



Colony composition, phasic reproduction, and queen–worker dimorphism of an oriental non-army ant doryline *Cerapachys sulcinodis* species complex in northern Thailand

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Abstract

The ant subfamily Dorylinae consists of the true army ants and non-army ant genera. The biology of these non-army ants is important in discussing the evolution of the army ant adaptive syndrome. We report on the colony composition, morphological characteristics, and phasic reproduction of a non-army doryline ant *Cerapachys sulcinodis* species complex collected in northern Thailand. Colony size was up to 1850 workers, which is larger than that of other non-army doryline ants. Unlike the true army ants, most colonies were polygynous. The queens were ergatoid (permanently wingless) and distinctively larger than the workers, but morphological specialization was not as strong as in army ant queens. The workers showed large variation in body size, but they have no distinctive subcastes like many genera of true army ants. These biological characteristics indicated that the *C. sulcinodis* complex has an atypical biology which are reminiscent of the army ant adaptive syndrome.

Keywords Non-army ant doryline · Cerapachyinae · Ergatoid queen · Caste dimorphism · Phasic reproduction

Introduction

True army ants of the subfamily Dorylinae are dominant predators in tropical and subtropical terrestrial ecosystems (Gotwald 1995, Kronauer 2020). They have some common biological features as follows: large colony size, specialized morphology of the wingless queen (dichthadiigyne),

monogyny, spontaneous mass raid, nomadism, and dependent colony foundation (i.e., DCF, colonial fission) (Kronauer 2009, 2020). The last three features (spontaneous mass raid, nomadism, and DCF) are collectively called the army ant adaptive syndrome. Specialized predation on ants and other social insects to some degree is also a notable characteristic of army ants, even though generalist predators also exist (Gotwald 1995, Hoenle et al. 2019). In addition, all true army ants except *Aenictus* and some species of *Neivamyrmex* show remarkable worker polymorphism (Gotwald 1995). Non-army doryline genera are phylogenetically related to these true army ants, but their life history seems to be different from true army ants (Wilson 1958, Hölldobler 1982). The biology of these non-army ants is valuable in discussing the evolution of the army ant adaptive syndrome. However, knowledge of the basic biology of these ants is still inadequate because most of them are very rare or cryptic, making them difficult to study. The only exception is *Ooceraea biroi* showing thelytokous parthenogenesis by workers (Tsuji and Yamauchi 1995), which is an unusual life history in ants. Among non-army ants, three genera, *Cerapachys*, *Chrysapace*, and *Yunodorylus*, are placed in a sister clade of Old-World true army ants containing *Dorylus*, *Aenictogiton* and *Aenictus* (Borowiec 2019). Here, we propose calling

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them the CCY clade. Investigating the biology of CCY is important because no studies have been carried out to date.

Colony composition data represent fundamental information in understanding the life history of ant societies (Tschinkel 2011). The colony size of true army ants is extremely large: *Dorylus wilverthi* makes colonies with 10–22 million workers (Raignier and van Boven 1955), which seems to be the largest recorded colony size in monodomous ants. Monogyny is common in army ants, except for two species of *Neivamyrmex*, *N. carolinensis* and *N. kiwopache*, that are polygynous (Rettenmeyer and Watkins 1978, Kronauer and Boomsma 2007, Snelling and Snelling 2007). Such basic information on most non-army doryline ants is still scarce.

Queen winglessness is widespread, known in 12 ant subfamilies, and the morphological and biological features of wingless queens (=ergatoid queens) are quite diverse among species (Peeters 2012). There are roughly two trends in ergatoid queen characters based on their morphological differentiations from their workers. (1) Ergatoid queens that have distinctive morphological characters from their workers (=strong caste dimorphism) and specialized for reproduction (=“sole-purpose” ergatoid queen), which is represented by true army ant dichthadiigyne. (2) Ergatoid queens show more or less similar morphology to their workers (=weak caste dimorphism), and some are known as “multi-purpose” ergatoid queens that can work as colony laborers (e.g., *Leptogenys diminuta* group, Ito and Ohkawara 2000; *Mystrium* spp., Molet et al. 2009).

The production of ergatoid queens is associated with colony foundation and dispersal strategy (Peeters and Ito 2001). In some ant species, ergatoid queens are produced with conspecific alate queens, as a combination of independent colony foundation (=ICF, flight dispersal by alate queens) and dependent colony foundation (=DCF, walking dispersal by ergatoid queens and workers), as reported in *Leptothorax* sp. A (Heinze and Buschinger 1989) and *Technomyrmex brunneus* (= *albipes*) (Tsuji et al. 1991, Yamauchi et al. 1991). On the other hand, some species produce only ergatoid queens; consequently, their colonies are obligately reproduced by DCF (e.g., *Leptogenys* spp., Ito 1997). True army ants represent this obligate DCF lifestyle, which is explicitly related to their group predation. Therefore, the colony life history characterized by the combination of (1) obligate DCF, and (2) morphologically specialized sole-purpose ergatoid queens is an important aspect of the army ant adaptive syndrome. Some non-army doryline ants, including the species studied in this paper, have ergatoid queens (Brown 1975, Borowiec 2016), but their morphological characteristics have not been studied in detail.

We found that a non-army doryline ant *Cerapachys sulcinodis* species complex is common in some areas of northern Thailand. The complex represents unique material to study the basic biology of non-army doryline ants in discussing

the evolution of the army ant syndrome. In this paper, we describe the colony composition, phasic reproduction, morphological caste difference between queens and workers, and morphometric analysis of the two castes.

Materials and methods

Study sites

The ant colonies were collected at two study sites in Chiang Mai Province, northern Thailand: Chiang Dao (alt. 500–600 m, hereafter CD) and Omkoi (alt. 950–1000 m, OM). The two study sites are about 180 km apart and both are dry dipterocarp forests, belonging to the *Aw* type of Köppen's climate classification, with distinct dry and rainy seasons. In the dry season from mid-November to mid-April, precipitation is very low (sometimes almost no rain for a month), and the diurnal range in temperature is wider (10–34 °C). Details of temperature and precipitation at the Omkoi study site are shown in our previous publication (Mizuno et al. 2019).

Ants

The specimens collected in northern Thailand were identified as *Cerapachys sulcinodis* Emery, 1889, based on the key and diagnosis of the worker caste provided by Brown (1975). We found two different types of ergatoid queen that can be easily discriminated by their external morphology (Fig. 1, S1). Each colony contained one of the two queen types. The coexistence of two queen types in the same colony has never been found. Based on the results of DNA barcoding analysis, these are two putative cryptic species (see Result section) although the workers of both species are similar, and it is difficult to distinguish them by their external morphology. In addition, we obtained three *Cerapachys* species in northern Thailand. These species are also recognized by queen morphology, but it is difficult to distinguish them based on worker morphology. Furthermore, we do not have specimens of *Cerapachys* from Myanmar, the type locality of *C. sulcinodis*. Therefore, at present, we cannot determine which is the true *C. sulcinodis*, or whether both are new species. In this paper, we tentatively refer to them as *C. sulcinodis* L (the tentative species with larger ergatoid queens, hereafter CsL) and *C. sulcinodis* S (smaller ergatoid queens, hereafter CsS) and refer to them together as the *C. sulcinodis* species complex.

The ergatoid queens of CsS have dense decumbent pilosity on the “cheek” (gena to malar area), but CsL ergatoid queens have only sparse erect to suberect hairs and lack decumbent hairs (Fig. 1). The dorsal surface of the petiole is smooth and shiny in CsL but appears

Fig. 1 Lateral view of an ergatoid queen, a large worker (1.15 mm HW), and a small worker (0.89 mm HW) of *Cerapachys sulcinodis* L (left column) and *C. sulcinodis* S (right column). The queen's abdominal tip was removed for dissection. Close-up image of queens' "cheek" are shown. Legs were removed for visibility. Scale bar 5 mm



longitudinally costate in CsS (Fig. S1c, f). Brown (1975) notes that the specimen series of *C. sulcinodis* collected in Doi Suthep, Thailand (about 60 km from CD, 120 km from OM) includes some exceptionally large wingless individuals, which have broader heads, three ocelli, clear dorsal truncal sutures, and wide “laterally stretched out” petiolar nodes. This description fits the ergatoid queens of our CsL specimens.

