

# Evolution of a soldier caste specialized to lay unfertilized eggs in the ant genus *Crematogaster* (subgenus *Orthocrema*)



Christian Peeters<sup>a,\*</sup>, Chung-Chi Lin<sup>b</sup>, Yves Quinet<sup>c</sup>, Glauco Martins Segundo<sup>c</sup>, Johan Billen<sup>d</sup>

<sup>a</sup> CNRS UMR 7625, Laboratoire Ecologie & Evolution, Université Pierre et Marie Curie, 7 quai Saint Bernard, Paris 75005, France

<sup>b</sup> Department of Biology, National Changhua University of Education, Changhua 50007, Taiwan, ROC

<sup>c</sup> Laboratório de Entomologia, Universidade Estadual do Ceará, 1700 Avenida Paranjana, 60714-903 Fortaleza-CE, Brazil

<sup>d</sup> Laboratory of Entomology, K.U. Leuven, Naamsestraat 59, B-3000 Leuven, Belgium

## ARTICLE INFO

### Article history:

Received 28 November 2012

Accepted 11 February 2013

### Keywords:

Soldier caste  
Workers  
Repletes  
Spermatheca  
Flight sclerites  
Mosaic

## ABSTRACT

Among social Hymenoptera, only some ant genera have more than one morphological kind of non-reproductive adults. Individuals that are bigger than ordinary workers can function for defence and/or food storage. In *Crematogaster* (*Orthocrema*) *smithi* from Arizona, a third caste exists in addition to winged queens and workers; it is intermediate in size, weight and morphology, and individuals lay many unfertilized eggs that are mostly eaten by larvae (Heinze et al., 1995, 1999). We studied another three species belonging to the subgenus *Orthocrema*: *Crematogaster pygmaea* from Brazil, *Crematogaster biroi* and *Crematogaster schimmeri* from Taiwan. Using scanning electron microscopy and ovarian dissections, we show that 'intermediates' are a patchwork of queen-like and worker-like traits, just as in *C. smithi*; importantly the combinations differ across species. 'Intermediates' are numerically few in the colonies, and in *C. pygmaea* they are produced seasonally. Using histology we confirmed the lack of a spermatheca, thus they are not ergatoid queens. Based on the similarity of their mosaic phenotypes with those in other ant lineages, we suggest that *Orthocrema* 'intermediates' are a soldier caste with a specialized trophic function. This soldier caste has been reported in other *Orthocrema* species from Madagascar, Guinea and Costa Rica, suggesting that it is widespread in this subgenus.

© 2013 Elsevier Ltd. All rights reserved.

## 1. Introduction

Division of labour is especially efficient in ant societies because of a marked morphological specialisation among female nestmates. In addition to winged queens (adapted for colony foundation and elevated fertility) and wingless workers (adapted for maintenance activities on the ground), additional morphological castes occur sporadically across different lineages. Ergatoid (permanently wingless) queens reproduce in many species that have shifted away from independent colony foundation (Peeters, 2012). Soldiers bigger than ordinary workers exist in unrelated genera and have a variety of functions that include colony defence, food storage or seed-milling (Molet et al., 2012). Since both ergatoid queen and soldier castes evolved repeatedly, their morphological characteristics are highly heterogeneous.

*Crematogaster* is the third largest genus of ants (478 current species; subfamily Myrmicinae), and is a common and conspicuous component of tropical faunas on all continents. Workers are monomorphic or exhibit continuous size polymorphism (Longino, 2003). Wingless individuals with a big gaster were reported long ago in *Crematogaster minutissima* (Holliday, 1903) and *Crematogaster biroi* (Bingham, 1903 p. 138). Heinze et al. (1995, 1999) showed in *Crematogaster smithi* that a 'third caste' ('neither queens, nor workers') is intermediate in size, weight and morphology between workers and winged queens. These intermediates do not perform foraging, maintenance, or defensive duties. They are never inseminated, but they lay viable eggs that are mostly eaten by larvae. Similar wingless intermediates are known in other species of the subgenus *Orthocrema* (Bernard, 1952; Longino, 2003; Hamidi, 2010; Blaimer, 2012a), but the use of a variety of terms ("large workers", "ergatoids", "intercastes", "ergatogynes", "intermediate workers") has prevented a comparative perspective.

The subgenus *Orthocrema* (108 species) is strongly divergent and sister to all other *Crematogaster* (Blaimer, 2012b). We compared

\* Corresponding author. Tel.: +33 146592763; fax: +33 144273516.

E-mail addresses: [cpeeters@snv.jussieu.fr](mailto:cpeeters@snv.jussieu.fr) (C. Peeters), [cdlin@cc.ncue.edu.tw](mailto:cdlin@cc.ncue.edu.tw) (C.-C. Lin), [yvesq@terra.com.br](mailto:yvesq@terra.com.br) (Y. Quinet), [glaucombssegundo@hotmail.com](mailto:glaucombssegundo@hotmail.com) (G. Martins Segundo), [Johan.Billen@bio.kuleuven.be](mailto:Johan.Billen@bio.kuleuven.be) (J. Billen).

the morphology of ‘intermediates’ in *Crematogaster pygmaea* from Brazil, and *C. biroi* and *Crematogaster schimmeri* from Taiwan. We show that ‘intermediates’ are mosaics of queen-like and worker-like morphological traits. Importantly, they all lack a spermatheca, hence they cannot reproduce sexually and are not ergatoid queens. Based on comparisons across ant lineages, we argue that these ‘intermediates’ are a soldier caste with an exclusively trophic function.

