Colony composition, arthropod egg predation, and antennal structure of the ant *Discothyrea sauteri* (Hymenoptera: Formicidae)

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ABSTRACT. In an evergreen, broadleaf forest in central Japan, we collected 21 colonies of the cryptic ant, *Discothyrea sauteri*, which nests in small cavities in the soil (*ca.* 100 mm depth), rotting logs, and dead branches lying on the forest floor. The largest colony contained 132 workers, and one third of the collected queenright colonies were functionally polygynous, as revealed by dissection of the queens. Ant nests collected between April and November contained many globular, arthropod eggs. These eggs did not hatch in the laboratory, but we concluded that they are spider eggs, because some colonies nested in woven silk egg-sac structures and many others nested in cavities lined with silk filaments. Moreover, SEM observations showed strong similarities of these silk filaments to those of spider egg sacs. The extremely enlarged terminal segment of the antennal funiculus, the antennal club, which characterizes *Discothyrea*, has a group of short, hollow sensilla near its end. As these sensilla firmly contact the surface of globular prey eggs when ants hold them, the antennal shape and the distribution of these sensilla may be related to lifting a prey egg using the antennae.

INTRODUCTION

The ant subfamily Proceratiinae Emery, 1895 comprises three genera, *Discothyrea* Roger, 1863, *Probolomyrmex* Mayr, 1901, and *Proceratium* Roger, 1863 (Bolton 2020). Brown (1957, 1958) noted that *Discothyrea* and *Proceratium* are specialized predators of arthropod eggs, especially spider eggs, one of the most remarkable specializations in ant predation. Specialization for spider egg predation by *Discothyrea* was first documented in the field for an African species,

D. oculata Emery, 1901 (Dejean & Dejean 1998; Dejean et al. 1999), whereas the Japanese species, *P. itoi* (Forel, 1918), preys on eggs of various arthropod taxa, including eggs of Hemiptera, Chilopoda, and Opiliones (Masuko 2019b). On the other hand, *Probolomyrmex* is a specialized predator of polyxenid millipedes (Ito 1998).

The genera *Discothyrea* and *Proceratium* are closely related (Ward & Fisher 2016) and are diagnosed as having "abdominal tergite IV enlarged and strongly vaulted, tergite hypertrophied with respect to sternite IV, which is re-

duced" (Bolton 2003, p.49). This gives them the peculiar body form with an anteriorly oriented abdomen, which is related to different behaviors such as predation, nest defense, and thanatosis (see below). Worldwide, 49 and 86 extant species have been described for *Discothyrea* and *Proceratium*, respectively (Bolton 2020). Yet, because these ants are cryptic and collection of entire colonies has been infrequent, detailed data on their behavior and ecology are limited to a few species (Dejean & Dejean 1998; Dejean et al. 1999; Masuko 2019b).

In the present study, 21 colonies of the Japanese species, *Discothyrea sauteri* Forel, 1912, were collected from a single locality in Kanagawa Prefecture, in central Japan, permitting us to obtain detailed information on colony population, social structure, and colony founding, based on field data and laboratory rearing. Also, morphological traits likely related to egg predation were examined using scanning electron microscopy (SEM) and histological sections.

MATERIALS AND METHODS

Study area and ant sampling

Ants were collected in a small forest (*ca.* 35 ha) located at Cape Manazuru (35.144° N, 139.154° E), Kanagawa Prefecture, from 1978 to 1984. The natural vegetation of this area is evergreen broadleaf forest dominated by *Castanopsis sieboldii* (Makino) Hatus. ex T. Yamaz. et Mashiba (Miyawaki et al. 1969). In this forest, most *D. sauteri* nests were excavated close to the soil surface (*ca*. 100 mm depth), but some were sheltered in dead branches or rotting logs lying on the forest floor (see below). As there was no effective way to locate nests from the surface, nests were encountered haphazardly by digging in soil or by dismantling dead branches and rotting logs. When a nest was found, as many ants as possible were collected using an aspirator, and taken to the laboratory, where they were sorted under a stereomicroscope. In addition to the ants and their brood, eggs of unidentified arthropods, likely stored as prey, were frequently noted and carefully collected.

After censusing in the laboratory, ants were then preserved in 80% ethanol or Kahle's solution (Barbosa 1974) or reared, depending on the intended purpose. Arthropod eggs gathered in the field were also preserved in these fixatives, or used as food for ants reared in the laboratory. Some dealate gynes (queens) and workers were dissected to examine insemination, ovary development, and the number of ovarioles.

Ant rearing

For behavioral observations in the laboratory, seven colonies were reared in polystyrene containers covered with a transparent glass and kept at 21–26°C. Container bottoms were covered with gypsum mixed with activated carbon powder. When a colony population (ants plus arthropod eggs) was small, a brood chamber was excavated in the center of the plaster floor that the ants selected for nesting. For large colonies, ants and prey eggs were not confined in such a chamber, but were placed directly in a terrarium. In addition to arthropod eggs recovered from ant field nests, eggs, or hatchling spiders and centipedes were given as prey to these laboratory colonies (see below for their taxonomy).

SEM and histology

SEM (Keyence VE-8800) and histological studies were conducted on 20 workers obtained from colonies collected from 1981 to 1984. These were first preserved in Kahle's solution and then transferred to 80% ethanol. SEM specimens were air-dried, mounted on stubs, and coated with gold-palladium. The same procedure was used for silk-like filaments found inside *D. sauteri* nests. For histological examination of worker antennae, preserved specimens were further dehydrated in an ethanol series with propylene oxide, before embedding in Agar Low Viscosity Resin (Agar Scientific). Sections (0.5–0.8 μm) were cut using a diamond knife, and then stained in a 1% methylene blue / 1% borax solution. Sections were examined using a compound microscope (Olympus Vanox) and morphological details were imaged with a digital camera (Shimadzu Moticam-2500).
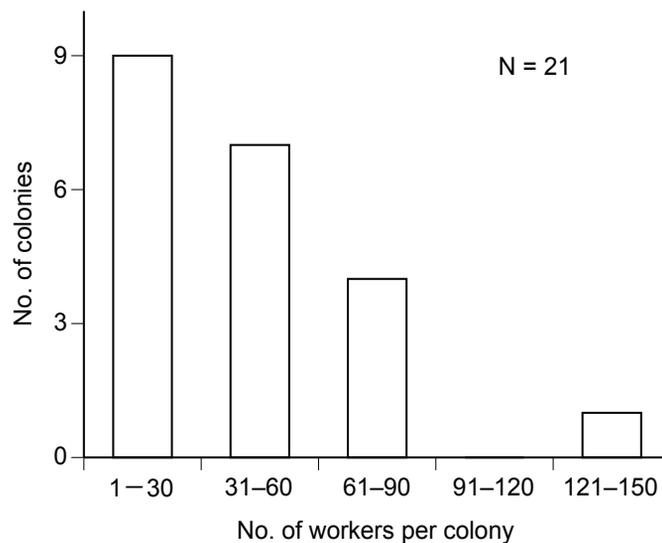
RESULTS

