furukawa et al. 2012) and is a known host of myrmecophiles (Iwai et al. 2016). *P. lamellidens* queens allow their host workers to care for their parasitic brood (eggs, larva, and pupa), resulting in a temporary period during which host-reared *P. lamellidens* workers and host workers inhabit the same colony (Kohriba

1963; Sakai 2000; Japanese Ant Database Group 2003). This mixed colony transitions to a *P. lamellidens*-only colony as the number of host workers gradually decreases (Japanese Ant Database Group 2003). Several species (*Camponotus japonicus*, *Camponotus obscuripes*, and *Camponotus kiusiuiensis*) have been suggested to be hosts of *P. lamellidens* (Yano 1911; Kohriba 1963, 1966; Kubota 1974; Sakai 1990, 1996, 2000; Japanese Ant Database Group 2003; Furukawa et al. 2012). *C. japonicus* has been confirmed as a host species according to observations of mixed colonies of *P. lamellidens* and *C. japonicus*. In mixed colonies in the field, the two different species coexisted without any aggressive behavior towards one other (Kohriba 1966). In addition, Kohriba demonstrated the early conditions of parasitic life in a laboratory environment and even observed brood production by *P. lamellidens* (Kohriba 1963).

There have been reports of new queens of *P. lamellidens* exhibiting an initial performance (the behavior of straddling host workers, which is speculated to promote invading the host colony) towards *C. obscuripes* workers and inhabiting *C. obscuripes* colonies (Sakai 1996; Furukawa et al. 2012). These findings suggest that *C. obscuripes* serves as a host

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for the temporary social parasite *P. lamellidens*. However, to accept this hypothesis, the following three factors must be examined and confirmed: parasite brood production, nestmate discrimination, and parasitism style. Parasite brood production is a primary event required to complete the social parasitism cycle (Hölldobler and Wilson 1990; Buschinger 2009; de la Mora et al. 2020). If *C. obscuripes* serves as the host, then *P. lamellidens* workers and brood must be identified along with *C. obscuripes*. However, *P. lamellidens* workers and brood have not yet been found in *C. obscuripes* colonies in the field or under artificial rearing conditions. Nestmate discrimination, i.e., whether *C. obscuripes* workers accept the parasites as nestmates must also be verified. Since a parasite and its host inhabit the same colony, they must recognize each other as nestmates while exhibiting aggressive behavior towards nonnestmates. Furthermore, it is necessary to show that *P. lamellidens* utilizes *C. obscuripes* as a host for temporary social parasitism and not other types of parasitism. In temporary socially parasitic ants, such as *P. lamellidens* and *C. japonicus* mentioned above, temporary coexistence between parasites and hosts occurs after killing the host queen.

The coexistence of parasites and hosts has also been shown in other social parasite strategies, such as xenobiosis and inquilinism, but these strategies do not result in the killing of a host queen by the parasite (Hölldobler and Wilson 1990; Buschinger 2009; de la Mora et al. 2020). For example, *Polyrhachis lama* (Formicidae: Formicinae) and *Polyrhachis loweryi* (Formicidae: Formicinae) have been identified as socially parasitic species (xenobiosis) of *Polyrhachis* species other than *P. lamellidens* (Maschwitz et al. 2000, 2003), and they do not kill the host queen. Dulotic species also kill the host queen; however, the subsequent coexistence with the host colony is permanent (because host workers are continually supplied by slave raiding), and they do not eventually replace the colony as some temporary socially parasitic ants do (Hölldobler and Wilson 1990; Buschinger 2009; de la Mora et al. 2020). Dulotic species are strictly divided into obligatory dulosis and facultative dulosis, in which the former worker, lacks the working ability while the latter possesses it. If *P. lamellidens* utilizes *C. obscuripes* as a host, then it is necessary to show that the *C. obscuripes* queen is killed by the *P. lamellidens* queen and that *P. lamellidens* workers are able to survive in the absence of their host. Therefore, exploration of mixed colonies in the field, behavioral tests, and rearing experiments are necessary

to show that *C. obscuripes* is a host of temporary social parasites.