## 2. Methods

### 2.1. Colony sampling

All three species nest in the ground. Colonies of *C. pygmaea* are highly polygynous and polydomous, with dozens of nests (simple 20 cm vertical tunnels connecting 1–4 horizontal chambers) connected by trails (Quinet et al., 2009). A total of 136 nests were excavated in Fortaleza and Eusébio (State of Ceará, Brazil) in December 2010–February 2011 (end of dry season), and mid-April 2011 (rainy season) (Table S1). The habitat was open areas in the savanna-like “tabuleiro”, adjacent to the littoral zone. Ten nests were also excavated in February 2011, in Serra das Almas Reserve, 300 km south of Fortaleza, in the “caatinga” zone, a seasonal xerophilous thorn shrubland that prevails on the semi-arid lowlands and covers most of Ceará State. Workers were not counted but Quinet et al. (2009) found a queen/worker ratio of 1:100.

Twenty-five queenright nests of *C. biroi* were collected in Taiwan, in Hushan (Yunlin county), Lienhuachih and Huisin (Nantou county), and Chuchih (Taipei county), during 2010–2011 (Table S1). Nests occur in rotten wood, in soil, and under stone in broad-leaved forests. In these same habitats (Hushan, Chichi (Nantou county) and Luyeh (Taitung county)), we also collected six queenright nests of *C. schimmeri*.

### 2.2. Histology

The posterior half of the gasters of 5 ‘intermediates’ of *C. biroi* and *C. pygmaea* were fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose. Tissues were postfixed in 2% osmium tetroxide in the same buffer, dehydrated in a graded acetone series and embedded in Araldite. Serial semithin sections with a thickness of 1 µm were made with a Leica EM UC6 ultramicrotome and viewed with an Olympus BX-51 microscope to check for the eventual presence of a spermatheca.

### 2.3. Other morphological observations

Specimens of the three castes of *C. pygmaea* (5 queens, 5 workers, 2 ‘intermediates’), *C. biroi* (3 queens, 5 workers, 3 ‘intermediates’), *C. schimmeri* (2 queens, 5 workers, 5 ‘intermediates’) were measured (total length from middle of clypeus to tip of gaster; width of first gaster segment) and weighed. Specimens of the three species were coated with a thin gold layer by sputtering, and examined with a JEOL JSM-6360 scanning electron microscope. Gasters were dissected in all three castes of the three species, to describe ovaries, yellow bodies, and check for eventual presence of a spermatheca.

## 3. Results

### 3.1. Occurrence of ‘intermediates’ in colonies

Seventy-one nests belonging to one *C. pygmaea* colony excavated in April (rainy season) yielded 14 ‘intermediates’, present in eight of the 25 more populous nests (with two or more queens). In

contrast, 75 nests (from 4 colonies) excavated a few months earlier (end of dry season) yielded only four ‘intermediates’ (Table S1).

*C. biroi* colonies ( $n = 25$ ) were monogynous with 12–479 workers and 1–5 ‘intermediates’ (26 in total). Colonies of *C. schimmeri* ( $n = 6$ ) were also monogynous with 314–626 workers and 14–23 ‘intermediates’ (103 in total) (Table S1).

### 3.2. External morphology of ‘intermediates’

Winged queens and workers of all species are highly divergent in total size, and the ‘intermediates’ fall in between (Fig. 1). Both linear measures and weights were intermediate (Table 1). Size differences in head and thorax are clearly illustrated in Figs. 2 and 3.

In *C. pygmaea*, ‘intermediates’ have less protruding eyes than winged queens, with a number of ommatidia ( $130 \pm 6$ ) midway between workers ( $54 \pm 5$ ) and queens ( $267 \pm 6$ ; Table 1). Similarly in *C. biroi* and *C. schimmeri*, ommatidia numbers of ‘intermediates’ are in-between queens and workers (Table 1). Compared to queens, the ocelli in ‘intermediates’ are reduced although very distinct (lens and small patch of photoreceptors) in *C. pygmaea* and *C. biroi*, while they are not seen in *C. schimmeri* (Fig. 2, Table 1).

Ant queens have a flight thorax in which the second segment (mesonotum) is greatly enlarged because it functions to attach the considerable wing muscles. The first segment (pronotum) is strongly reduced, unlike in workers where winglessness causes it to be more prominent (Keller et al., submitted for publication). *Orthocrema* queens have indeed a large raised mesonotum that completely hides the pronotum in dorsal view (Fig. 3). In workers, although pronotum and mesonotum are fused, the former is obviously bigger. In ‘intermediates’, despite winglessness, the mesonotum is bigger than in workers but the pronotum is visible dorsally (Fig. 3). Moreover, ‘intermediates’ exhibit striking interspecific differences in thorax structure. In *C. biroi*, a suture (i.e. line or groove indicating the junction of two formerly articulated sclerites) cannot be seen between pronotum and mesonotum. In *C. schimmeri*, the pro-mesonotal suture is distinct and importantly, the mesonotum is less reduced in size (Fig. 3). Too few specimens were examined by SEM to assess intraspecific variability in ‘intermediates’.

### 3.3. Internal morphology of ‘intermediates’

Dissections of ‘intermediates’ revealed striking differences in numbers of ovarioles: in-between queens and workers in *C. pygmaea* and *C. biroi*, but same as queens in *C. schimmeri* (Table 1). Yolk oocytes were present (Fig. 4A–C) and eggs were laid before dissections in *C. biroi*. Dark yellow bodies were conspicuous at the base of ovarioles in ‘intermediates’, indicating that many eggs had been laid earlier. Unlike in queens, a spermatheca was not found in ‘intermediates’, and this was confirmed by both transverse and longitudinal histological sections (Fig. 4D–E).