A colony of another related species *Cerapachys* RM sp. 6 was used for morphological analysis. This is an undescribed species which is very similar to the *C. sulcinodis* complex, and the range of worker body size in *C. RM* sp. 6 almost overlapped with that in the *C. sulcinodis* complex (queen HW 1.20 mm, worker HW 0.76–0.91 mm). Unlike the ergatoid queens of the *C. sulcinodis* complex, the queen of *C. RM* sp. 6 had clear wing bases, suggesting that she had wings (=dealate queen). The colony (author's personal colony code RM-53) of this species was collected in Kun Chan Kian, Chiang Mai province, Thailand, in July 2016. The result of DNA barcoding suggested that *C. RM* sp. 6 is a different species from CsL and CsS (see Result section); therefore, this specimen is not a conspecific queen polymorphism of the *C. sulcinodis* complex. The colony composition of *C. RM* sp. 6 will be reported in another paper (Mizuno et al. in prep.).

Voucher specimens were deposited in Thailand Natural History Museum.

DNA barcoding

DNA barcoding analysis among CsL, CsS, three *Cerapachys* ants (*C. RM* sp. 5, sp. 6 and sp. 8) and *Lioponera RM* sp. 4 was done to confirm the phylogenetic relationship. DNA extraction, PCR amplification, and sequencing of standard DNA barcoding region (658 bp) of mitochondrial cytochrome oxidase subunit I (COI) gene were performed by following protocols used in Satria et al. (2015). Successful assembly of each COI sequence was confirmed by translating it to the amino acid sequence and checking the absence of frame shifts caused by erroneous indels. A total of 23 sequences were submitted to the International Nucleotide Sequence Database (INSD) via DNA Data Bank of Japan (DDBJ); the list of sequenced samples and accession numbers is shown in Table S1. All additional materials for DNA barcoding were also collected in Chiang Mai province.

The final COI alignment of 620 bp length was used for analyses. Genetic divergences based on K2P distance (Kimura 1980) were calculated by pairwise comparison in MEGA X (Kumar et al. 2018). A neighbor-joining (NJ) tree was then reconstructed based on the K2P distances using MEGA X with 1000 bootstrap replicates. In addition, Maximum likelihood (ML) phylogenetic analysis was performed using IQ-TREE 2.1.3 (Minh et al. 2020), with 1000 replicates of ultrafast bootstrap approximation (UFBoot; Hoang et al. 2018); the optimal substitution model (TPM2+I) was

selected based on BIC using ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE.

Colony collection

The colonies were collected from November 2013 to July 2019, but collection was almost impossible during the dry season because no foraging individuals were found and we could not find any nest sites. Therefore, colony composition data were not obtained during the dry season (December to April). The colonies nested in preexisting spaces such as under a rotting log or stone or in an underground space that may have been made by other animals or arthropods. In total, 40 colonies were collected, of which 26 and 10 were CsL and CsS, respectively. The remaining four colonies were not identified because they did not contain a queen. The number of queens, males, workers, mature pupae, young pupae, prepupae, larvae, and eggs in each colony were counted. All data in this study were analyzed using R software version 3.6.1 (R Core Team 2019). The Wilcoxon rank sum test with *wilcox.test* and Spearman's rank correlation with *cor.test* were used to analyze colony composition. Hereafter, the name and version of the packages used are shown in each paragraph, and the name of the functions used is shown in the "Results" section in italics.

Colony rearing under laboratory conditions

In total, 22 captive colonies (16 CsL, 6 CsS) were attempted to rear in the laboratory. Half of them (8 CsL, 3 CsS) showed a typical phasic reproduction, and detailed observation was carried out in three of them (shown in the following paragraph). Another half of captive colonies (8 CsL, 3 CsS) never reproduced any adult ants in the laboratory (eggs never be laid or the eggs never hatched), maybe due to unusual colony condition under the laboratory. The captive colonies were reared in plastic boxes (235 × 312 × 80 mm) with a plaster floor. They were kept under constant temperature (25°C) and day length (12L:12D). Two-to-four small plastic boxes with plaster floors were provided as nesting chambers for the ants. The chamber boxes were large (87 × 125 × 18 mm) or small (53 × 83 × 13 mm) according to the number of ants in each colony. The ants feed not only on ant brood like other non-army ant dorylines but also other small arthropods (Mizuno et al. in prep.); therefore, mealworms (larvae of *Tenebrio molitor* Linnaeus, 1758) or nymphs of Turkestan cockroach (*Blatta lateralis* Walker, 1868) were given as prey every two days during the observation period.

Brood composition (= approximate number of eggs, larvae, prepupae, and pupae) in three colonies (CsL, colony FI14-77, FI14-81; CsS, colony FI14-97) were counted principally every day for a year (29 October 2014 to 29 October 2015). Details of the results of the laboratory observations will be

reported in separate publications (Mizuno et al. in prep.). The duration of four developmental stages of brood was presumed from daily change in brood composition as follows: *egg stage*, from the appearance of the first egg to the hatching of the first larva; *larval stage*, from the appearance of the first larva to the appearance of the first prepupa; *prepupal stage*, from the appearance of the first prepupa to the appearance of the first pupa; and the *pupal stage*, from the appearance of the first pupa to the emergence of the first callow worker. If the day of the appearance of the first individual was not determined because of infrequent observation, the data were omitted from the analysis. The whole duration of a colony cycle was defined as follows: from the appearance of the first egg to the appearance of the first egg of the next batch. Multiple comparisons of duration of each developmental stage and an entire reproductive cycle among colonies were performed by pairwise comparisons using the Wilcoxon rank sum test with *pairwise.wilcox.test* in R.

Determination of reproductive status

All the queens in seven colonies of CsL (total 36 queens) and five colonies of CsS (total 27 queens) were dissected under a binocular microscope to examine their reproductive status. The number of ovarioles in each ovary, the number of developing oocytes, the presence or absence of sperm in the spermatheca, and the degree of yellow body accumulation were recorded. The queens in ten polygynous colonies were dissected just after collection to determine the existence of reproductive skew among the queens (CsL, 35 queens of colony FI13-168, FI14-75, RM-147, RM-310, RM-350, RM-367; CsS, 26 queens of colony FI13-167, FI14-82, RM-152, RM-425). The queens in two monogynous colonies were dissected after colony rearing (CsL, FI13-166; CsS, RM-198). In addition, seven queens in six colonies of CsL (RM-42, RM-198, RM-416, RM-426, RM-437, RM-550) and three queens in a colony of CsS (RM-415) were also dissected after laboratory observation. In total, the number of queens dissected was 43 and 30 for CsL and CsS, respectively. Eighty-four workers in four colonies of CsL and 20 workers in one colony of CsS were also dissected just after collection using the same method as that for the queens. The number of ovarioles in each species was compared using the Wilcoxon rank sum test with *wilcox.test*. The distribution of the number of ovarioles per queen was analyzed using the F-test with *var.test*, Shapiro–Wilk test with *shapiro.test*, and the R package "e1071" version 1.7–3 (the functions *kurtosis* and *skewness* in the package).

Morphological observation and morphometric analysis

Head width (HW) was measured to determine the size difference between two castes and body size variation in the

workers. Maximum head width (above compound eyes, eyes were excluded) was measured to the nearest 0.0166 mm using a binocular microscope (Olympus SZ-60) with an attached micrometer. All the queens and 400 randomly selected workers were measured from a colony each of the two species (CsL, colony FI13-168; CsS, colony FI14-82). To compare the variation in head width of the workers among localities and colonies in each species, additional workers were measured using the same method. Fifty or 100 workers in five colonies (total, 400 workers) of the two species were examined. The list of measured colonies and the number of individuals is shown in Table S2. The distribution of head width was analyzed using the Wilcoxon rank sum test with *wilcox.test*, Kruskal–Wallis rank sum test with *kruscal.test* and Kendall's rank correlation with *cor.test*.

Some workers and queens were photographed under a scanning electron microscope (SEM, JCM-7000; JEOL Ltd., Tokyo) to compare their head and mesosoma structures. A worker and an ergatoid queen each of CsL and CsS were used. Also, a worker and a dealate queen in one colony of *C. RM sp. 6* were photographed for comparison.

Morphometric analysis of the queens and workers was conducted to compare the growth rules underlying the body shape among the two species and castes. All the queens of the two examined colonies (CsL, colony FI13-168, 14 queens; CsS, colony FI14-82, 19 queens) and 35 workers of both colonies were selected for this analysis. These examined colonies were the same that were used for the head width measurement. The measured workers were selected evenly from the smallest to the largest based on their head width. Photographs of all the body parts were taken using a digital camera (Shimadzu moticam 2000) connected to a binocular microscope (Olympus SZX12) and measured using ImageJ software version 1.52 k (open source: <https://imagej.nih.gov/ij/>). Head width (HW), head length (HL), pronotum width (PrW), and IV abdominal segment width (AW) were measured for the analysis. To assess the allometric relationship among the body parts, all the measurement data were transformed to common logarithm. First, correlations among the pairs of each measurement of body parts were assessed. When the two measured values correlated, then their regression line (slope and y intercept) and p-value of multiple comparisons among them were computed. Standardized major axis regression (SMA) was used for the analysis with R package “smatr” version 3.4–8 (Warton et al. 2012). When the slope of the allometric coefficient is significantly different from 1, it was concluded that they show allometric growth.