Colony composition

Among the 21 *D. sauteri* colonies collected, four were judged to be incomplete samples (Table 1). The number of workers per colony (including the incomplete samples) ranged from 1–132 and the median was 34 (n = 21; mean ± SD = 40.4 ± 32.1) (Fig. 1); thus, *D. sauteri* colonies are rather small. Only one queenless colony (coded 81-285) was noted among 17 complete or nearly complete samples. Among the other 16 colonies, 11 were monogynous and 5 were polygynous (numbers of queens ranged from 1–14). Seventeen queens from 3 monogynous and 3 polygynous colonies were dissected (Table 2).

Dissections of 16 queens (one dissection failed) revealed that all were inseminated and had corpora lutea (yellow bodies) in their ovaries. Corpora lutea are remnants of follicle cells and their presence indicates that these queens had laid eggs (Torre-Bueno 1989). Moreover, the degree of ovary development, based on ovariole length, was the same in two queens of colony 83–49 and among ten queens of colony 82–60 (Fig. 2a, b). These observations confirm that polygyny in *D. sauteri* is functional, since multiple queens in the same nest were equally fertile. A statistically significant positive correlation was obtained between queen and worker numbers per colony (Fig. 3), which is consistent with functional polygyny. When two polygynous colonies, 81-77 with 14 queens and 82-245 with 2 queens, were reared in the laboratory, there was no evidence of aggressive interactions among the queens. In all dissected queens, with a single exception in colony 82–60, the number of ovarioles per ovary was three (Table 2), and the same was true for two workers that were dissected.

Colony foundation

In functionally polygynous ants, colony budding, in which one or more inseminated queens accompanied by workers disperse from a natal nest, is common (Hölldobler & Wilson 1990). Due to small sample sizes, the nature of *D. sauteri* colony foundation remains unconfirmed. In the present study, no solitary foundress was collected, while an incipient colony (82–290) was collected in early August, composed of one queen, one worker, and four eggs (Table 1). Larvae and pupae were absent. Since pupal gynes and males were observed in *D. sauteri* nests collected in mid or late July (Table 1), this small colony might be a fraction just split from a mother colony, containing a newly inseminated queen. The short ovaries of this queen indicated that her egg-laying had

Fig. 1. Frequency distribution of the colony worker population in *D. sauteri*, based on 21 collections made at Manazuru between 1978 and 1984.

just started (Fig. 2c). However, it is also possible that the queen started her colony independently during the previous year and had produced only one worker in this second year. Another small colony (82-366), collected in mid September, was also monogynous, having only seven younger larvae and four workers, of which three were callows. One of them was totally pale, suggesting that it had eclosed in that nest. Here, too, the queen's ovaries were short, but somewhat longer than those of queen 82–290 (Fig. 2c, d), suggesting that this colony might have started during the previous year. Because only two such inicipient colonies were collected in the present study, it remains uncertain whether independent foundation, colony fission, or both occur in *D. sauteri*.

Nesting habits and prey eggs

Among the 21 colonies collected, 14 nested directly in the soil, 6 nested in dead branches or rotting logs, and one in a soil-litter sample carried to the laboratory, so that its nesting site was uncertain (likely in the soil; colony 79–63; Table S1).

Table 1. Census of 21 colonies of *D. sauteri* collected in Manazuru, Kanagawa Prefecture, Japan. Colony collections (samples) were categorized as complete (C), nearly complete (N), or incomplete (I). No record exists for sex and caste of two pupae in 79-63; however, a male pupa and five worker pupae were confirmed later in laboratory rearing.

Colony code	Date of collection	Sample category	Queen	Worker	Egg	Larva	Prepupa	Pupa
78-45	5 Feb. 1978	C	1	84		41		
83-49	6 Mar. 1983	C	2	64		49		
79-36	25 Mar. 1979	C	1	40		14		
82-60	26 Mar. 1982	N	10	76		ca. 150		
78-112	9 Apr. 1978	C	1	46		31		
84-7	13 Apr. 1984	C	1	73		ca. 60		
81-77	6 May 1981	C	14	132				
81-109	27 May 1981	C	1	15		37		
79-63	24 June 1979	C	1	16	5	2	5	2
78-141	15 July 1978	I		33	ca. 25			1 gyne, 1 male
81-190	21 July 1981	N	1	13	24	10	22	7 workers, 9 males
82-245	23 July 1982	C	2	28	ca. 30	37	32	18 workers, 5 males
81-203	29 July 1981	I		3	ca. 10	8	3	13 workers
82-290	10 Aug. 1982	C	1	1	4			
82-366	19 Sept. 1982	C	1	4		7		
81-285	23 Sept. 1981	N		40	14	38		3 workers
81-347	25 Oct. 1981	C	1	50		ca. 70		
81-397	12 Nov. 1981	C	2	17		68		
82-419	25 Nov. 1982	C	1	51		57		
82-423	25 Nov. 1982	I		34		3		
78-238	9 Dec. 1978	I		28		ca. 40		

Fig. 2. Microscopy of whole ovaries of *D. sauteri* queens. All preserved specimens. (a, b) Ovaries of two of the 10 queens of colony 82-60. The ten queens were freshly dissected the day after field collection. All were inseminated and had corpora lutea, and their ovariole lengths were similar. (c) Ovary of the queen of an incipient colony 82-290 that contained only one worker. (d) Ovary of the queen of an incipient colony 82-366 that contained four workers. Scale bar 0.5 mm, common for (a) to (d).