## 4. Discussion

### 4.1. *Orthocrema* ‘intermediates’ are a mosaic of queen-like and worker-like traits

Our morphological data in *C. pygmaea*, *C. biroi* and *C. schimmeri* show that all have a third caste combining morphological traits from winged queens and workers. Individuals with intermediate size are very conspicuous (Fig. 1) given the striking size differences between queens and workers in the subgenus *Orthocrema*. The gaster of ‘intermediates’ is particularly big, and this reflects a higher number of ovarioles compared to workers (Table 1). Most individuals dissected had either active ovaries (i.e. many developing

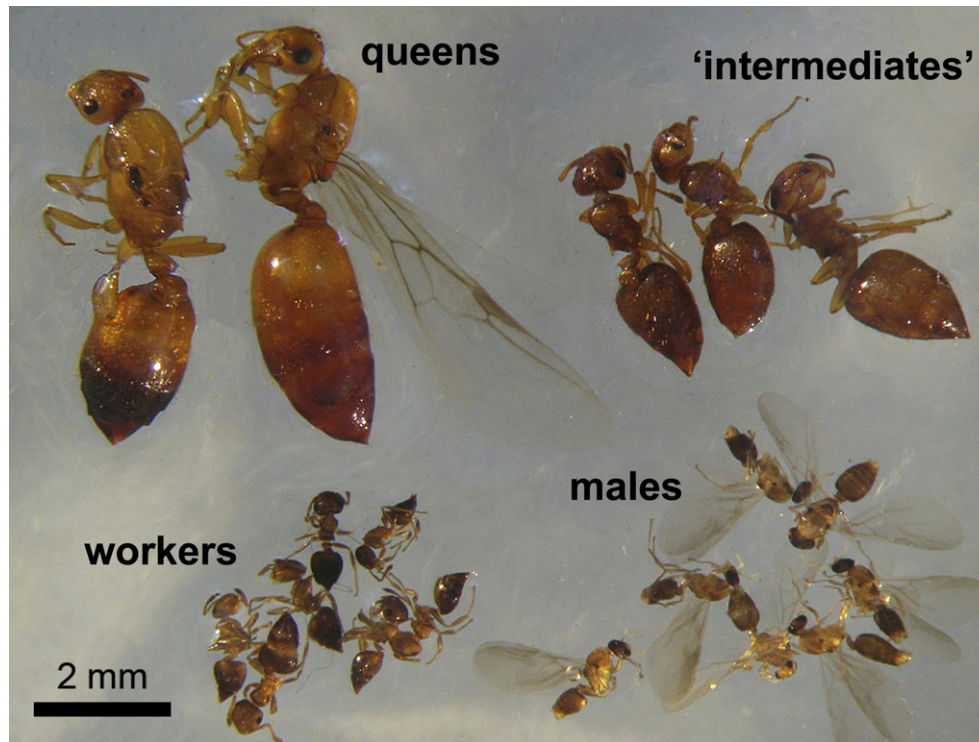


Fig. 1. Comparison of all three female castes as well as males in *C. pygmaea*, showing the large size differences.

yolky oocytes) or yellow bodies at the bases (indicating past egg-laying) (Fig. 4). Importantly, all 'intermediates' lacked a spermatheca (confirmed by histology), which means that they cannot store sperm to fertilize their eggs, hence they cannot produce diploid offspring. Therefore they are not ergatoid queens as found in many ants across all lineages (Peeters, 2012), or as suggested for *C. biroi* (Bingham, 1903 p. 138).

Winged queens typically have big compound eyes (many ommatidia) and three ocelli (involved in stabilization reflexes) as adaptations for flying. 'Intermediates' in all three species had fewer ommatidia while the ocelli were vestigial (or absent in *C. schimmeri*) (Table 1, Fig. 2). The thorax was bigger than in workers, the mesonotum exceeded the pronotum and other sclerites were incompletely fused (Fig. 3). Such an incomplete flight thorax lacks functional significance but reflects partial activation of a winged queen's developmental program. Similarly for ommatidia numbers that are midway between winged queens and workers: since 'intermediates' are not active outside the nest, bigger eyes are unlikely to bring any adaptive advantage, but it is another evidence

of a partially expressed queen phenotype. 'Intermediates' are a mosaic of several queen-like and worker-like traits. Importantly, traits such as ommatidia number, ocelli, thorax structure and ovariole number were not identical to those in the winged queen and worker castes (Table 1), and overall size of 'intermediates' was in-between queens and workers.

We emphasize that even though all intermediates had a mosaic nature, across species they differed in the pattern of association of queen-like and worker-like traits. For example, ovariole number in *C. biroi* was intermediate, but it overlapped with queens in *C. schimmeri*. The mesonotum was more queen-like in *C. schimmeri* than in the other species, but this was not true for ocelli. However, 'intermediates' always had larger ovaries and gaster than workers, presumably because such traits are adaptive for egg production. In the absence of a detailed phylogeny as well as life history data for more species, we cannot determine if *Orthocrema* 'intermediates' represent multiple independent evolutionary events, or a single evolution in a basal ancestor followed by gradual modifications.

Table 1

Comparison of morphological traits across three castes of three species of *Crematogaster* (*Orthocrema*). Each ommatidia count corresponds to a different individual. Linear measures and weights are averages (see Methods for sample sizes).

|                     | Total length/gaster width (mm) | Weight (mg) | Raised ocelli | Number of ommatidia         | Number of ovarioles (ants dissected) |
|---------------------|--------------------------------|-------------|---------------|-----------------------------|--------------------------------------|
| <i>C. pygmaea</i>   |                                |             |               |                             |                                      |
| Queens              | 6.59/1.78                      | 6.98        | Yes           | 260-263-265-269-276         | 20–26 (n = 7)                        |
| 'Intermediates'     | 5.18/1.57                      | 4.1         | Reduced       | 119-128-129-130-131-131-139 | 18–20 (n = 7)                        |
| Workers             | 2.1/0.66                       | 0.42        | No            | 49-49-54-55-59-61           | 2 (n = 10)                           |
| <i>C. biroi</i>     |                                |             |               |                             |                                      |
| Queens              | 4.83/1.16                      | 5.00        | Yes           | 120-122-124                 | 12–16 (n = 6)                        |
| 'Intermediates'     | 4.42/1.22                      | 3.59        | Reduced       | 84-86                       | 5–8 (n = 6)                          |
| Workers             | 2.15/0.6                       | 0.43        | No            | 36-38-42-45-46              | 2 (n = 12)                           |
| <i>C. schimmeri</i> |                                |             |               |                             |                                      |
| Queens              | 5.96/1.37                      | 5.47        | Yes           | 197-215-225                 | 12–20 (n = 3)                        |
| 'Intermediates'     | 4.47/1.49                      | 3.8         | No            | 101-113-120-128             | 12–16 (n = 5)                        |
| Workers             | 2.19/0.69                      | 0.48        | No            | 45-46-49-49-52-59           | 2 (n = 10)                           |