In this paper, we report the first record of a mixed colony consisting of *C. obscuripes* workers and a *P. lamellidens* queen, workers, and brood from Yamagata Prefecture, Japan. Two independent mixed colonies were found in the field, and the symbiotic relationship was confirmed by behavioral tests. We also confirmed through rearing experiments that *P. lamellidens* queens practice social parasitism against *C. obscuripes*. These observations indicate that *P. lamellidens* can complete the social parasitism cycle using *C. obscuripes* as a natural host.

Materials and methods

Sampling and rearing

Ant colony samples were collected in Yamagata and Niigata Prefecture, Japan (2019 to 2020). Two mixed colonies of *P. lamellidens* and *C. obscuripes* were sampled from a primeval beech forest in Oguni-machi, Yamagata Prefecture (N 37°55'13", E 139°40'54"; N 37°55'24", E 139°40'27") and named colony A and B, respectively. Each colony (A:B) consisted of a *P. lamellidens* queen, a worker, a larva, and a *C. obscuripes* worker in the following numbers: 1:1, 729:12, 200:244, and 31:112. None included the queen and larva of *C. obscuripes* (Table 1). The *P. lamellidens* and *C. obscuripes* workers used in the behavioral test and the rearing experiment were collected from free-living colonies. Two free-living *C. obscuripes* colonies not parasitized by *P. lamellidens* were collected from a primeval beech forest in Oguni-machi (N 38°08'48", E 139°50'35") and a mixed forest in Tsuruoka City, Yamagata Prefecture (N 38°33'49", E 139°55'31"); the average colony size consisted of one queen and 300 workers. One free-living *P. lamellidens* colony was collected from mixed forests (N 38°41'43", E 139°48'2"); the colony size consisted of 20 workers. Eight newly mated *P. lamellidens* queens were collected after finishing their nuptial flights from a mixed forest in Nagaoka City, Niigata Prefecture (N 37°25'51", E 138°52'53"). Each collected ant colony was reared separately in a plastic tub (10.0 cm in length, 10.0 cm in width, 2.9 cm in height, or 27.0 cm in length, 19.0 cm in width, 5.1 cm in height) in which plaster was laid and a feeding area was established

Table 1 Compositions of the two mixed colonies

	<i>P. lamellidens</i>			<i>C. obscuripes</i>		
	Queen	Workers	Larvae	Queen	Workers	Larvae
Colony A	1	729	> 200	0	31	0
Colony B	1	12	244	0	112	0

or in a plastic cup (9.5 cm in diameter, 4.5 cm in height) in which moistened tissue was laid. Newly mated *P. lamellidens* queens were reared individually in a plastic tub that was 4.5 cm in length, 2.5 cm in width, and 2.0 cm in height and contained moistened tissue. The ants were fed 5–5000 µl of 50% maple syrup (Maple Farms Japan, Limited, Osaka, Japan) every 4–10 days. Worker ants were also fed mealworms and/or 8–16 g of Pro Jelly for beetles and stag beetles (Wraios, Limited, Saitama, Japan). Mixed colonies and non-mixed colonies (*C. obscuripes* and *P. lamellidens*) were reared under either dark conditions at 15–25 °C or light:dark conditions (14L:10D) at 20–30 °C. The colonies experimentally invaded by *P. lamellidens* were reared in a cool incubator (Mitsubishi Electric Engineering Company, Limited, Tokyo, Japan) under dark conditions at 20–25 °C. Newly mated *P. lamellidens* queens were reared in a cool incubator under dark conditions at 15–20 °C. The identifications were performed based on morphological characteristics (referring to Japanese Ant Database Group 2003) and the mitochondrial genes encoding cytochrome oxidase subunits I and II (*COI/II*) in the NCBI database (<http://www.ncbi.nlm.nih.gov>). DNA was extracted from the legs by crushing them with a disposable homogenizer and treating them with proteinase K. Extracted DNA was amplified by PCR with the following conditions: 98 °C for 2 min, 30 cycles each of 98 °C for 10 s, 55 °C for 15 s, and 72 °C for 2 min with LifeECO Thermal Cycler (Bioer, China). The Sanger sequencing of the amplified DNA was performed by Eurofins Genomics (Tokyo, Japan). C1-J-1754F (CCACGTTTAAATAATATAAGATTTTGAC) and C2-N-3661R (CCACAAATTTCTGAACATTGACCA) were used as PCR primers (Degnan et al. 2004; Simon et al. 1994). Sequences from *P. lamellidens* and *C. obscuripes* from mixed colonies and free-living were confirmed by matching each species by alignment and clustering with MAFFT v7 (Kuraku et al. 2013).