We noted the presence of globular eggs in these ant nests (Fig. 4a, b, arrows; Fig. 5c). These were obviously different in shape and color from those of *D. sauteri*, which are oblong and grayish in color (Fig. 7b, arrowhead). Although no hatchlings emerged in the laboratory from these exogenous eggs, it is likely that they were spider eggs. Indeed, an entire spider egg-sac-like structure was observed in the field when colony 78-141 was discovered inside a dead bamboo internode (Fig. 5a), which contained approximately 250 globular, cream-colored eggs (Table S1; Fig. 5c). This structure also enclosed 33 workers and about 25 eggs of *D. sauteri* (Table 1), and those ant eggs were completely inter-mixed with the mass of prey eggs (Fig. 5b). This habit of jumbling prey eggs and the ant imma-ture brood, including larvae and pupae as well as eggs, was consistently observed in laboratory colonies (Fig. 7b).

The size of prey eggs, which ranged in diameter from 0.42 to 0.82 mm, varied slightly from one nest to the next (Table S1), suggesting that these eggs have different origins, perhaps pertaining to different spider species. It should be further noted that prey eggs were found in all colonies collected from mid-April through mid-November (Table S1). The few eggs that were discovered on March 6 in colony 83-49 were most likely left over from the previous season.

Table 2. Reproductive data from queens in monogynous and polygynous colonies of *D. sauteri*.

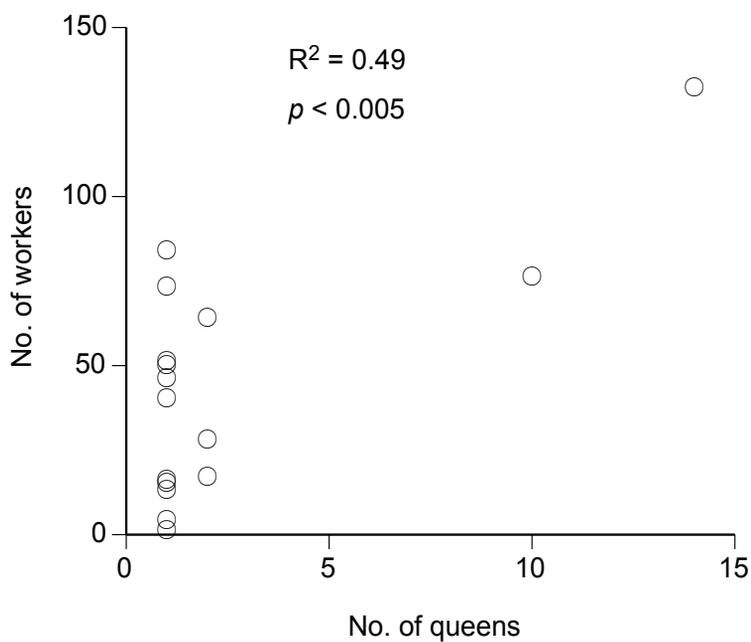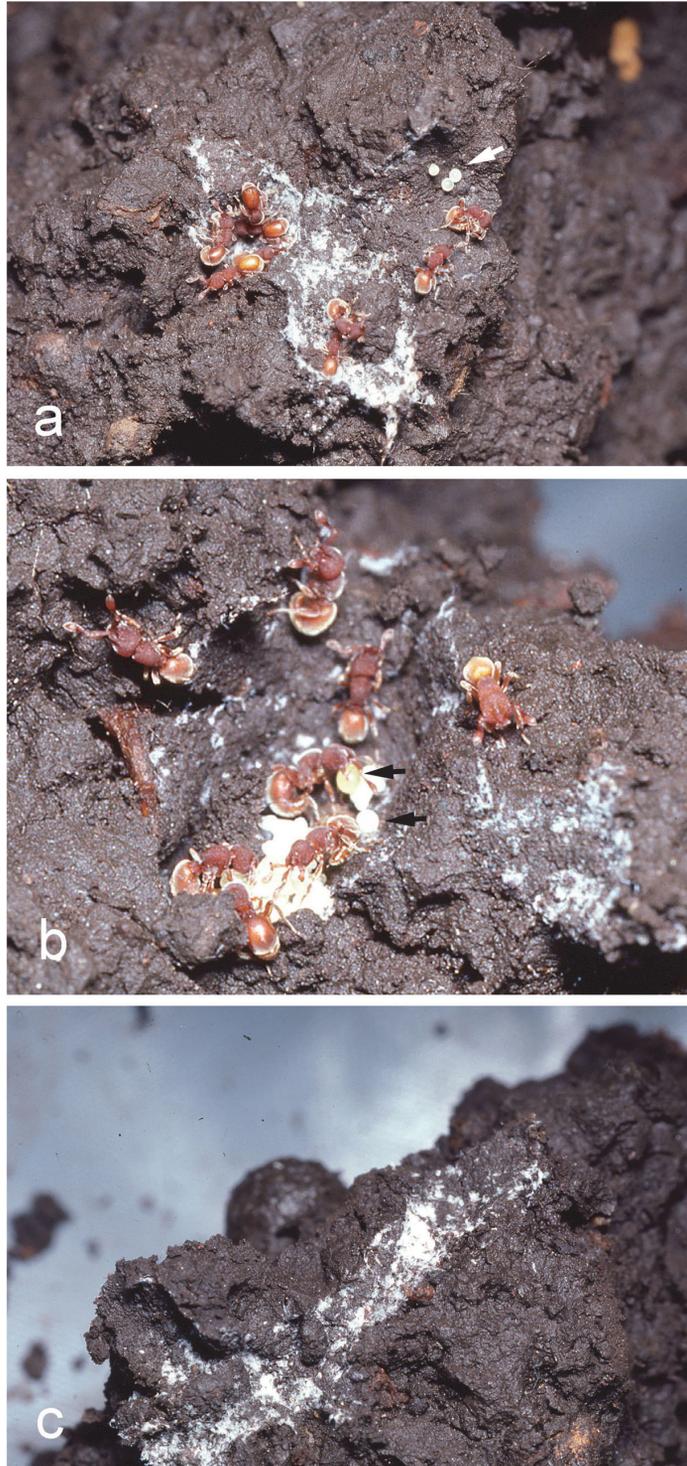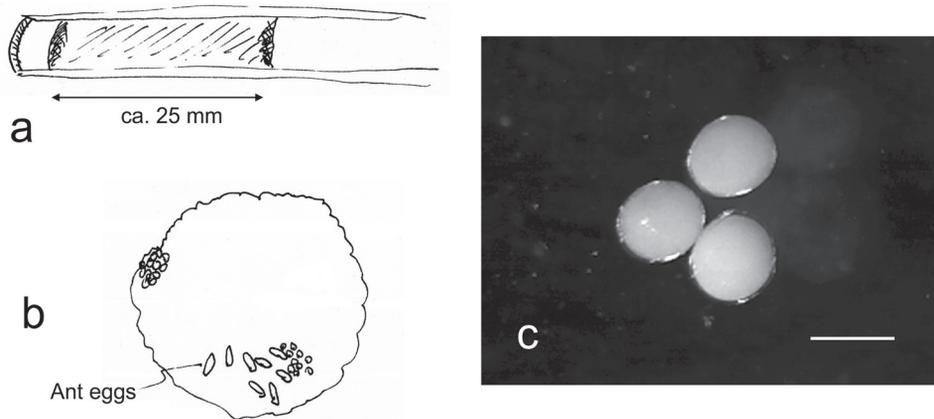| Colony code	Date of collection	No. of workers	No. of queens	Queen	Insemination	Corpora lutea	No. of ovarioles
83-49	6 Mar. 1983	64	2	a	Yes	Present	3+3
				b	Yes	Present	3+3
82-60	26 Mar. 1982	76	10	a	Yes	Present	3+3
				b	Yes	Present	3+3
				c	Yes	Present	3+3
				d	Yes	Present	3+3
				e	Yes	Present	3+3
				f	Yes	Present	3+3
				g	Yes	Present	3+3
				h	Yes	Present	3+3
				i	Yes	Present	3+4
				j	Yes	Present	3+3
82-245	23 July 1982	28	2	a	?	?	?
				b	Yes	Present	3+3
82-290	10 Aug. 1982	1	1		Yes	Present	3+3
82-366	19 Sept. 1982	4	1		Yes	Present	3+3
82-419	25 Nov. 1982	51	1		Yes	Present	3+3