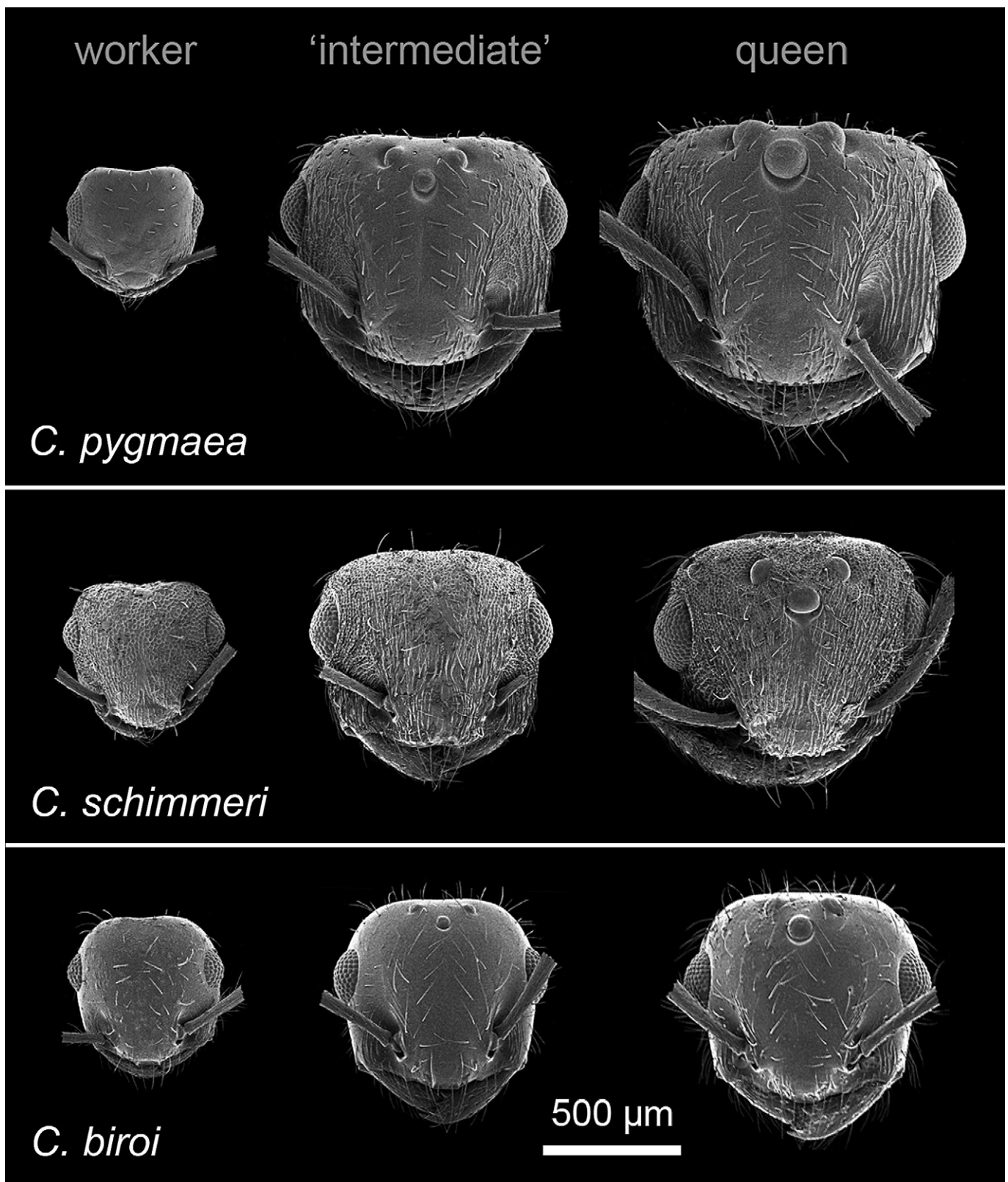
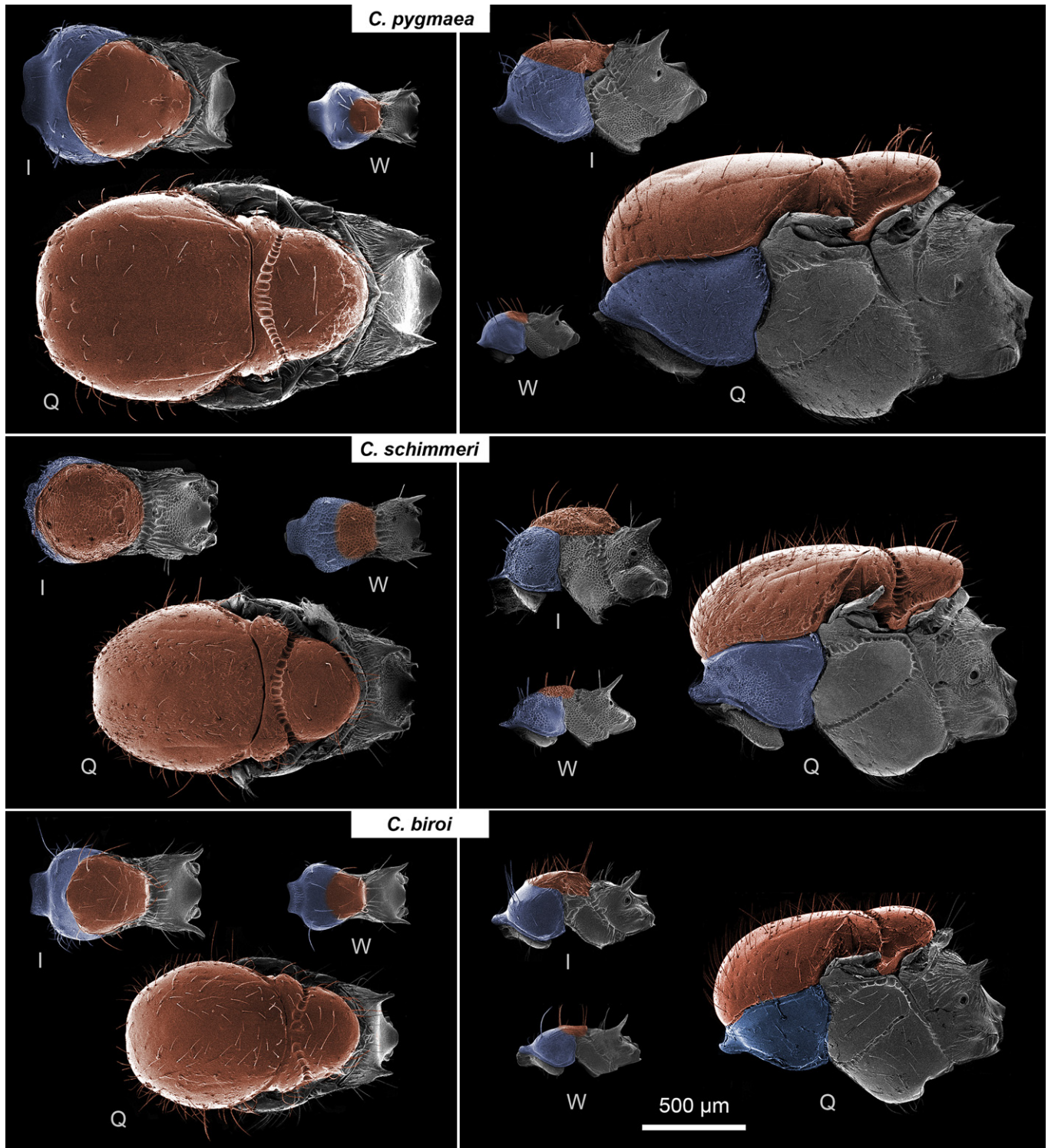