Behavioral tests

Behavioral tests were conducted in a plastic tub (8.0×4.0×3.0 cm), with one worker ant from each colony (the mixed Colony A, the free-living *C. obscuripes* colony, and the free-living *P. lamellidens* colony) encountering others. Mixed colony B was not used for behavioral tests because of the low number of *P. lamellidens* workers. The ants were selected at random and used only once in each test. The workers used for the test were transferred to another case and were not duplicated in subsequent tests. For 30 s, after introducing each ant into the case, a partition was used to prevent contact, reduce stress, and calm their behavior. The first behavior noted during the first

contact in less than 6 min after the removal of the partition was recorded according to the following definitions:

- Ignore: the ant does not respond aggressively to contact with the target.
- Threat: the ant opens its mandibles or keeps its distance from the target.
- Rush: the ant approaches and quickly bites the target.
- Bite: the ant continuously bites the target.

Among these behaviors, “Threat”, “Rush”, and “Bite” were considered aggressive behaviors.

Rearing experiments involving temporary social parasite

The study of temporary social parasitism in a laboratory environment was conducted based on previously reported procedures (Kohriba 1963). Assays to study initial contact between a newly mated *P. lamellidens* queen and a *C. obscuripes* worker were performed in well-ventilated tubes (1.0×8.0 cm). For eight newly mated *P. lamellidens* queens, one to twenty free-living *C. obscuripes* workers were placed in the tube every other day. After several days, when contact with more than 60 *C. obscuripes* workers was observed, the *P. lamellidens* queen was transferred to an empty plastic tub without the *C. obscuripes* queen (10.0×10.0×2.9 cm). Once it was confirmed that the *C. obscuripes* workers were not attacking the transferred *P. lamellidens* queen, she was allowed to contact the *C. obscuripes* queen. The *C. obscuripes* queen was subjected to anesthesia by freezing (4 °C for 2 min, – 20 °C for 10 min) before contact with a new *P. lamellidens* queen. A newly mated *P. lamellidens* queen is often attacked by the *C. obscuripes* workers or queens, as observed in rearing experiments in previous studies (Sakai 1996, 2000). Since the purpose of this rearing experiment was to learn whether the newly mated *P. lamellidens* queen would recognize the *C. obscuripes* queen as her host, to exclude the possibility of counterattack by the *C. obscuripes* queen, we conducted the anesthesia treatment.

Results

Field observations and mixed colony composition

Two mixed *P. lamellidens* and *C. obscuripes* colonies were found inside fallen beech trees in Yamagata, Japan. A queen, larvae, and workers of *P. lamellidens* and workers of *C. obscuripes* coexisted in the same colony (Fig. 1a; Table 1). The balance of worker numbers between *P. lamellidens* and *C. obscuripes* was biased towards *P. lamellidens* in Colony A and *C. obscuripes* in Colony B (Table 1). We used only

Fig. 1 **a** *P. lamellidens* and *C. obscuripes* were collected from the same colony (**a** Colony A). **b** *P. lamellidens* queen isolated from Colony A. This queen's left and right mid and hind legs were all severed at the base. **c** *P. lamellidens* and *C. obscuripes* perform trophallaxis (Colony A)

Colony A in the behavioral test because the number of *P. lamellidens* workers at Colony B was small. Only one *P. lamellidens* queen was found in each colony. In addition, the *P. lamellidens* queen found in Colony A possessed left and right mid- and hindlegs that were all severed at their bases (Fig. 1b). After collection, no signs of mutual aggression were observed between the two species in the mixed colonies in the field and under laboratory conditions. Under laboratory-rearing conditions, trophallaxis between *C. obscuripes* and *P. lamellidens* workers was observed (Fig. 1c). All *C. obscuripes* workers (host workers) belonging to Colony A died within approximately 2 months after collection. The *P. lamellidens* colony has persisted to the present day (as of October 2020). The ants in the colonies were identified as *P. lamellidens* and *C. obscuripes* by comparing the *COI/II* sequences of non-parasitized *C. obscuripes* and free-living *P. lamellidens*.