Fig. 3. Relationship between queen number and colony worker population, based on 16 queenright, complete, or nearly complete samples of *D. sauteri*.

Fig. 4. Inside of a nest of a *D. sauteri* colony (81-347) in the soil. (a-c) White fibrous material covers the inner surface of nest cavities. Arrows in (a) and (b) indicate prey eggs. Worker ants are about 2 mm long.

Fig. 5. (a) Field sketch of a split-half of a dead bamboo stem in which colony 78-141 was found. Near one end of the stem, the ants existed inside a fibrous structure that was about 25 mm long and seemed to be an egg sac cocoon. (b) Field sketch of a mass of about 250 globular prey eggs mingled with elongate ant eggs. (c) Prey eggs found there. Preserved specimens. Scale bar 0.5 mm.
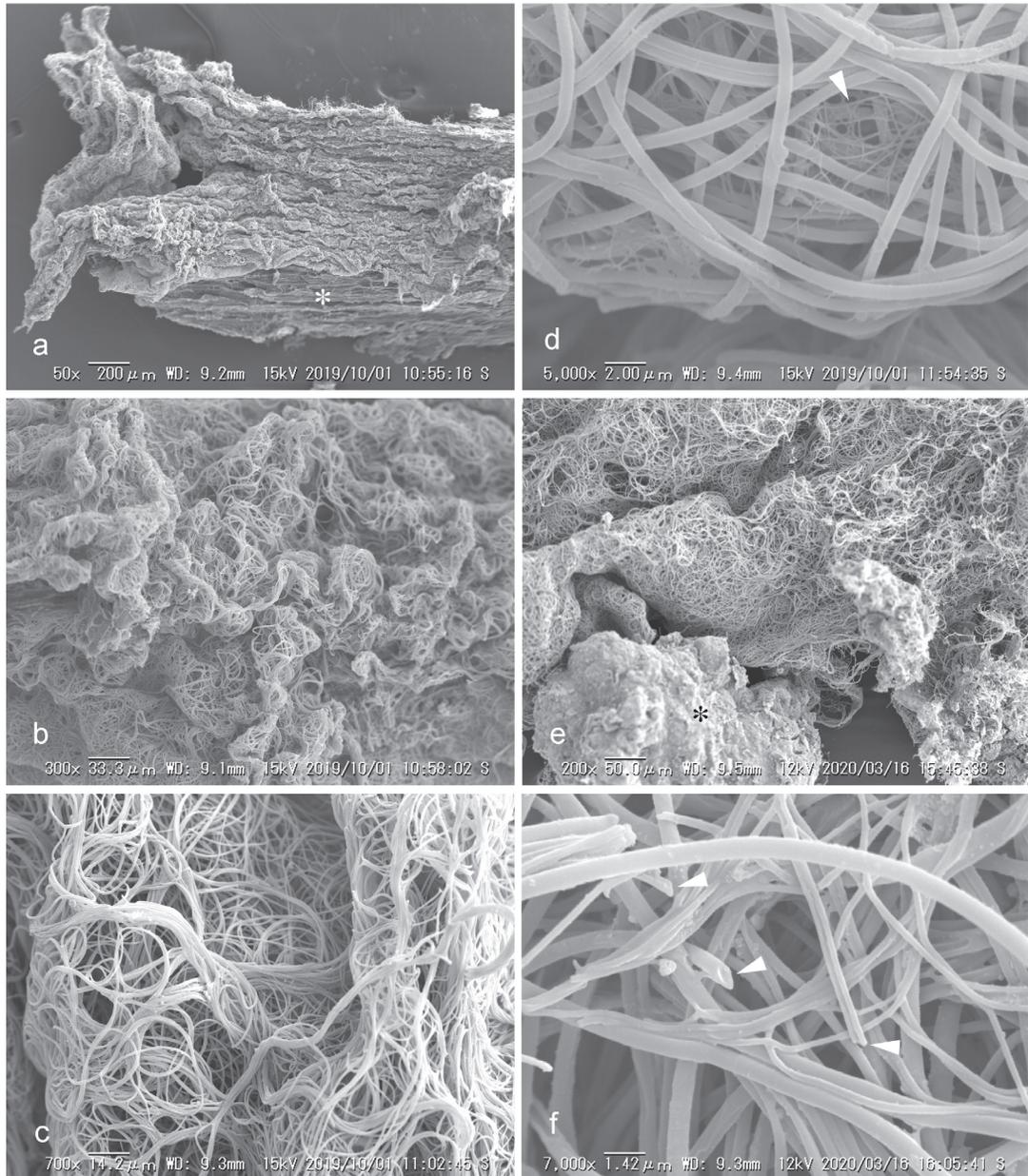
Silk filaments in *D. sauteri* nests