Fig. 2. Scanning electron micrographs of heads of three castes in 3 *Orthocrema* species. Note occurrence of ocelli and size of eyes. All printed at same scale.





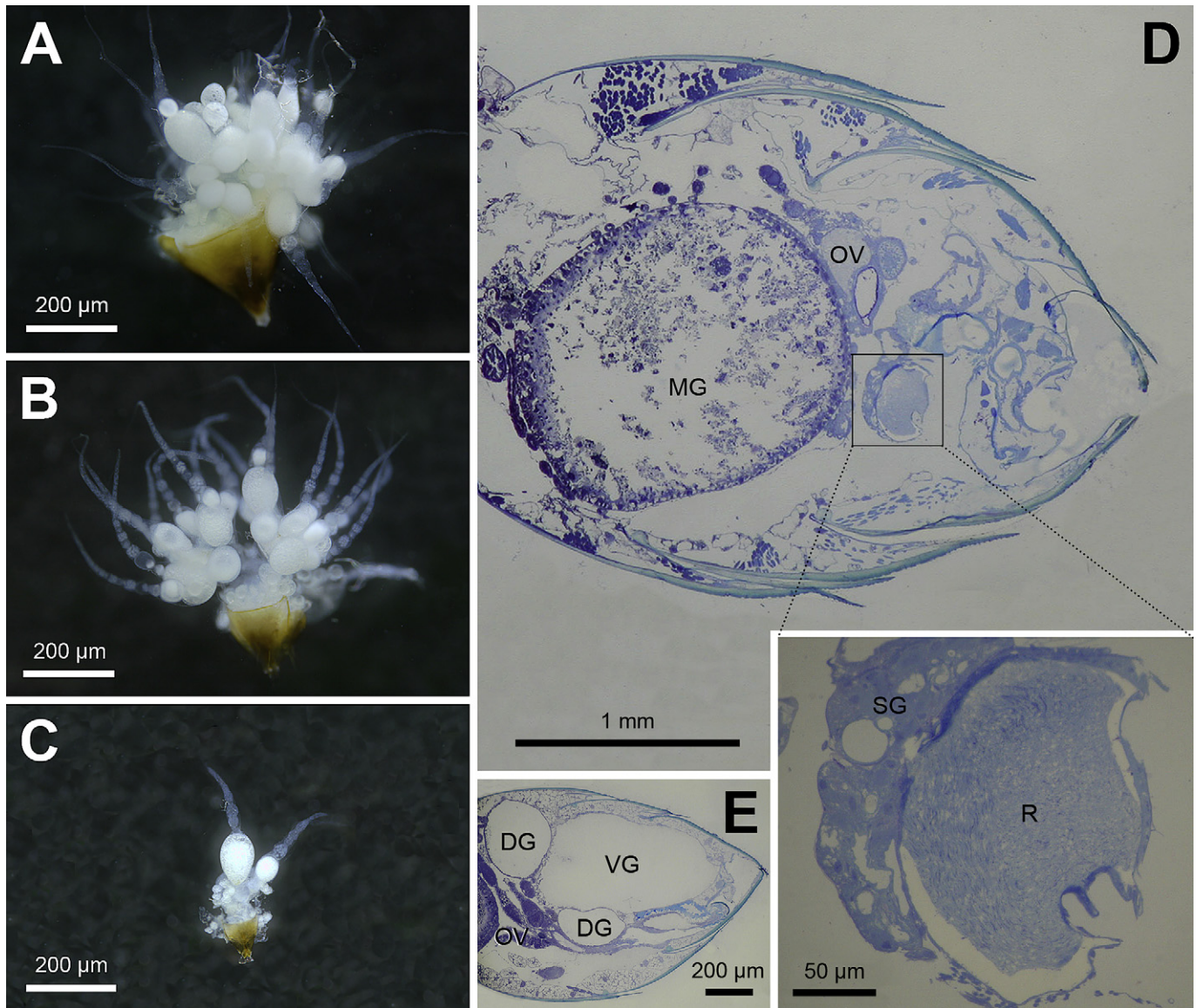
**Fig. 3.** Dorsal and lateral SEMs of thorax of three castes (Q: queen, I: 'intermediate'; W: worker) in 3 *Orthocrema* species. Note different sizes and degrees of fusion of flight sclerites. Pronotum (T1) coloured in blue, mesonotum (T2) in red. All printed at same scale.