Results

DNA barcoding

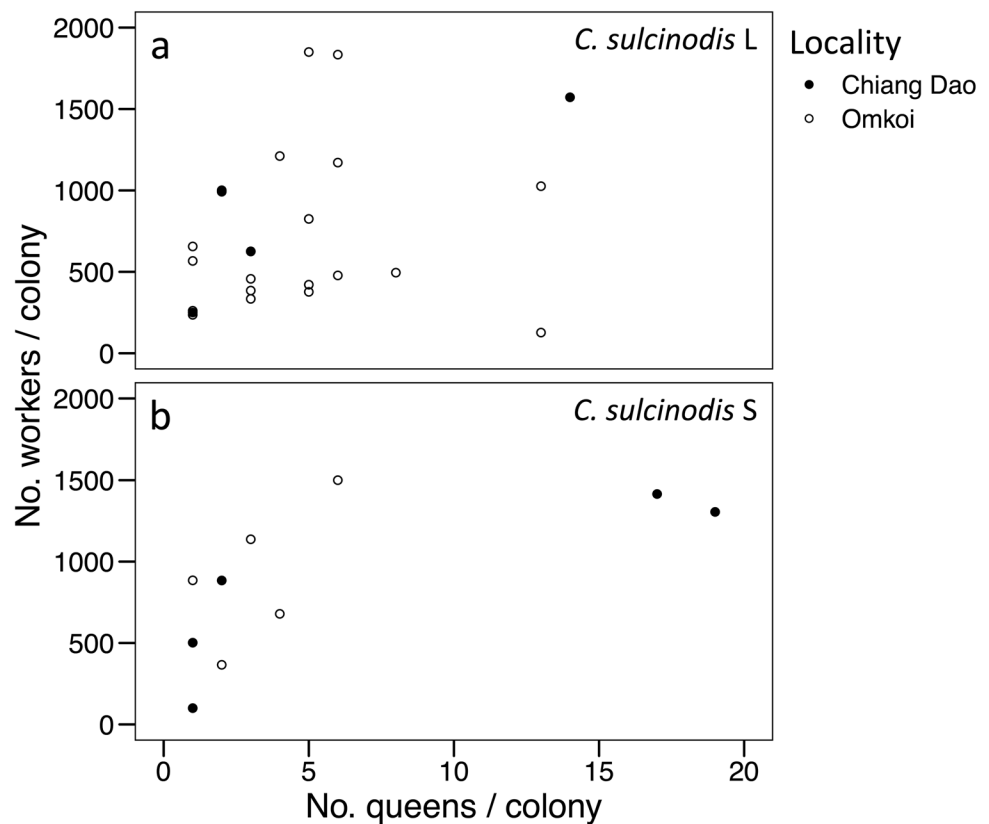
Neighbor-joining (NJ) and Maximum likelihood (ML) analyses yielded identical tree topology. *C. sulcinodis* species complex was separated into two distinct sympatric clades which are congruent with CsL and CsS distinguished by ergatoid queen morphology (Fig. S2). CsL and CsS clades were each strongly supported with $\geq 95\%$ of both bootstrap value in NJ analysis and UFBoot value in ML analysis. Genetic divergences between CsL and CsS clades were 12.5–15.4% in K2P distance and much larger than maximum intra-clade divergences observed in each clade (4.2% in CsL, 4.9% in CsS): in each of these clades, samples from the same locality had identical or nearly identical haplotypes whereas divergences of 2.8–4.9% were observed among those from different locality (Table S3). Other putative *Cerapahys* species included in the analysis (*C. RM sp.5*, *C. RM sp.6*, *C. RM sp.7*) were distinct from each other and from CsL and CsS, with 13.6–25.0% divergences.

Colony composition

The number of workers and queens in the colonies of the two species of the *C. sulcinodis* complex is shown in Fig. 2, Tables S4 and S5. Four queenless colonies, which contained approximately 50–400 workers, were omitted from the figure and tables. All the queens of both species were ergatoid (permanently wingless), and no winged queen (alate or dealate) was ever collected. The majority of the colonies were polygynous (19 of 24 colonies in CsL, 7 of 10 colonies in CsS). The maximum number of queens in CsL and CsS was 14 and 19, respectively. In both species, the number of queens per colony was not significantly different between the two localities (CsL: median number of queens in Omkoi = 5, range = 1–13, Chiang Dao = 3, range = 1–14, $W = 38.5$, $p = 0.5407$, Wilcoxon rank sum test, *wilcox.test*; CsS: median number of queens in Omkoi = 2.5, range = 1–6, Chiang Dao = 2, range = 1–19, $W = 10.5$, $p = 1$, Wilcoxon rank sum test). Therefore, data from both localities were combined for comparison between the two species. Between the two species, the median of queen number per colony was not significantly different (median of queen number of CsL = 3.5, range = 1–14, CsS = 2, range = 1–19, $W = 120.5$, $p = 0.6232$, Wilcoxon rank sum test).

The maximum number of workers of CsL and CsS was 1850 and 1500, respectively. The number of workers was, again, not significantly different between the two

Fig. 2 Relationship between the number of queens and workers (colony size) of *Cerapachys sulcinodis* L (a) and *C. sulcinodis* S (b). Four queenless colonies were omitted from the figures



localities in both species (CsL: median of worker number in Omkoi = 495, range = 127–1850, Chiang Dao = 626, range = 250–1572, $W = 54$, $p = 0.6793$; CsS: median of worker number in Omkoi = 1011, range = 366–1500, Chiang Dao = 884, range = 100–1415, $W = 8$, $p = 0.7302$, Wilcoxon rank sum test). The number of workers was not significantly different between the two species (median of CsL = 596, range = 127–1850, CsS = 885, range = 100–1500, $W = 86$, $p = 0.3916$, Wilcoxon rank sum test). The number of queens and workers were not significantly correlated in CsL ($\rho = 0.3446565$, $p = 0.09908$, $n = 22$, Spearman's rank correlation coefficient, *cor.test*). A significant positive correlation was observed in CsS ($\rho = 0.783156$, $p < 0.05$, $n = 10$, Spearman's rank correlation coefficient), but it was possibly caused by small sample size.

The brood composition of both species showed a typical phasic reproduction as is known in some other doryline ants (Fig. 3). Almost half the colonies had only larvae (CsL $n = 11$, CsS $n = 7$), and eggs were found with pupae in all but one colony (5 of 6 colonies with eggs had pupae together). One colony had eggs, many small larvae, and only one mature pupa, which seems to show exceptionally slow development. The colonies collected in November had only mature pupae (CsS, colony FI13-167) or no brood, suggesting that the reproductive cycle was interrupted during

the dry season from November. In May (start of the rainy season), only two CsL colonies were collected: one colony (RM-302, collected on 6 May 2017) included only larvae, and the other (RM-310, 7 May 2017) had mature pupae and small larvae, which were apparently a different batch from the mature pupae. A few males were collected in August in two colonies of CsL and one colony of CsS. No newly produced virgin ergatoid queen was ever found (see the result of reproductive condition of queens and workers).

Reproductive cycle under laboratory conditions

Three observed captive colonies completed a total of 13 reproductive cycles. Figure 4 shows the successive change in brood composition in laboratory-reared colonies of the two species. As suggested from the field colonies, a typical phasic reproductive cycle was observed in both species. In both species, the brood became adults by feeding exclusively on non-ant preys (mealworms and cockroaches). The duration of each brood developmental stage and an entire reproductive cycle (from the start of egg-laying to the start of the next egg-laying) are shown in Table 1. The duration of the egg stage was markedly variable from 8 to 38 days, whereas the duration of other developmental stages was rather constant. During the colony rearing, sometimes the eggs did not hatch for an unusually long period (occasionally more

Fig. 3 Schematic illustration of phasic reproduction. Brood composition of collected colonies of *Cerapachys sulcinodis* species complex and its presumed phase. *PP* prepupae