In addition to the whole egg-sac-like structure in which a *D. sauteri* colony sheltered (see above), the inner walls of 12 ant nests were almost entirely covered with silk (Fig. 4a–c, Table S1), the origin of which remains to be determined. However, SEM examination of silk structure revealed common characteristics: the filaments formed bundles (Fig. 6c, d, f) and included multiple fiber types of different diameters (Fig. 6d, arrowhead). As the bundling indicates that these fibers must have been spun at the same time, the most plausible source of such fibers are the tubuliform and aciniform silk glands of spiders (Hayashi, personal communication) because an individual spider has multiple sets of these glands and is able to simultaneously produce multiple fibers. Also, tubuliform and aciniform glands are known to produce egg sac silks (Foelix 2011). Compared to tubuliform gland silks, aciniform glands contribute fibers of smaller diameter (Moon 2003; Correa-Garhwal et al. 2019). Therefore, as filaments from *D. sauteri* nests showed both of these characteristics, they are most likely silks of spider egg sacs (Hayashi, personal communication). Moreover, among fibers lining the subterranean cavities of *D. sauteri* nests, many had cut ends, which were frequently straight (Fig. 6f, arrowheads). Apparently, the ants severed the filaments with their mandibles (see below).
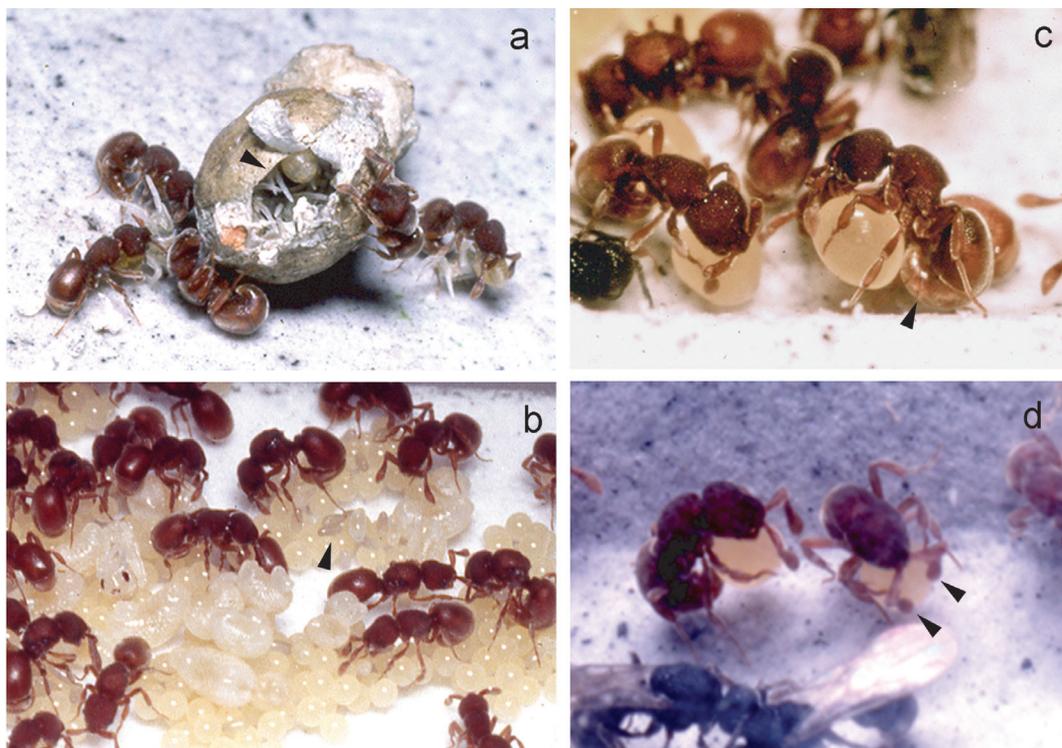
Predatory behavior in the laboratory

Seven queenright colonies were reared and observed in the laboratory. Durations of laboratory maintenance varied, but the longest was only six months because it was difficult to continuously supply ants with spider eggs of appropriate size.