#### 4.2. *Orthocrema* 'intermediates' are specialized to lay unfertilized eggs

Unlike solitary insects that forage for their own needs, social insects can take in and process excess food that is later shared among colony members. This key adaptation can also overcome any seasonal trophic stress, because food can be stored during times of

plenty. Two mechanisms of food storage and exchange involve living ants – trophallaxis and egg consumption – both of which can be enhanced by morphological specialization (Wheeler, 1994). First, repletes in Myrmicinae and Formicinae have a highly expanded crop and gaster (Hölldobler and Wilson, 1990). Second, eggs are packages of proteins and energy, and bigger ovaries are an alternative to the adaptations of repletes. Both the digestive capabilities





**Fig. 4.** Developed ovaries of queen (A), 'intermediate' (B) and worker (C) of *C. schimperi*, and longitudinal sections through posterior abdomen of queen (D) and 'intermediate' (E) of *C. pygmaea*, both shown at same scale. Note absence of spermatheca in 'intermediate'. Inset in D shows enlargement of filled spermatheca of queen. DG: Dufour gland, MG: midgut, OV: ovaries, R: spermatheca reservoir, SG: spermatheca gland.

of workers or larvae and the dietary regime can influence which of these two methods of food redistribution occurs (Wheeler, 1994).

In *C. smithi*, both workers and 'intermediates' lay eggs that are the major protein source for larvae. Heinze et al. (1995, 1999) suggested that  $10 \pm 2$  ovarioles in 'intermediates' (vs. 2 in workers) give them much higher fecundity. This was confirmed by laboratory observations: an isolated 'intermediate' laid up to 20 eggs in 72 h, compared to <18 eggs in groups of 50 workers. Moreover, *C. smithi* 'intermediates' are highly efficient egg-layers, averaging 0.97 eggs per day and per milligram fresh weight, compared to 0.05 eggs for workers (Heinze et al., 1995, 1999). In *C. smithi* some unfertilized eggs can develop into males (Heinze et al., 2000), which may also give a fitness advantage after the death of the founding queen.

We lack data on behaviour and oviposition rates for 'intermediates' of the Brazilian and Taiwanese species, but their morphological similarity to *C. smithi* leads us to speculate that 'intermediates' are also trophic specialists. *C. smithi* 'intermediates'

were constantly surrounded and groomed by a court of workers (Heinze et al., 1999), and this was also observed in *C. pygmaea* (Hamidi, 2010); such behaviour is consistent with emission of a fertility signal linked to oogenesis (Peeters and Liebig, 2009). When liquid food with a red dye was given to *C. birroi*, after four days most of the first and second instar larvae had red stomachs, unlike most of the third and fourth instar larvae. This suggests that young larvae receive liquid food from the workers, while older larvae eat eggs. In *C. smithi*, larvae are not fed with pieces of solid prey and eggs appear to be the sole food for larvae (Heinze et al., 1995, 1999). The behaviour of 'trophic specialists' needs to be studied further in our three species.

#### 4.3. *Orthocrema* 'intermediates' correspond to a soldier caste

The third caste in *Orthocrema* shares many characteristics with the soldier caste in other ant genera. To avoid multiplying terms each time a new phenotype is described across the ants, we suggest

that “soldier caste” is appropriate, even though there is no defensive function (see below). The term “soldier” has a long history in ant biology (e.g. Wheeler, 1910) to describe wingless individuals that are neither queens (with or without wings) nor workers and that have a restricted behavioural repertoire (specialized colony-maintenance functions include defense, food storage or seed-milling). Irrespective of function, soldiers always have an intermediate size between winged queens and workers, and in many species their head is enlarged with bigger mandible muscles and specialized mandible shapes (see Molet et al., 2012).

A soldier caste without a defensive function is known in other ants, e.g. soldiers mill seeds in *Acanthomyrmex ferox* (Gobin and Ito, 2000) and various *Pheidole* (Wilson, 1984). In other genera they are known to function as repletes, especially when young, e.g. *Colobopsis* (Hasegawa, 1993; Wilson, 1974), *Cataglyphis bombycina* (Molet et al., in prep.), *Pheidole ryukyensis* (Tsuji, 1990). However, there are also many genera with size-polymorphic workers, and the larger workers (‘majors’) can function as repletes (Hölldobler and Wilson, 1990).