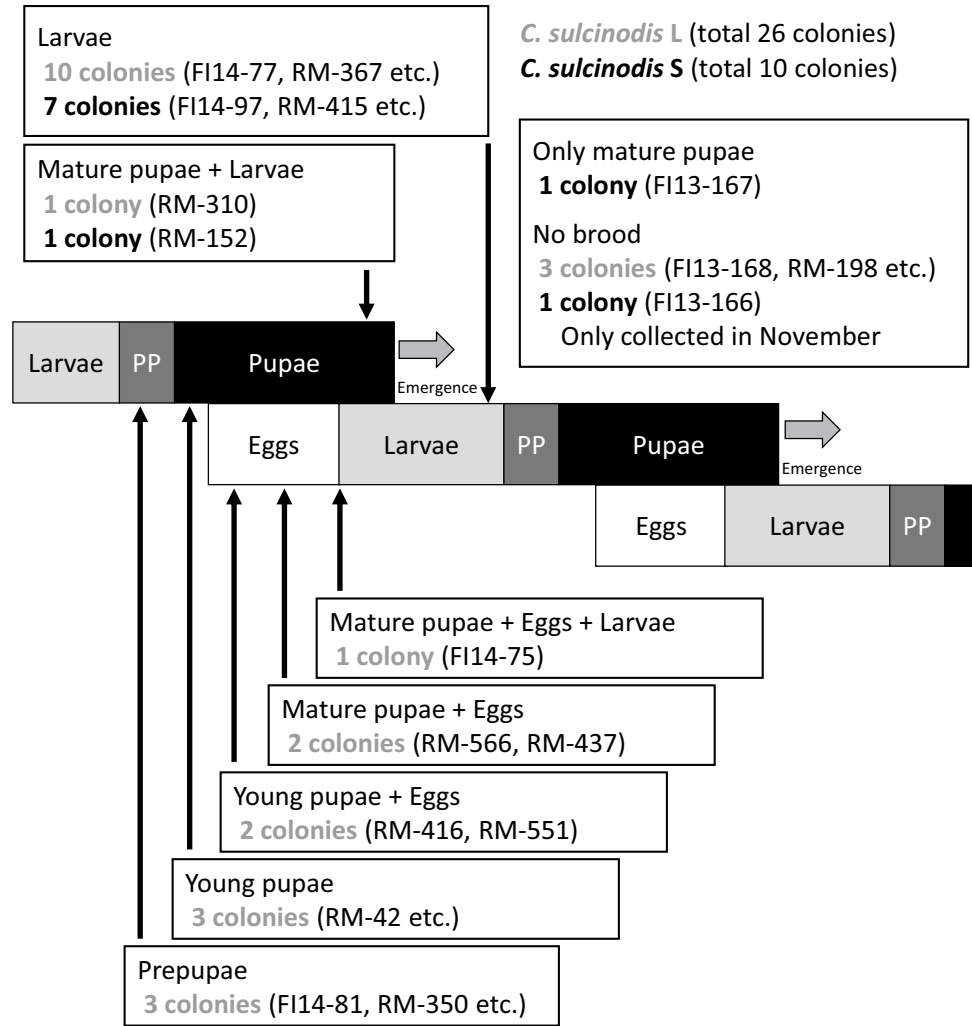


Fig. 4 Successive change in brood composition in the laboratory colonies of *Cerapachys sulcinodis* L (a, colony FI14-77) and *C. sulcinodis* S (b, colony FI14-97). * The colony was not observed for these eight days

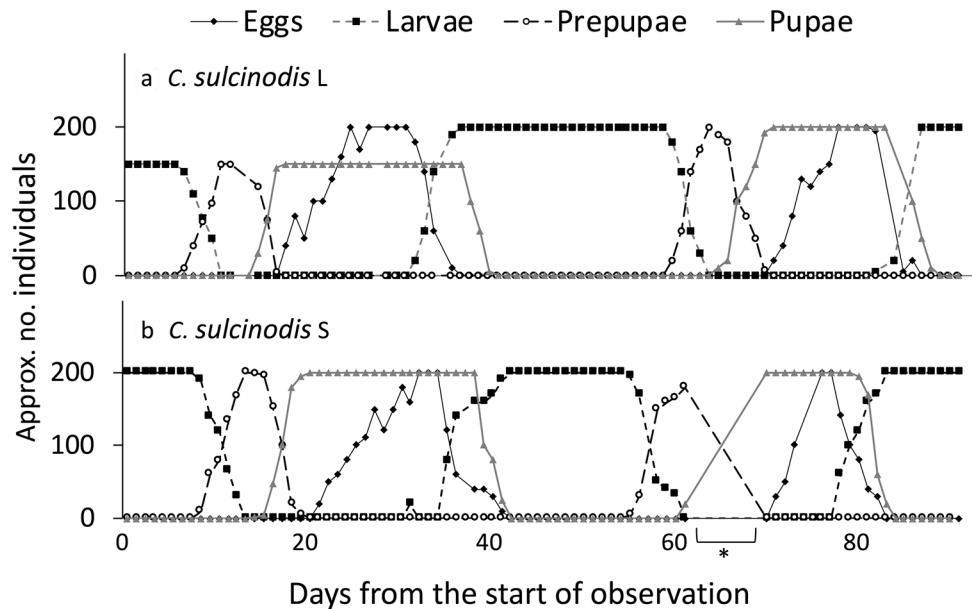


Table 1 Estimated days of each developmental stage and an entire colony cycle of three reared colonies

| Colony code | sp. | Approx. colony size ^a | Egg stage | | Larval stage | | Prepupal stage | | Pupal stage | | An entire cycle | |
|-------------|-----|----------------------------------|-------------------------|---|-------------------------|---|----------------------|---|-------------------------|---|--------------------------|---|
| | | | Average ± SD (Range) | n | Average ± SD (range) | n | Average ± SD (range) | n | Average ± SD (range) | n | Average ± SD (range) | n |
| FI14-77 | CsL | 1000 | 14.50 ± 5.45 (20–21) | 4 | 27.00 ± 6.08 (34–42) | 3 | 6.33 ± 0.58 (6–7) | 3 | 22.00 ± 3.61 (18–25) | 3 | (42, 64) | 2 |
| FI14-81 | CsL | 300 | 14.60 ± 6.80 (9–26) | 5 | 26.00 ± 2.65 (24–29) | 3 | (7) | 2 | (19, 21) | 2 | 41.75 ± 6.99 (34–51) | 4 |
| FI14-97 | CsS | 1500 | 16.60 ± 12.22 (8–38) | 5 | 26.60 ± 1.52 (24–28) | 5 | 5.67 ± 2.89 (4–9) | 3 | (20, 21) | 2 | 56.25 ± 11.30 (49–73) | 4 |

Average days of each stage and an entire cycle were not significantly different among the colonies in all stages (pairwise comparisons using Wilcoxon rank sum test)

CsL *Cerapachys sulcinodis* L; CsS *Cerapachys sulcinodis* S

^aApproximate number of workers in the colony when observation started

than a month), probably because of unsuitable rearing conditions. The length of each developmental stage and an entire reproductive cycle were not significantly different among the colonies ($p > 0.1$ in pairwise Wilcoxon rank sum test with Bonferroni correction, *pairwise.wilcox.test*). The abdomens of the queens were not physogastric during the egg-laying periods (CsL; 13 egg-laying periods of three queens, CsS; 6 egg-laying periods of 16 queens).

Reproductive conditions of queens and workers

All the dissected ergatoid queens of both species had mated ($n = 43$ CsL, 30 CsS), and the degree of accumulation of yellow bodies did not vary among the queens in the polygynous colonies, indicating that they were functionally polygynous where most of the queens lay eggs without marked reproductive skew. In 10 colonies which included larvae (CsL, 6 colonies; CsS, 4 colonies), developing oocytes were not detected in almost all the queens. This result corresponds to their phasic reproduction as suggested from the result of colony composition and laboratory rearing of the colonies. Developing oocytes were found in only three queens of one colony (colony RM-350) of CsL, which included many prepupae. These three queens had 58–74 developing oocytes in their ovaries. The number of ovarioles per queen varied from 32 to 56 in CsL and 29–51 in CsS, and there was no significant difference in the number of ovarioles between the two species (Fig. S3, S4, median of CsL = 42, CsS = 41, $W = 573.5$, $p = 0.6361$, Wilcoxon rank sum test, *wilcox.test*; all the data of the dissected queens are shown in Table S6, S7). The distribution of the number of ovarioles of CsS showed a smaller variance than that of CsL (CsL = 37.91441, CsS = 18.03695, $F = 2.102$, num $df = 36$, denom $df = 28$, $p = 0.04541$, F test, *var.test*). That of the CsL queens was not significantly different from normal distribution ($p = 0.485$, $W = 0.97266$, Shapiro–Wilk test, *shapiro.test*), while it was different in CsS ($p = 0.01503$, $W = 0.90767$, Shapiro–Wilk test). The kurtosis of the distribution of the number of ovarioles of CsS was higher than that of CsL (kurtosis, CsL = -0.4544339 , CsS = 2.649909, computed with *kurtosis*) with skew (CsL = 0.3347738, CsS = -0.6394706 , computed with *skewness*).