Aside from the unidentified arthropod eggs collected from ant field nests, the following organisms that were supplied as prey for laboratory colonies were retrieved by the workers and consumed by their larvae: eggs and/or hatchlings of the spiders *Nephila clavata* L. Koch, 1878 (Araneidae)*, Parasteatoda tepidariorum* (L. Koch, 1841) (Theridiidae)*,* and *Pardosa astrigera* L. Koch, 1878 (Lycosidae)*,* and eggs of centipedes, undetermined species *of Scolopocryptops* Newport, 1844 (Scolopendromorpha) and Geophilomorpha (genus undetermined). On the other hand, *D. sauteri* colonies ignored eggs of Polydesmida (genus undetermined) and the burrower bug, *Macroscytus japonensis* (Scott, 1874) (Hemiptera) that were collected from field nests of the ant, *Proceratium itoi* (see Masuko 2019b). Honey solutions were licked by workers, but modestly, and afterward, no stomodeal trophallaxis was observed among workers during the study period.
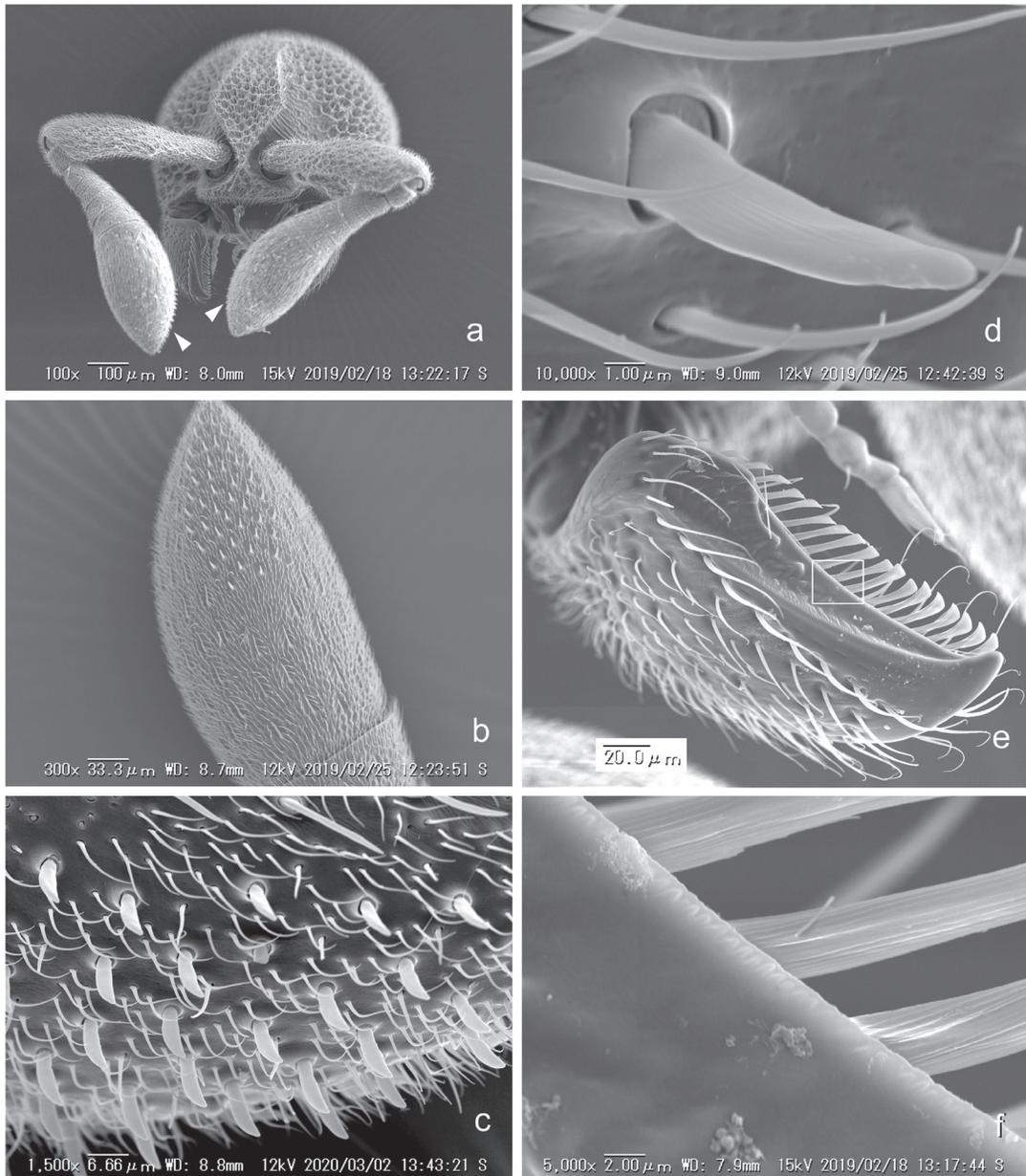
Fig. 6. Silk filaments found in nests of two *D. sauteri* colonies collected at Manazuru. (a) Sheet of an egg sac lining the cavity of a dead branch where *D. sauteri* colony 82-419 was found. The uncovered surface of the dead branch is indicated by an asterisk. (b–d) Enlarged images of the surface of a silk filament sheet shown in (a). Filaments form bundles, and in addition to these thick filaments, there is another type of filament, much smaller in diameter (d, arrowhead). (e) Sheet of silk filaments lining the cavity of a *D. sauteri* nest in the soil (colony 84-7). The soil surface is indicated by an asterisk. (f) Enlarged image of the filaments shown in (e). There are two types of filaments of different diameters, and some filaments have straight cut ends (arrowheads).
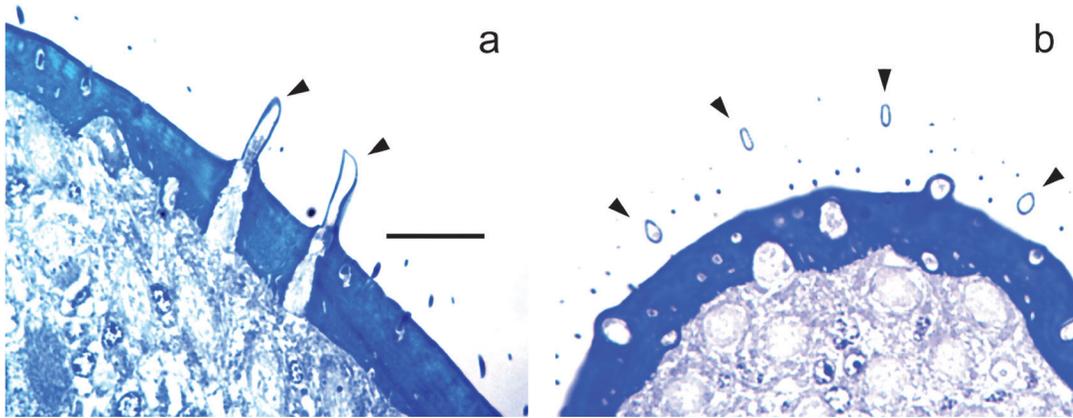
Fig. 7. Predation on spider eggs in laboratory colonies of *D. sauteri*. (a) An egg sac of the spider, *Pardosa astrigera*, given intact to the ants. It was then cut open and the spiderings inside were taken out by *D. sauteri* workers. Cut ends of the opening are smooth (arrowhead). (b) Eggs of the spider, *Parasteatoda tepidariorum*, (spherical and yellowish), mixed with larvae, pupae, and eggs (arrowhead) of *D. sauteri*. Several *D. sauteri* queens are seen on these mixed heaps. (c) A *D. sauteri* worker holding and licking an egg of *P. astrigera* that is supported by its antennae and abdominal tip. Note that the two antennae and the abdomen form a stable "tripodal" support for the egg. Arrowhead indicates the posterior edge of the 4th abdominal tergite. (d) Two workers of *D. sauteri* holding and licking eggs of *P. astrigera.* Arrowheads point the antennal funiculi applied to the egg surface.