Behavioral tests

Behavioral tests were performed to quantitatively determine whether the mixed colony composition was a result of temporary social parasitism by *P. lamellidens*. When

C. obscuripes and *P. lamellidens* workers from the same mixed colony contacted one another, no aggressive behaviors were observed (Table 2). To exclude the possibility that these workers obtained from the mixed colonies were deficient in the ability to recognize their nestmates, each worker was placed in a container with a worker from a different unparasitized colony. Both species showed clear aggressive behaviors towards the other species (Table 2). These results indicate that *P. lamellidens* and *C. obscuripes* inhabiting a mixed colony are able to recognize each other as nestmates.

Artificial social parasitism rearing experiment

To observe the initial stages of parasitism by *P. lamellidens* among *C. obscuripes* in a laboratory environment, we conducted rearing experiments. All newly mated *P. lamellidens* queens collected from the field were reared in the laboratory for more than 6 months. First, we allowed eight newly mated *P. lamellidens* queens to contact non-parasitized *C. obscuripes* workers in a well-ventilated tube individually. As in previous studies, the newly mated *P. lamellidens* queen straddled the host worker and rubbed their body with their legs (Kohriba 1963; Kubota 1974; Sakai 1990, 2000;

Table 2 The presence of aggressive behaviors in each colony ($n = 10$)

	<i>P. lam</i> (mixed colony) vs <i>C. obs</i> (mixed colony)	<i>P. lam</i> (normal colony) vs <i>C. obs</i> (normal colony)	<i>P. lam</i> (mixed colony) vs <i>C. obs</i> (normal colony)	<i>P. lam</i> (normal colony) vs <i>C. obs</i> (mixed colony)
Aggressive behavior				
Threat	0	9	4	1
Rush	0	1	5	6
Bite	0	0	0	3
Non-aggressive behavior				
Ignore	10	0	1*	0

All tests showed common tendencies in both species to initiate or not initiate aggressive behavior. We used a mixed colony (Colony A) and normal colonies without hosts or parasites (*P. lamellidens*, *C. obscuripes*) in the behavioral tests

P. lam *P. lamellidens*, *C. obs* *C. obscuripes*

*In only one out of ten tests, aggressive behavior was initiated on the second contact, not the first

Japanese Ant Database Group 2003). Although no quantitative records exist, some newly mated *P. lamellidens* queens died while contacting the host workers. As a result of this phenomenon that appears to host's attack, five out of eight newly mated *P. lamellidens* queens survived the contact process with *C. obscuripes* workers. After sufficient contact

with *C. obscuripes* workers, one of the five surviving newly mated the *P. lamellidens* queen met the non-parasitized *C. obscuripes* queen. The newly mated *P. lamellidens* queen immediately bit the *C. obscuripes* queen's neck (Fig. 2a). This biting behavior continued for 3 days, and eventually, the *C. obscuripes* queen's head was severed (Fig. 2b). This

Fig. 2 The early stage of the parasitism cycle. **a** The new *P. lamellidens* queen bites the neck of the *C. obscuripes* queen. **b** The *C. obscuripes* queen after being attacked for 3 days. **c** Eggs laid by the

parasite queen (*P. lamellidens*) in the host (*C. obscuripes*) colony. *P. lamellidens* eggs were laid after the killing of the *C. obscuripes* queen. **d** *P. lamellidens* eggs were gathered by *C. obscuripes* workers

killing behavior against the *C. obscuripes* queen was distinctly different from common bite attacks (as described in “Materials and methods”). The newly mated *P. lamellidens* queen started laying eggs four days after killing the *C. obscuripes* queen (Fig. 2c). More than ten eggs were confirmed within a week of the first oviposition. *P. lamellidens* eggs were collected and cared for by *C. obscuripes* workers (Fig. 2d). Between the killing of the host queen and oviposition, *C. obscuripes* workers did not show any aggressive behaviors towards the newly mated *P. lamellidens* queen.

Discussion

C. obscuripes has been suggested as a possible host for *P. lamellidens* (Sakai 1996; Furukawa et al. 2012), but there was no conclusive evidence. This paper is the first record of a mixed colony of *C. obscuripes* and *P. lamellidens* in the field, and *P. lamellidens* parasitism of *C. obscuripes* colonies was confirmed in a rearing experiment.