All the dissected workers of both species had two ovarioles (1–1) but no spermatheca ($n = 84$ in CsL, $n = 20$ in CsS). Some workers of both species had a few developing oocytes.

Head width distribution and caste dimorphism

Figure 5 shows the distribution of head width of each colony of the *C. sulcinodis* species complex. The head width of the queens was conspicuously larger than that of the workers in both species (CsL colony FI13-168, median queen head width = 11.508 mm,

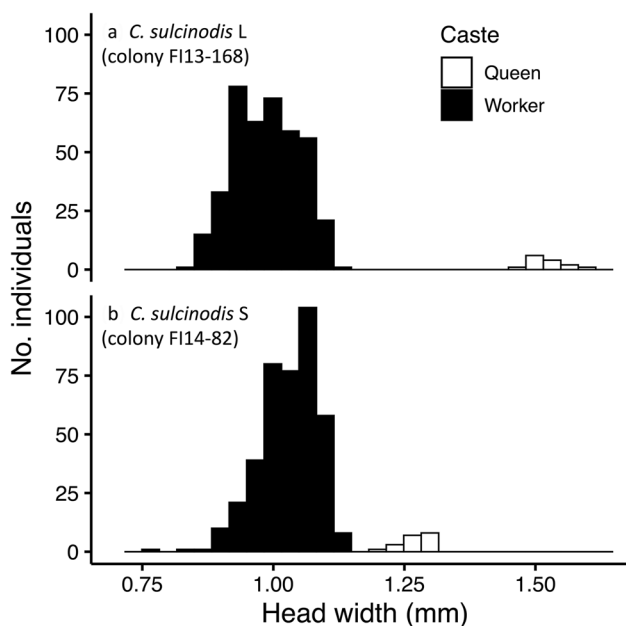


Fig. 5 Distribution of the head width of the queens and workers of *Cerapachys sulcinodis* L (a, colony FI13-168) and *C. sulcinodis* S (b, colony FI14-82). Head width is significantly different between the queens and workers in both species ($p < 0.001$, Wilcoxon rank sum test)

range = 1.467–1.583 mm, median worker head width = 0.9833 mm, range = 0.8333–1.1167 mm, $W = 5600$, $p < 0.001$, Wilcoxon rank sum test by *wilcox.test*; CsS colony FI14-82, median queen head width = 1.267 mm, range = 1.200–1.300 mm, median worker head width = 1.0333 mm, range = 0.7667–1.1333 mm, $W = 7600$, $p < 0.001$, Wilcoxon rank sum test). The head width of the queens of CsL was larger than that of CsS ($W = 266$, $p < 0.001$, Wilcoxon rank sum test). In both species, the head of the largest queen was approximately 1.08-times wider than that of the smallest queen. The relationship between the distribution of the worker head width and colony sizes were assessed (Fig. S5). Colony size had a significant effect on the distribution of head width in both species (CsL, chi-squared = 7863.4, $df = 10$, $p < 0.001$; CsS, chi-squared = 7842.8, $df = 10$, $p < 0.001$, Kruskal–Wallis rank sum test by *kruscal.test*). The correlation between colony size and worker head width of the CsL colonies was weakly negative (tau = -0.2374015, $z = -8.6836$, $p < 0.001$, Kendall's rank correlation, *cor.test*), whereas that of CsS was weakly positive (tau = 0.06094081, $z = 2.2334$, $p = 0.02552$, Kendall's rank correlation). The head width of a total of 800 workers from six colonies was not significantly different between the two species (median of CsL, 0.9833 mm, range = 0.8176–1.1500 mm; CsS 0.9833 mm, range = 0.7667–1.1333 mm, $W = 317,682$, $p = 0.8013$, Wilcoxon rank sum test, *wilcox.test*). In both species, the head of

the largest worker was approximately 1.4-times wider than that of the smallest worker.

Comparison of head and mesosomal morphology

The workers of the three *Cerapachys* species lacked ocelli (Fig. 6a, b, c). On the other hand, all the queens of the three species had three ocelli (Fig. 6d, e, f). The dorsal mesosomal sclerites of the workers of all three species were completely fused into one part without clear sutures (Fig. 6g, h, i, m, n, o). The ergatoid queens of both species of the *C. sulcinodis* complex had a similar simple mesosomal structure to their workers, but the striate between the pronotum and mesonotum (promesonotal suture) was clearly visible (Fig. 6j, k, p, q). The ergatoid queens did not have tegulae and wing bases. Unlike these ergatoid queens, the dorsal mesosomal sclerites of an alate queen of *C. RM sp. 6* were separated into five parts (i.e., pronotum, mesonotum, scutellum, metanotum, and propodeum) and also had tegulae and wing bases (Fig. 6l, r). In dorsal view, the mesonotum width of the ergatoid queens of the *C. sulcinodis* complex was narrower than the pronotum width (Fig. 6p, q), which was similar to their workers (Fig. 6m, n, o). On the other hand, in the alate queen of *C. RM sp. 6*, the mesonotum width was roughly the same as the pronotum width (Fig. 6r). Related to mesonotum width, the mesopleuron of the alate queen was laterally swollen but that in the ergatoid queens was flattened. The antero-posterior length of the meso- to metanotum (total length of mesonotum, scutellum, and metanotum on the midline) was longer in the *C. RM sp. 6* alate queen and shorter in the ergatoid queens of the *C. sulcinodis* complex. The pronotum was relatively longer in the ergatoid queens but shorter in the alate queen.

Figure 7 shows the relationship between pronotum width and head width and between pronotum width and abdomen width in both the queens and workers of the *C. sulcinodis* complex and an alate queen of *C. RM sp. 6*. The plots of the queens and workers were completely separated in both species of the *C. sulcinodis* complex. The head width of the CsS queens was smaller than that of the CsL queens, but their abdominal width was similar to each other. As described above, the alate queen of *C. RM sp. 6* had a wider pronotum than the ergatoid queens of the *C. sulcinodis* complex because of the wider mesonotum and wings. The head width of this individual was not much different from the ergatoid queens of CsS, but the abdominal width was narrower than that in the ergatoids of the *C. sulcinodis* complex.

Morphometric analysis

Table 2 shows a summary of the morphometric analysis of both castes of the *C. sulcinodis* complex. A significant correlation between pronotum width and both head width and