When an intact egg sac from the spider, *P. astrigera*, was placed in the terrarium of a laboratory nest, the worker that discovered it immediately started antennation on its surface. Soon afterward the ant returned to the brood chamber, wherein it walked around in excited, rapid gait, which was not observed during its walk to the chamber. Concurrently, the ant that discovered the egg sac frantically palpated other workers with its antennae or gently bit them with its mandibles. After several repetitions of this behavior, the worker again returned to the egg sac. After being stimulated, other workers dispersed into the terrarium (tandem running and trail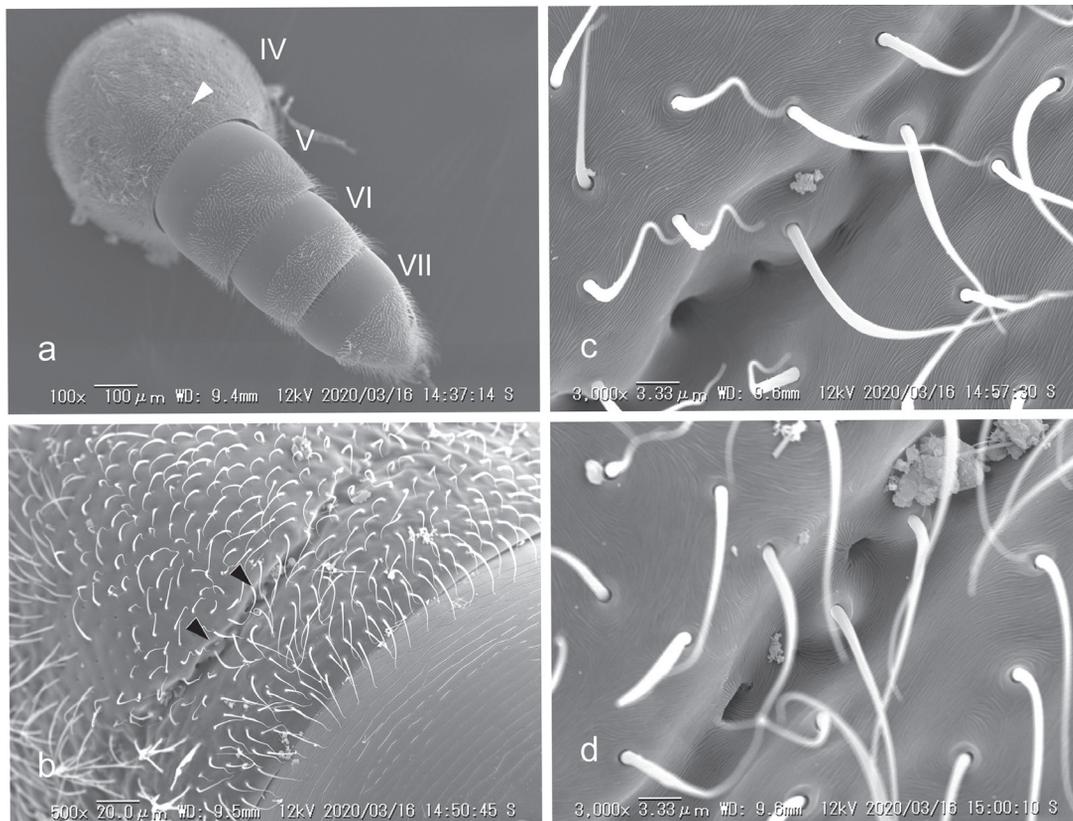 following were not seen) and a hole was made in the egg sac at its "equator" during the subsequent 30 min. Egg sacs of *P. astrigera* are lenticular in shape, being composed of two sheets comprising the upper and lower sides. The seam occurs at the "equator" where the two sheets meet (Fujii 1978). The opening was made at that point because it seemed structurally weak. From this opening, a spidering was removed by a *D. sauteri* worker (Fig. 7a). Its retrieval into the ant brood chamber occurred 30 min later. The cut ends on the egg sac surface appeared straight and smooth (Fig. 7a, arrowhead), suggesting that *D. sauteri* workers used their mandibles, which have edentate masticatory margins, like scissors (Fig. 8e, f).

Fig. 8. (a) Frontal view of the head of a *D. sauteri* worker. From this angle, a group of specialized, dagger-like sensilla are located on the medio-posterior surface near the distal end of each antennal funiculus (arrowheads). (b) The distal segment of the right funiculus of a *D. sauteri* worker. (c) Lateral view of the dagger-shaped sensilla on the right funiculus. (d) Enlarged image of a dagger-shaped sensilla. No porous structures on the surface. (e) Frontal view of the right mandible of a *D. sauteri* worker. (f) Straight edge of the inner margin of the mandible. Enlarged from the location boxed in (e).

Fig. 9. A longitudinal section (a) and a cross-section (b) of the dagger-shaped sensilla (arrowheads) on antennal funiculi of a *D. sauteri* worker. Scale bar 15 μm, common for (a) and (b).

Fig. 10. (a) Posterior view of the 4th to 7th abdominal segments (marked as IV–VII) of a *D. sauteri* worker. The arrowhead points to the transverse groove on the tergite of the 4th abdominal segment. (b) Transverse groove on the 4th abdominal tergite. Arrowheads point to pores enlarged in (c) and (d).

After retrieval, intriguingly, prey eggs and the ant immature brood were mixed together, and adults, especially the queens, remained upon such piles (Fig. 7b). Also, in the field, this placement of ant immatures together with prey eggs was usually observed. Worker behavior regarding prey eggs is of further interest. In the laboratory, *D. sauteri* workers frequently cleaned prey eggs (Fig. 7c, d). During this activity, an ant raised the anterior half of its body, standing on its middle and hind legs, while holding an egg at three points. The posterior end of the ant's abdomen, curved anteriorly, supported the egg from behind, while its two thick, club-shaped antennal funiculi held it from the front (Fig. 7c, d, arrowheads). By this means, the prey egg was lifted off the substrate. The ant did not use its legs to support the egg, but instead it used its forelegs to continuously roll the egg while licking its surface with its lower mouthparts (Fig. 7c). After being offered as prey, stored spider eggs changed in appearance, becoming filled with droplets, which indicates changes in embryos inside eggs (see Discussion).

Thanatosis and adult transport

Being disturbed with forceps in the laboratory, *D. sauteri* workers immediately feign death, or perform thanatosis, by curling up in an immobile, compact shape. Also, when they confront other dead insects, they turn round, directing their gasters to the objects motionlessly.