A bigger gaster is an important adaptation for food storage, to accommodate a distended crop filled with liquid (mostly glucose), hypertrophied fat bodies, or more ovarioles for higher egg-laying capability. In *Crematogaster* and many other myrmicine ants, size dimorphism between winged queens and workers is considerable (Fig. 1), and there is thus an empty morphospace with potential new functions. Workers have only two ovarioles, thus they can lay very few eggs (Fig. 4C), but a wingless third caste with more ovarioles can be adaptive for enhanced food redistribution. The intermediate size and mosaic nature of ant soldiers support the idea that parts of the developmental programs of existing castes are combined to produce a phenotype that is both wingless and bigger than workers (Molet et al., 2012).

Another characteristic of ant soldiers is that they constitute a tiny proportion of adult population in colonies. This is also true in *Orthocrema*. In *C. smithi*, half of all colonies had between one and ten soldiers (Heinze et al., 1999). Similarly in *C. biroi* and *C. pygmaea*, only small numbers of soldiers were found (they were more numerous in *C. schimmeri*, Table S1). *C. pygmaea* is affected by a semi-arid climate, with rainfall restricted to three consecutive months during summer. The rainy season triggers an increase in queen number, together with colony growth and expansion into additional nests (Hamidi, 2010). Such temporal polydomy allows tracking fluctuating food sources, i.e. seasonal vegetation with nectaries or homopterans (Quinet et al., 2009). Nests sampled before and at the beginning of the rainy season mostly lacked soldiers (Table S1), but one month later soldiers were found. Hamidi (2010) reported a maximum of 5–10 soldiers per nest collected during the rainy season. It is possible that *C. pygmaea* soldiers function to store abundant but highly seasonal food resources and redistribute this as eggs that can be stored over several weeks. Similarly, *C. smithi* occurs at 2000 m elevation in Arizona, which corresponds to an arid, strongly seasonal habitat (Heinze et al., 1999). However, there is no seasonal fluctuation in Taiwan: foragers of *C. biroi* and *C. schimmeri* remained active throughout the year, and we collected soldiers in the majority of nests notwithstanding of season. Both Taiwanese species are monogynous, in contrast with polygyny in *C. pygmaea*, indicating that trophic specialists are selected for across a broad range of reproductive systems and colony sizes.

#### 4.4. Comparative overview of soldier caste in *Orthocrema*

A third caste has been reported in several species of the subgenus *Orthocrema*. In *Crematogaster pulchella* from Guinea, Bernard (1952 p. 230) described an “ergatoid gyne” twice as long as

workers, with two posterior ocelli and a gaster proportionally bigger than in winged queens and workers (Fig. 5). In Costa Rica, soldiers (“ergatogynes”) were reported to be common in *Crematogaster bryophila*, *Crematogaster curvispinosa* and *Crematogaster nigropilosa* (Longino, 2003). Given monogyny and polydomy in the latter species, nests can be found without the functional (dealate) queen, but with one or more soldiers. Many nests only had soldiers, workers and brood, but in other nests soldiers occurred with a single physogastric queen. Data are needed about ovariole number in all these species. In *C. curvispinosa* from Mexico, Heinze et al. (1999) reported soldiers having more ovarioles than workers.

In Madagascar, a maximum of 8 soldiers (“intermediate workers”, “intermorphs”) were found in four queenright colonies of *Crematogaster rasoherinae* (Blaimer, 2012a). A cuticular outgrowth occurs where wings normally attach. Similarly in *Crematogaster madecassa*, soldiers had intermediate measurements and a more complex thorax than in workers (bigger mesonotum, wing attachment sutures) (Blaimer, 2012a). Interestingly, *Crematogaster volamena* (also from Madagascar) has individuals bigger than ordinary workers, with a distinctly enlarged head and more powerful mandibles. They do not share the attributes of the intermediates in *C. rasoherinae* and *C. madecassa*, such as presence of ocelli and a queen-like thorax (Blaimer, 2012a). No information is available about ovariole number in *C. volamena*, but this may be a soldier caste with a different phenotype, i.e. a distinct patchwork of queen-like and worker-like traits selected for defence.

Until now a third caste has been reported in 11 species of *Orthocrema*, but none in the other subgenera of *Crematogaster*. We currently ignore whether any *Orthocrema* species lacks soldiers. Across several continents, *Orthocrema* soldiers share an increased gaster volume, but more data are needed on ovariole numbers. Behavioural studies of food storage in *Crematogaster* (*Orthocrema*) must investigate the link with big queen-worker dimorphism in

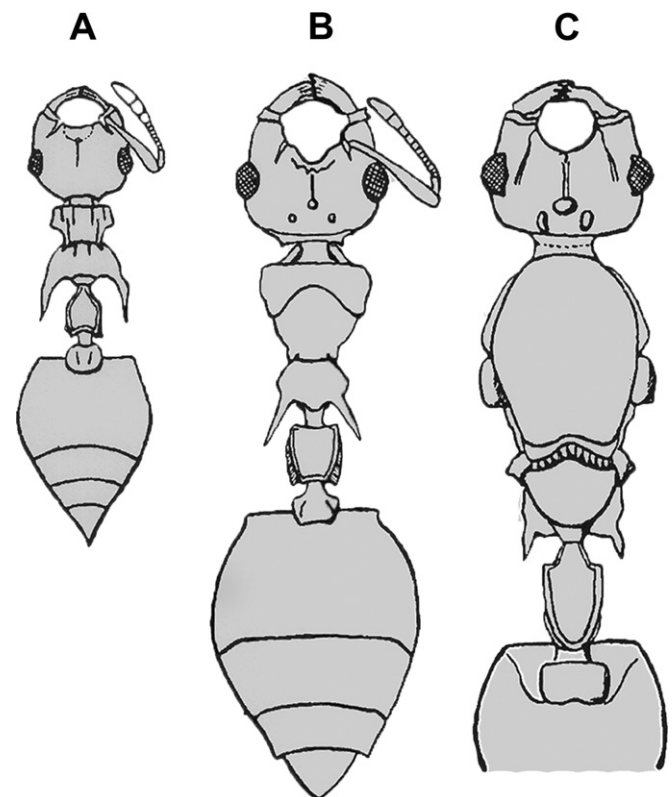


Fig. 5. Schematic drawing of the three female castes in *C. pulchella* from Guinea (West Africa): worker (A), soldier (B) and dealate queen (C). Reproduced from Bernard (1952).



size, as well as the adaptive benefits in seasonally constrained environments.