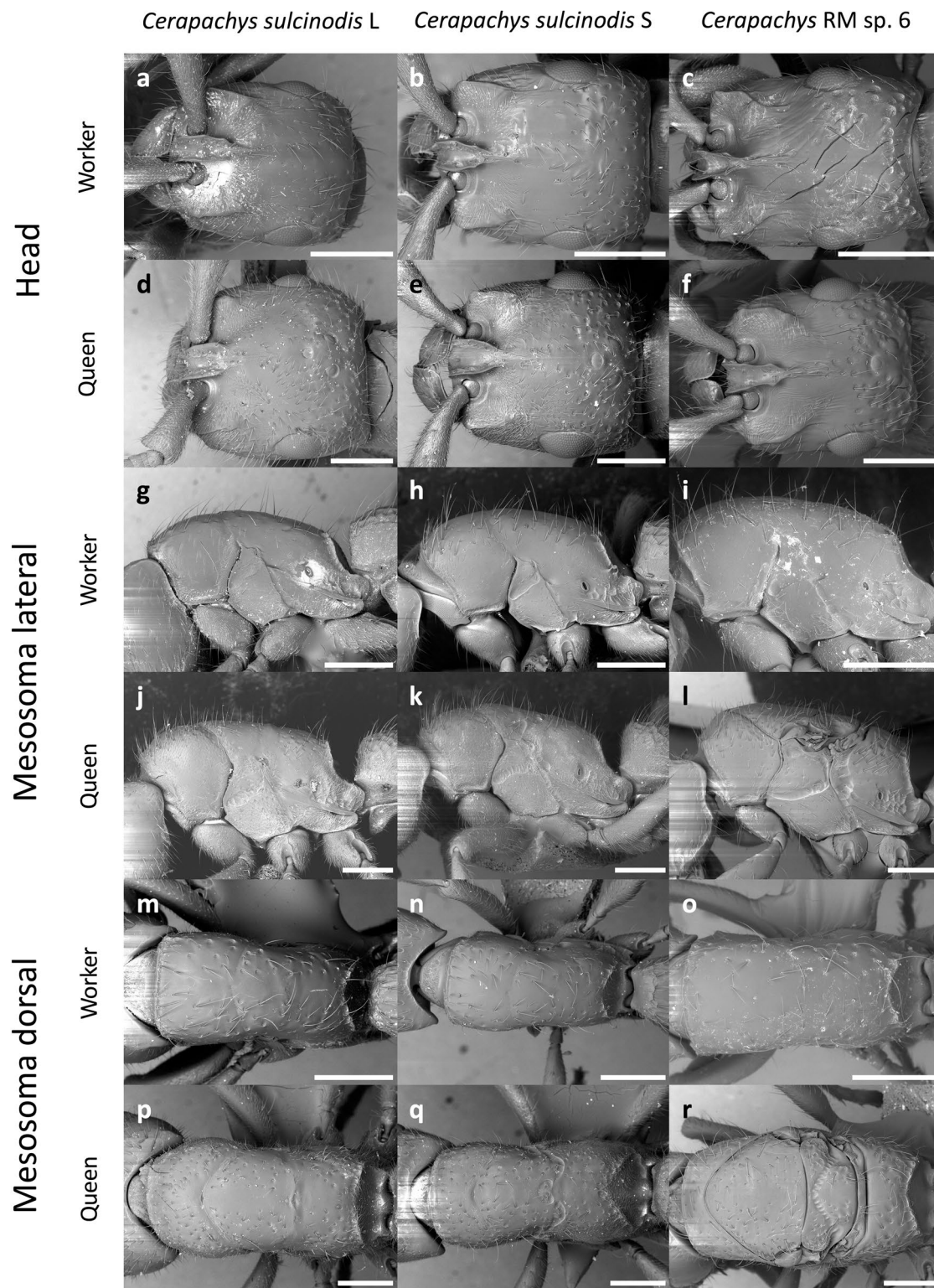


Fig. 6 SEM images of the head (a–f), and mesosomal lateral (g–l) and dorsal (m–r) views of the workers and queens of the *Cerapachys sulcinodis* species complex and a related species *Cerapachys* RM sp. 6. Scale bar 500 μ m

Fig. 7 Relationship between pronotum width and head width (a), and IV segment of the abdomen (b) in the queens and workers of the *Cerapachys sulcinodis* species complex and a queen of *Cerapachys* RM sp. 6. Both axes are logarithmic scale

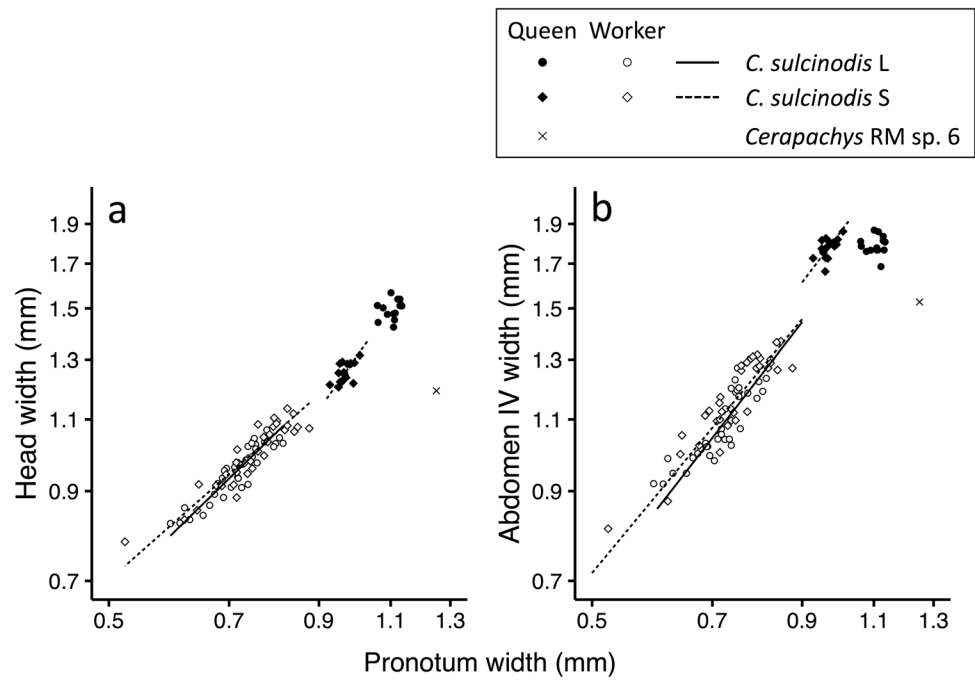


Table 2 Morphometric analysis between pairs of log-transformed morphological measurements (pronotum width vs. head width, pronotum width vs. abdominal width, and head width vs. head length) with these comparisons between castes and species

| Species | Caste | N | Correlation analysis | | Regression line | | Test against isometry | | |
|------------------------------------|--------|----|----------------------|--------|--|-----------------------|-----------------------|--------|------------------------|
| | | | Pearson's r2 | p | Slope = allometry coefficient ^a | Intercept* | r | p | Conclusion |
| Pronotum width vs. head width | | | | | | | | | |
| CsL | Queen | 14 | 0.0793 | 0.3295 | – | – | – | – | – |
| | Worker | 35 | 0.8397 | <0.001 | 0.9699 ^{ab} | 0.1197 ^a | –0.0761 | 0.6639 | Isometry |
| CsS | Queen | 19 | 0.3673 | <0.01 | 1.4077 ^a | 0.1181 ^a | 0.4015 | 0.0884 | Isometry |
| | Worker | 35 | 0.8491 | <0.001 | 0.8724 ^b | 0.1096 ^a | –0.3323 | 0.0511 | Iso/negative allometry |
| Pronotum width vs. abdominal width | | | | | | | | | |
| CsL | Queen | 14 | 0.0002 | 0.9590 | – | – | – | – | – |
| | Worker | 35 | 0.7493 | <0.001 | 1.2546 ^a | 0.2164 ^c | 0.4156 | <0.05 | Positive allometry |
| CsS | Queen | 19 | 0.3309 | <0.01 | 1.3238 ^a | 0.2684 ^a | 0.3282 | 0.1701 | Isometry |
| | Worker | 35 | 0.8339 | <0.001 | 1.1806 ^a | 0.2183 ^b | 0.3786 | <0.05 | Positive allometry |
| Head width vs. head length | | | | | | | | | |
| CsL | Queen | 14 | 0.4468 | <0.01 | 1.2603 ^a | –0.0182 ^{ac} | 0.2994 | 0.2984 | Isometry |
| | Worker | 35 | 0.8531 | <0.001 | 0.7577 ^b | 0.0752 ^a | –0.5913 | <0.001 | Negative allometry |
| CsS | Queen | 19 | 0.2108 | <0.05 | 0.9156 ^{ab} | 0.0492 ^{ac} | –0.0989 | 0.6869 | Isometry |
| | Worker | 35 | 0.9216 | <0.001 | 0.9709 ^a | 0.0676 ^b | –0.1048 | 0.5490 | Isometry |

CsL *Cerapachys sulcinodis* L; CsS *Cerapachys sulcinodis* S

*Results of the multiple comparison; different letters mean significant difference ($p < 0.05$)

abdominal width was not observed in the ergatoid queens of CsL; therefore, allometry was not analyzed. The slope of the regression line (= allometric coefficient) between the log-transformed pronotum width and head width was significantly different between the queens and workers in CsS. However, the allometric coefficient was not different from 1

(= isometric) in all the examined species and castes except the queens of CsL.

On the other hand, the allometric coefficient between the pronotum width and abdominal width was not significantly different among all the examined species and castes. The allometric coefficient was significantly different from 1

(= positively allometric) in the workers of both species but not in the queens of CsS (= isometric). The intercept of the regression lines was significantly different.

The allometric coefficient between the head width and head length was significantly different between the castes of CsL. The intercept of the regression lines was significantly different between the castes of CsS. Negative allometry on head shape was observed in the workers of CsL, but that in CsS was isometric. Figure 8 shows the relationship between the head width and length of the *C. sulcinodis* complex. The plots of the workers of the two species overlapped in the range of the larger individuals (head width 0.9 to 1.1 mm in Fig. 8) but tended to be separate in the smaller workers (head width < 0.9 mm). A comparison of the head shape of the two species is also shown in Fig. 8. The head shape of the large workers was similar to each other, but the smaller workers of CsL had a slightly more slender head than the smaller workers of CsS. Such small workers were not a majority in the colonies of this species (only 53 of a total of 800 measured CsL workers).