In *D. oculata*, recruitment of nestmates to spider eggs using a scent trail was observed (Dejean et al. 1999), while in *D. sauteri*, recruitment behavior was observed, but scent marking could not be confirmed. On the other hand, in the laboratory, adult transport was observed in *D. sauteri*, in which a worker (transporter) gaped widely and grasped the sides of the head of another worker (transportee) with its mandibles while the body of transportee curled over the head of transporter. This body placement by transporter and transportee seems very similar to that reported in *Rhytidoponera metalica* (Smith, 1858) (Möglich & Hölldobler 1974).

SEM and histological studies of worker antennae and abdomens

Apparently to facilitate egg handling, the apical segment of each antennal funiculus of a worker has a cluster of short, flat sensilla near its distal end (Fig. 8a, b). These sensilla are located medio-posteriorly (Fig. 8a, arrowheads), and firmly contact a prey egg when the ant holds and licks it (Fig. 7c, d). The sensilla are, more precisely, dagger-shaped (Fig. 8c, d), and their number on each side was approximately 50 (46, 47, 50, and 51; n = 4 workers). They are, however, absent from all other areas of the antennal surface (both funiculus and scape). Closer SEM examination at high magnification showed a complete absence of porous structures on the surface (Fig. 8d) and histological sections revealed that they are hollow inside (Fig. 9a, b). Still, they may also function as mechanoreceptors, though innervation was not evident in the sections.

In contrast to the antennae, no specialized structures such as setae, nor specialized surface structures of the integument, were observed on the dorsal area of the distal abdominal segments that contact a prey egg (Figs. 7c and 10a). However, the fourth abdominal segment, counting the *propodeum* as the first abdominal segment (Torre-Bueno 1989), has a transverse groove near the posterior edge of its tergite (Fig. 10a, b, arrowheads). In this groove, several pores align (Fig. 10b–d), and appear to be outlets of exocrine glands located beneath the integument. The presence of such exocrine glands in the vicinity of the posterior margin of the 4th abdominal tergite has already been suggested for ants of the genus *Proceratium* (Baroni Urbani & de Andrade 2003). Contents of these putative exocrine glands remain totally unknown. Histological sectioning of *D. sauteri* worker abdomens revealed that there are tissues inside, which connect to the surface pores, thus being putatively secretory (Masuko, unpublished observation).

DISCUSSION

There are quite a few studies reporting general predator-prey relationships between ants (predators) and spiders (prey) (e.g., Vieira & Höfer 1994; Halaj et al. 1997; Nelson et al. 2004); however, in the Formicidae, predatory specialization on either adult spiders or their eggs, is only known in *Discothyrea* (Hölldobler & Wilson 1990; Dejean & Dejean 1998; Dejean et al. 1999). Recently, Cushing (2012) summarized spider-ant associations, citing many examples of predation on ants by spiders, but none for ant predation on spiders. For ants, spider eggs are undoubtedly excellent food sources because they are weakly chorionated, and thereby easy to consume, and they occur in large numbers in a single egg sac. However, spider egg sacs are difficult to cut open, because their silken sheets are firm and tough, and have multiple layers (Gheysens et al. 2005; Foelix 2011), so that special "tools" are needed. For this purpose, the masticatory margins of the mandibles in *Discothyrea* are edentate and rather straight (Fig. 8e, f), which contrasts with the dentate mandibles of *Proceratium* (Brown 1958, p.250; Baroni Urbani & de Andrade 2003, Table Ia, d; but see Hita-Garcia et al. (2019) for a few species of *Discothyrea* having dentate mandibles). Moreover, this masticatory border is "lined with a very even, close rank of minute peg-like bristles" (Brown 1958, p.250) (Fig. 8e), which might serve as a comb to arrange, or thin out, silk filaments. Similar comb-like structures are found on the tibial spurs of the forelegs of *D. oculata* (Dejean et al. 1999).

How *Discothyrea* ants find egg sacs and retrieve them from the adult female spiders is completely unknown. *Discothyrea oculata* preys on spider eggs of the family Segestriidae (Dejean & Dejean 1998), which dwell in tubular webs constructed in crevices, within which they place their egg sacs (Preston-Mafham 2011). In the segestriid genus, *Ariadna*, females accompany egg masses and also young spiders for short periods after hatching (Beatty 1970, p. 437; Yamakawa & Kumada 1973). Therefore, in order to retrieve segestriid eggs, *Discothyrea* ants must confront the defenses of female spiders in their burrows. Adult *Discothyrea* have tough, thick in-teguments. Thanatosis, which had already been observed in other *Discothyrea* (Brown 1958, p.252; Katayama 2013), and the gaster-directing behavior might be used to deter spiders from attacking. In *Proceratium*, workers and queens have also been observed to turn around, using their vaulted gasters to block the entrance or passageway to a nest (Poldi 1963; Masuko 2019b).

The reason why nest cavities of *Discothyrea* are lined with exogenous silk remains unknown. In some cases, such silk linings may be inherited, if the ants simply adopt the shelter of a spider. However, transport of silk separated from egg sacs and its use in lining a glass tube were observed in *D. oculata* laboratory nests (Dejean et al. 1999); therefore, this elaboration must have certain advantages for nesting by *Discothyrea*. Beyond using silk as a lining, ants of the genus *Polyrhachis* Smith, 1857 use spider silk to construct arboreal nests (Collart 1932; Dwyer & Ebert 1994).

Finally, comparisons of *Discothyrea* and *Proceratium* might be meaningful. First, although quantitative data are only available for *Proceratium itoi*, members of this genus may be generalists for arthropod eggs (Masuko 2019b), whereas *Discothyrea* specialize in spider eggs. In the cladistic analysis by Baroni Urbani & de Andrade (2003, Table I), important morphological differences between these genera relate to the dentition of mandibles and shapes of antennal funiculi. Both of these, i.e., edentate mandibles and enlargement of the apical antennal segment in *Discothyrea*, are related to predation on spider eggs. Second, Katayama (2013) reported changes in appearance of spider eggs stored by *D. kamiteta* Kubota & Terayama, 1999. Exactly the same phenomenon occurs in *D. sauteri*. This change may explain why hatchlings have not been obtained from eggs stored in nests of these two *Discothyrea* species. In contrast, hatchlings did emerge from eggs stored in nests of *P. itoi* (Masuko 2019b). Third, nutrition of *Proceratium* queens depends totally on hemolymph of their own larval progeny (Masuko 1986, 2019a), whereas this behavior, termed larval hemolymph feeding, has not been observed in *Discothyrea*; however, other nutritional sources for *Discothyrea* queens remain unknown.

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