In the behavioral tests, *P. lamellidens* and *C. obscuripes* workers from the same mixed colony showed aggressive behavior towards workers from other colonies. Nevertheless, they recognized each other as nestmates, even though they were different species (Table 2). However, non-parasitized *C. obscuripes* exhibited distinct aggressive behaviors towards *P. lamellidens*. These results suggest that both species inhabit the mixed colony as nestmates. It is thought that a newly mated *P. lamellidens* queen will kill the host queen in the early stages of social parasitism according to the previous knowledge of *C. japonicus* as its host (Japanese Ant Database Group 2003). We carried out rearing experiments to verify that *C. obscuripes* is affected by social parasitism. In our rearing experiment, we observed that the *P. lamellidens* queens started laying eggs after killing the host queen (Fig. 2). Thus, we anesthetized the *C. obscuripes* queen before contacting the newly mated *P. lamellidens* queen in the rearing experiment. However, since the newly mated *P. lamellidens* queen demonstrated the same killing process toward *C. japonicus*, as shown only in the case of a *C. obscuripes* queen in previous studies (Japanese Ant Database Group 2003), we believe anesthesia does not affect the hypothesis verification of this experiment. Host-queen killing and oviposition in the host colony are common features of temporary socially parasitic and dulotic ants, not xenobiosis and inquiline (Hölldobler and Wilson 1990; Buschinger 2009; de la Mora et al. 2020). *P. lamellidens* persisted after the death of the host workers in mixed Colony A. Temporary coexistence with the host and sustaining the colony after the host's death are unique characteristics in temporary socially parasitic ants, not dulotic ants (with some exceptions, e.g., facultatively dulotic ants) (Hölldobler and Wilson 1990; Buschinger 2009; de la Mora et al. 2020).

Based on these observations, the two colonies obtained from the field provide evidence of *P. lamellidens* completing the temporary social parasitism cycle in a *C. obscuripes* colony, and we have clear evidence that *C. obscuripes* is a natural host for *P. lamellidens*. The above experiments and observations verified that *P. lamellidens* is not an obligatorily dulotic ant, as they could persist after the death of the host workers. However, the possibility of facultative dulosis has not yet been rejected. None of the reports of raiding behavior in the field or a few records of the mixed colony with its hosts suggests that *P. lamellidens* is an unlikely facultatively dulotic ant; thus, further verification is needed.

The balance of worker numbers in the two mixed colonies was different (Table 1). We speculate that this reflects the difference in the time that has elapsed since the host queen was killed. Probably, Colony A had been around longer than Colony B since the killing of the host queen; therefore, it had fewer workers of *C. obscuripes* than *P. lamellidens*. The left and right mid- and hindlegs of the *P. lamellidens* queen in Colony A were all severed at their bases (Fig. 1b). In past fieldwork (unpublished) in another region [Nirasaki City, Yamanashi Prefecture (N 35°42'49", E 138°29'12")], we observed an injured *P. lamellidens* queen from a free-living *P. lamellidens* colony. After the nuptial flight, a newly mated *P. lamellidens* queen invades a host colony to establish a new colony, but she is often attacked by some host workers at first contact, as observed in rearing experiments in previous studies (Kohriba 1963; Sakai 1996, 2000). In this rearing experiment, a newly mated *P. lamellidens* queen, probably killed by an attack from its host workers, has been confirmed. We believe that the *P. lamellidens* queen of Colony A succeeded in usurping the host colony before being killed by attack by host workers in the early stages of social parasitism.

C. obscuripes workers, members of the collected mixed colony, and those whose queen was killed in the rearing experiment did not show aggressive behaviors towards *P. lamellidens*, and both species coexisted as nestmates. This phenomenon suggests that *P. lamellidens* may have some involvement in nestmate discrimination by *C. obscuripes*. It is known that some socially parasitic ants alter host-like cuticular hydrocarbon (CHC) profiles or express almost no CHCs, which promotes coexistence with their host (Dettner and Liepert 1994; Johnson et al. 2001; Lenoir et al. 2001; Akino 2008; de la Mora et al. 2020). *P. lamellidens* queens and workers in mixed colonies may utilize similar strategies. The behavior of straddling host workers was observed in *P. lamellidens* queens. As a previous study speculated (Kohriba 1963), *P. lamellidens* queens may disguise their CHC profiles by performing this behavior, enabling invasion of the host colony.