## Acknowledgements

We thank Jean-Christophe de Biseau and Rachid Hamidi for stimulating our interest in the third caste of *C. pygmaea*. An Vandoren and Alex Vrijdaghs assisted with tissue preparation for histology and scanning microscopy. Roberto Keller helped to interpret thorax morphology and Fig. 3. We are grateful to J.-C. de Biseau, R. Keller and M. Molet for comments on this manuscript.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.asd.2013.02.003>.

## References

- Bernard, F., 1952. La réserve naturelle intégrale du Mt Nimba. XI. Hyménoptères Formicidae. Mémoires de l'Institut Français d'Afrique Noire 19, 165–270.
- Bingham, C.T., 1903. The fauna of British India, including Ceylon and Burma. In: Hymenoptera. Ants and Cuckoo-wasps, vol. II. Taylor and Francis, London, p. 506.
- Blaimer, B.B., 2012a. Taxonomy and species-groups of the subgenus *Crematogaster* (*Orthocrema*) in the Malagasy region (Hymenoptera, Formicidae). ZooKeys 199, 23–70.
- Blaimer, B.B., 2012b. Acrobat ants go global – origin, evolution and systematics of the genus *Crematogaster* (Hymenoptera: Formicidae). Molecular Phylogenetics and Evolution 65, 421–436.
- Gobin, B., Ito, F., 2000. Queens and major workers of *Acanthomyrmex ferox* redistribute nutrients with trophic eggs. Naturwissenschaften 87, 323–326.
- Hamidi, R., 2010. Structure sociale et stratégies de reproduction chez la fourmi hautement polygyne *Crematogaster pygmaea*. Unpublished thesis, Université Libre de Bruxelles, Belgium.
- Hasegawa, E., 1993. Caste specialization in food storage in the dimorphic ant *Colobopsis nipponicus* (Wheeler). Insectes Sociaux 40, 261–271.
- Heinze, J., Cover, S.P., Hölldobler, B., 1995. Neither worker, nor queen: an ant caste specialized in the production of unfertilized eggs. Psyche 102, 173–185.
- Heinze, J., Foitzik, S., Oberstadt, B., Ruppel, O., Hölldobler, B., 1999. A female caste specialized for the production of unfertilized eggs in the ant *Crematogaster smithi*. Naturwissenschaften 86, 93–95.
- Heinze, J., Strätz, M., Pedersen, J.S., Haberl, M., 2000. Microsatellite analysis suggests occasional worker reproduction in the monogynous ant *Crematogaster smithi*. Insectes Sociaux 47, 299–301.
- Hölldobler, B., Wilson, E.O., 1990. The Ants. Harvard University Press, Cambridge, Massachusetts.
- Holliday, M., 1903. A study of some ergatogynic ants. In: Zoologische Jahrbücher, vol. 19. Abteilung für Systematik, Geographie und Biologie der Tiere. pp. 293–328.
- Keller, R.A., Peeters, C., Beldade, P. Thorax morphology in ant queens and workers shows divergent specialization for behaviours on the ground, submitted for publication.
- Longino, J.T., 2003. The *Crematogaster* (Hymenoptera, Formicidae, Myrmicinae) of Costa Rica. Zootaxa 151, 1–150.
- Molet, M., Wheeler, D., Peeters, C., 2012. Evolution of novel mosaic castes in ants: modularity, phenotypic plasticity, and colonial buffering. The American Naturalist 180, 328–341.
- Molet, M., Maicher, V., Peeters, C. Bigger helpers in the ant *Cataglyphis bombycina*: increased worker polymorphism or novel soldier caste? In preparation.
- Peeters, C., 2012. Convergent evolution of wingless reproductives across all sub-families of ants, and sporadic loss of winged queens (Hymenoptera: Formicidae). Myrmecological News 16, 75–91.
- Peeters, C., Liebig, J., 2009. Fertility signaling as a general mechanism of regulating reproductive division of labor in ants. In: Gadau, J., Fewell, J. (Eds.), Organization of Insect Societies: From Genome to Socio-complexity. Harvard University Press, Cambridge, pp. 220–242.
- Quinet, Y., Hamidi, R., Ruiz-Gonzalez, M., de Biseau, J.-C., Longino, J.T., 2009. *Crematogaster pygmaea* (Hymenoptera: Formicidae: Myrmicinae), a highly polygynous and polydomous *Crematogaster* from northeastern Brazil. Zootaxa 2075, 45–54.
- Tsuji, K., 1990. Nutrient storage in the major workers of *Pheidole ryukyensis* (Hymenoptera: Formicidae). Applied Entomology & Zoology 25, 283–287.
- Wheeler, D.E., 1994. Nourishment in ants: patterns in individuals and societies. In: Hunt, J.H., Nalepa, C.A. (Eds.), Nourishment and Evolution in Insect Societies. Westview Press, Boulder, Colorado, pp. 245–278.
- Wheeler, W.M., 1910. Ants: Their Structure, Development and Behavior. Columbia University Press, New York.
- Wilson, E.O., 1974. The soldier of the ant *Camponotus (Colobopsis) fraxinicola*, as a trophic caste. Psyche 81, 182–188.
- Wilson, E.O., 1984. The relation between caste ratios and division of labor in the ant genus *Pheidole* (Hymenoptera: Formicidae). Behavioral Ecology and Sociobiology 16, 89–98.