Discussion

Social structure, seasonality, and phasic reproduction

The colony composition of known army and non-army ants in the subfamily Dorylinae is summarized in Table 3. The

colony size of all known army ants is more than tens of thousands of individuals per colony, while the majority of non-army ants show a smaller colony size of around 100 workers per colony. The colonies of non-army ants with more than 1,000 workers were rarely recorded, with the single exception of a colony of *Lioponera* mg02 consisting of one queen and 2409 workers (Fisher and Peeters 2019). The colony size of *Zasphinctus steinheili* is relatively large for a non-army ant, but does not exceed 500 workers per colony. The estimated colony size of the *Ooceraea biroi* studied by Tsuji and Yamauchi (1995) was 150–600. This colony is relatively large, but their unusual clonal colony organization should be considered. Except for these species, non-army doryline ants have colonies with fewer than a few hundred workers. The colony size of the *C. sulcinodis* complex (up to 1850 workers per colony) is larger than that of other known non-army dorylines but quite smaller than that in the true army ants. Also, the number of ovarioles per queen in the *C. sulcinodis* complex is apparently larger than that in other non-army dorylines, while quite smaller if compared with that in the army ants.

Unlike the majority of army ants that exhibit monogyny, polygynous colonies were dominant in the *C. sulcinodis* complex. Polygyny is reported in some non-army ants (*Lioponera* spp., Idogawa and Dobata 2018, Ito et al. 2018; *Z. steinheili*, Buschnger et al. 1989), but data on the number of queens in non-army dorylines are still scarce. Functional polygyny by the ergatoid queens with a relatively large colony size found in the *C. sulcinodis* complex is similar to that of *Z. steinheili* (Buschnger et al. 1989). Polygyny might be an adaptation for increasing colony fecundity to gain a larger colony size, instead of the large queen fecundity with extreme morphological specialization in army ant queens. It is possible that the lack of virgin queens in our dissection dataset is related to the intra-colony mating. But the intra-colony mating has never been observed in the laboratory colonies (Mizuno unpublished), and low sampling frequency in September (the possible season of sexual production, see following paragraph) may be one of the possible reasons for the absence of virgin ergatoid queens in our samples.

All but one colony collected in November did not include any brood, suggesting that the colony reproductive cycle in the *C. sulcinodis* complex was interrupted during the dry season. This result corresponds to their very low foraging activity in the dry season (Mizuno et al. 2019). The colony collected in early May (RM-310) included mature pupae and small larvae, which were apparently a different batch from the mature pupae, indicating that at least a second batch was found in early May. In the laboratory, the average length of one reproductive cycle was ca. 5–8 weeks. Thus, egg production may start from mid- or late March, at the end of the dry season. Virgin queens and a newly producing sexual brood were not found in our colonies. A few colonies collected in

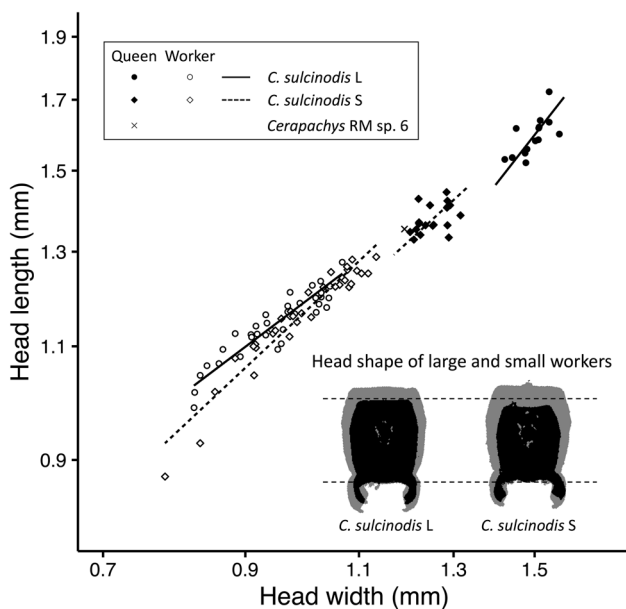


Fig. 8 Relationship between head width and head length in the queens and workers of the *Cerapachys sulcinodis* species complex and a queen of *Cerapachys* RM sp. 6. Both axes are logarithmic scale

Table 3 Colony composition, phasic reproduction, and number of ovarioles of known army ants and non-army ants

| Species | Colony size | Colony size | Queens per colony | Phasic reproduction | Queen morphology ^a | Ovarioles per queen | Ovarioles per worker | Worker sub-castes | References |
|--|-------------------------|-------------|-------------------|---------------------|-------------------------------|---------------------|----------------------|-------------------|--|
| Army ants | | | | | | | | | |
| <i>Aenictus laeviceps</i> | 6–11 × 10 ⁴ | | 1 | + | DG | ? | 2 | – | Schneirla and Reyes (1966) |
| <i>Dorylus wilverthi</i> | 10–22 × 10 ⁶ | | 1 | – | DG | ? | 2 | + | Raignier and Van Boven (1955) |
| <i>Eciton burchellii</i> | 5–20 × 10 ⁵ | | 1 | + | DG | 2400 | 2–6 | + | Hagan (1954), Schneirla (1957), Rettenmeyer (1963), Whelden (1963) |
| <i>E. hamatum</i> | 10–50 × 10 ⁴ | | 1 | + | DG | 2400 | 2–6 | + | Hagan (1954), Rettenmeyer (1963), Whelden (1963) |
| <i>Neivamyrmex nigrescens</i> | 8–14 × 10 ⁴ | | 1 | + | DG | 1000 | 0 | – | Schneirla (1958), Holiday (1904) |
| Non-army ants | | | | | | | | | |
| <i>Cerapachys sulcinodis</i> L | 127–1850 | | 1–14 | + | EQ | 32–56 | 2 | – | Present study |
| <i>Cerapachys sulcinodis</i> S | 100–1500 | | 1–19 | + | EQ | 29–51 | 2 | – | Present study |
| <i>Lioponera cohici</i> | ca. 100 | | ? | – | ? | ? | ? | ? | Wilson (1958) |
| <i>L. daikoku</i> | 1–44 | | 1–3 | – | AQ | 4 | 2 | – | Idogawa and Dobata (2018) |
| <i>L. dumbletoni</i> | 200 | | 1 | – | EQ | ? | ? | ? | Wilson (1958) |
| <i>L. punctatissima</i> | 35 | | 1 | ? | AQ | ? | ? | ? | Wilson (1958) |
| <i>L. suscitata</i> | 14–40 | | 1 | – | AQ | 4 | 2 | – | Ito et al. (2018) |
| <i>Lioponera</i> sp.1 (<i>L. suscitata</i> group) | 12 | | 2 | – | AQ | 4 | 2 | – | Ito et al. (2018) |
| <i>Ooceraea biroi</i> | 150–600 | | – ^b | + | – ^b | – ^b | 2–6 | – | Tsuji and Yamauchi (1995), Ravary and Jaisson (2004) |
| <i>Parasyscia</i> cf. <i>cribrinodis</i> | 236 | | 1 | + ^c | EQ | 25 | 2 | – | Villet et al. (1991) |
| <i>P. opaca</i> | ca. 100 | | ? | + ^d | ? | ? | 1 | ? | Wilson (1958, 1959a, 1959b) |
| <i>P. polynikes</i> | 20 | | ? | ? | ? | ? | ? | ? | Wilson (1959a) |
| <i>Syscia humicola</i> | 4–21 | | 1–9 ^e | + | EQ | 2–5 | 2 | – | Masuko (2006) |
| <i>Zasphectus steinhelli</i> | 80–400 | | 9–20 | + | EQ | 4–11 | 2 | – | Buschinger et al. (1989) |

^aDG dichthadiigyne, EQ ergatoid queen, AQ alate queen

^bClonal species without queen caste

^cThe colony included only larvae but no other brood, and the queen had no developing oocytes despite accumulated yellow bodies (Villet et al. 1991)

^dWilson (1959b) described larvae collected from an emigration trail as “approximately the same growth stage”

^eFunctional monogyny is suggested from the result of dissection (Masuko 2006)

August included males, indicating that their nuptial flight occurs in August. A theoretical study suggests that a DCF society in ants might show a male-biased sex ratio (Bulmer 1983, Pamilo 1991), and in fact, the male production rate of army ants is notably high (Schneirla 1956). In contrast, males were collected only rarely in our field collection (six

males from two colonies of CsL, one male from a colony of CsS). Sex investment in the *C. sulcinodis* complex seems to be exceptional, but our dataset suffers from low sampling frequency in September.

The brood composition of the field colonies suggests that the *C. sulcinodis* complex shows phasic reproduction like

other army and non-army doryline ants, which was confirmed by the laboratory-reared colonies. Borowiec (2016) noted that the colony composition of *Cerapachys antennatus* indicated synchronized brood development. In addition, we also confirmed phasic reproduction by rearing 10 colonies of eight *Cerapachys* species under laboratory conditions (Mizuno et al. in prep.). Phasic reproduction might be a common biological character of this genus, which is also common in non-army doryline ants except for a few non-phasic species in *Lioponera*, *Tanipone*, and *Simopone* (Borowiec 2016, Idogawa and Dobata 2018, Ito et al. 2018).