The host *Diacamma* sp. and *Rhytidoponera* sp. workers (Formicidae, Ponerinae) of *Polyrhachis lama* and *Polyrhachis loweryi* do not rear parasites' brood (Maschwitz et al.

2003; Witte et al. 2009). It is speculated that the reason for the lack of brood care by the host is that the parasite/host is distantly related at the subfamily level, and there are discrepancies in larval feeding methods (Formicinae larvae receive liquid nourishment that is preprocessed by workers, whereas Ponerinae larvae are provided unmodified prey particles by workers); thus, the rearing style of the host species is not compatible with the parasite brood (Witte et al. 2009). Conversely, in the case of *P. lamellidens*, the hosts *C. japonicus* and *C. obscuripes* were observed rearing *P. lamellidens* brood (Fig. 2c, d; Kohriba 1963). *P. lamellidens* and its host ants are relatively closely related, and although they differ at the genus level, they both belong to the subfamily Formicinae (Brady et al. 2006) and probably share a common feeding method. The differences in the presence or absence of brood care by the host, even among socially parasitic species in the genus *Polyrhachis*, may be related to common brood feeding methods between parasites and hosts, as described above.

The nesting styles of *P. lamellidens* and its host differ. Initial nesting sites of *P. lamellidens* (*P. lamellidens* changes its nesting site at the end of the social parasitism cycle) are tree hollows, while *C. japonicus* nests in soil and *C. obscuripes* nests in decaying wood (Japanese Ant Database Group 2003). Thus, *P. lamellidens* is relatively unusual as a socially parasitic ant. The exact explanation for why *P. lamellidens* utilizes *Camponotus* as its host is unclear, but it is likely that *P. lamellidens* has high adaptability to various environments that harbor *Camponotus*.

Morphological similarities (e.g., body size) between socially parasitic ants and their hosts has been reported; one explanation for this phenomenon is that a socially parasitic ant has to adapt to the host's colony environment (e.g., tunnel size) (Fischer et al. 2020). This phenomenon is probably also true for *P. lamellidens* and its hosts. The workers of ants in the subgenus *Camponotus* (*C. japonicus* and *C. obscuripes* belong to this subgenus) are 7–12 mm long, which is similar to or longer than *P. lamellidens* (7–9 mm for workers, 10 mm for the queen) (Japanese Ant Database Group 2003; Kohout 2014). Conversely, workers of the subgenera *Myrmamblys* and *Myrmentoma* (belonging to the genus *Camponotus*), which are not hosts of *P. lamellidens*, are less than 5 mm in total body length (Japanese Ant Database Group 2003).

This study revealed that *P. lamellidens* utilizes multiple ant species as a host (*C. japonicus* and *C. obscuripes*). In addition to *P. lamellidens*, socially parasitic ants that parasitize numerous hosts (e.g., *Lasius umbratus*) are known regardless of parasitism style. The common feature of these species is that the socially parasitic ants and their hosts tend to be closely related species, and the hosts belong to a specific taxonomic group at the subfamily level (Emery 1909; Hölldobler and Wilson 1990; Japanese Ant Database Group 2003; Huang and Dornhaus 2008; de la Mora et al. 2020).

The reason why the hosts are closely related to the parasitic ants and are restricted to few taxa is thought to be because it is easy for the parasitic ants to disguise their CHC profile (Blatrix and Sermage 2005; Huang and Dornhaus 2008; de la Mora et al. 2020); the same is probably true for *P. lamellidens*. Moreover, as mentioned, another factor in establishing social parasitism is that the host is closely related to the parasite species and can take care of the parasite's brood.

Our findings provide evidence, for the first time, of temporary social parasitism involving *P. lamellidens* and *C. obscuripes*. The methodology presented in this investigation will contribute to, and accelerate, the study of social insects.

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Author contributions HI, YK, and NK designed the project. HI performed all behavioral tests and rearing experiments. YK and NK performed molecular species identification. HI and YK collected ant specimens. The first draft of the manuscript was written by HI, and all the authors commented on previous versions of the manuscript. NK, MT, and KA managed the experimental environment. All the authors read and approved the final manuscript.

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Data availability The sequence data of cytochrome oxidase subunits I and II (*COI/II*) of *P. lamellidens* and *C. obscuripes* will be made available in Figshare (<https://doi.org/10.6084/m9.figshare.13711843>).

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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