Caste dimorphism and morphology of the ergatoid queens

The degree of caste dimorphism in the *C. sulcinodis* complex is well developed in both species. The queens have a notably large body, large number of ovarioles, and a spermatheca. Their body shape was allometrically different from that of their workers. As summarized in the introduction, caste dimorphism and specialization of DCF (= produce ergatoid queens with conspecific winged queens or not) are important characters of the ergatoid queen and the colony reproductive strategy. As shown above, all the collected queens of both species of the *C. sulcinodis* complex were ergatoid, and no conspecific alate queen was found. Johnson (2010) reported the independent colony foundation by flightless queens in harvester ant *Pogonomyrmex* spp., and argued that the existence of flightless queen is not the strong evidence of DCF. However, the smallest colony of *C. sulcinodis* complex included at least 100 workers, and no incipient colonies was collected in our colony sampling. Also, solitary foraging ergatoid queen was never be observed during the field observation (Mizuno et al. 2019). Hence, the colonies of the *C. sulcinodis* complex are very likely to be reproduced by DCF obligately. The social structure of the *C. sulcinodis* complex shows obligate DCF with developed ergatoid queens, in the same pattern as is known for true army ants.

In spite of their large body size, the ergatoid queens of the *C. sulcinodis* complex have a narrow and simple mesosoma like their workers, rather than the dealate queen of a related species, *C. RM* sp. 6. The ergatoid queens lack wings and have fused thoracic sclerites. The abdominal width of the CsS ergatoid queen is wider than that of the dealate queen of *C. RM* sp. 6, even though their head width is almost the same, suggesting morphological specialization for reproductive function. Under laboratory conditions, the ergatoid queens of both species do not engage in brood care, nest maintenance, or foraging (Mizuno, unpublished). These morphological and behavioral characteristics of the ergatoid queens in the *C. sulcinodis* complex indicate that they are “sole-purpose” ergatoid queens.

Unlike the ergatoid queens of CsS, clear correlations between pronotum width and head width, and pronotum width and abdomen width were not observed in the queens of CsL, probably because of their relatively narrow body size range and/or unstable body proportion. However, the range in distribution of the head width of the queens is not considerably different between CsL and CsS (the ratio of the largest and smallest head width was 1.08 in both); therefore, the proportion of the body parts in the CsL queens seems to be more unstable than that in the CsS queens. A similar trend is also observed in the distribution of the number of ovarioles per CsL queen that shows larger variance and weaker kurtosis (= wide and blunt distribution).

In spite of conspicuous caste dimorphism and morphological specialization in the mesosoma and abdomen, the ergatoid queens of the *C. sulcinodis* complex are not dichthadiiagnous. The abdomen of the queens of the *C. sulcinodis* complex is not extremely elongate and is never physogastric. Other morphological characters that are commonly found in army ant dichthadiiagnines (e.g., subglobular head shape, lack or small compound eyes, short mandible, and wide petiole, Gotwald 1995) are not conspicuous in the ergatoid queens of the *C. sulcinodis* complex. Accordingly, the degree of morphological specialization in the ergatoid queens of the *C. sulcinodis* complex is weaker than that of the army ant dichthadiiagnine. Queen winglessness is commonly found in many other non-army doryline genera like *Eburopone*, *Eusphinctus*, *Leptanilloides*, *Lioponera*, *Ooceraea*, *Parasyscia*, *Simopone*, *Sphinctomyrmex*, *Syscia*, *Tanipone*, and *Zasphinctus* (Brown 1975, Masuko 2006, Borowiec 2016). Notably, a subdichthadiiagnine is known in two species of the genus *Yunodorylus* (*Y. eguchii*, Eguchi et al. 2016; *Y. doryloides*, Satria et al. 2018), which constitutes the CCY clade with *Cerapachys* and *Chrysapace*.

In non-army doryline ants, colony size was reported in four ergatoid queen species (Table 3). In the genus *Lioponera*, the ergatoid queen species *L. dumbletoni* and *L. mg02* (Fisher and Peeters 2019) have larger colony sizes than that of the congeneric winged queen species *L. daikoku*, *L. punctatissima*, *L. suscitata*, and *Lioponera* sp.1. However, *Sy. humicola* has ergatoid queens but extremely small colony sizes (the largest colony consists of two ergatoid queens and 21 workers, Masuko 2006). These results suggest that queen winglessness is not directly related to the increase in their colony size in non-army dorylines. This situation is also the case in ponerine ants with ergatoid queens. For example, in *Leptogenys*, colony size variation among ergatoid species is less than 20 to more than 10,000 (Maschwitz et al. 1989, Ito 1997). Villet (1989) suggested that queen winglessness is led by difficulty of non-claustral colony foundation, especially in dietary specialized ponerine ants. He also pointed out that queen winglessness in some non-army dorylines (formally called

tribe Acanthostichini and Cerapachyini in the subfamily Ponerinae) might fit this hypothesis because of their dietary specialization for ants and termites. Reports on solitary colony foundation of the subfamily Dorylinae are scarce. Idogawa and Dobata (2018) collected four single foundress dealate queens of *L. daikoku*, two of which contained a *Monomorium* brood as prey in their nest chambers, suggesting non-claustral colony foundation. Keller et al. (2014) indicated that the configuration of the queen's 1st and 2nd thoracic segment reflects their way of colony foundation (i.e., claustral or non-claustral independent foundation) because of the skeletomuscular adaptation for foraging. They also show that the queens of the non-army ant doryline *Parasyscia nitidulus* (= *Cerapachys nitidulus*) and *Cylindromyrmex brevitarsus* have a relatively longer 1st thoracic segment, suggesting non-claustral colony foundation. The queen of our *C. RM* sp. 6 has a longer 1st thoracic segment ($T1/T2 = 0.32$), also supporting non-claustral colony foundation. Foraging for an ant brood by solitary queens might be difficult during the colony founding phase. The widespread occurrence of queen winglessness in non-army dorylines seems to be related to the intense difficulty of their independent colony foundation and might be a pre-adaptation for army ant evolution in the doryline lineage.

Worker polymorphism

The workers of the *C. sulcinodis* complex show a relatively wide variation in body size with allometric growth (= polymorphic), but they have no distinctive subcastes. From the result of morphometric analysis, the abdominal width of the workers is positively allometric to their pronotum width, in which the larger workers have a wider abdomen (= smaller workers have a slender abdomen) in both species. A similar pattern in which larger individuals have wider body parts was observed in the heads of the CsL workers (head length was negatively allometric to head width), but not in CsS.

Some army ant genera (e.g., *Dorylus*, *Eciton*, and *Labidus*) show well-developed subcastes and polyethism (Gotwald 1995). Unlike army ants, almost all non-army ant doryline genera have monomorphic workers (Borowiec 2016), but the workers of some species have a relatively wide body size range and allometric growth (Brown 1975). Wilson (1959b) described a small worker of *Parasyscia polynikes* having a more slender body than the larger workers, suggesting allometric growth. Borowiec (2016) wrote that *Cerapachys* and *Yunodorylus* workers are "monomorphic to moderately polymorphic", unlike other non-army dorylines. Our study shows that the smaller workers of the

C. sulcinodis complex have a more slender body, which is the same trend as pointed out in previous studies.

Conclusion

The biological and morphological characteristics investigated in this paper showed that the *C. sulcinodis* complex has an atypical biology which are reminiscent of the army ant adaptive syndrome. The biology of many non-army doryline genera has still not yet been studied. Comparative studies of non-army dorylinae, especially among the CCY clade, are necessary to discuss the evolutionary history of true army ants in the Old-World. Molecular phylogeny research suggests that true army ants independently occurred twice in the New- and Old-World (Borowiec 2019). Comparison of the basic biological knowledge of non-army ants in the New-World, especially sister genera of true army ants, such as *Acanthostichus*, *Cylindromyrmex*, *Leptanilloides*, *Neocerapachys*, and *Sphinctomyrmex*, is also needed to discuss the two-alternative evolution of the army ant adaptive syndrome in the Old- and the New-World.

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Author contributions FI designed the research; RM, FI and WJ identified ant species; RM, FI, WJ and PS performed field study; AY performed DNA barcoding. RM analyzed the data and wrote the primarily manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Availability of data and material Data deposited as electronic supplementary materials.

Code availability R code for morphometric analysis is available as electronic supplementary material.

Declarations

Conflicts of interest Not applicable.

Ethical approval Not applicable.

Consent to participate (include appropriate statements) All authors have approved for contribute on the research of this manuscript.

Consent for publication (include appropriate statements) All authors have approved for submission of this manuscript.

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