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„Phenotypic integration and caste characterization in the ant tribe Camponotini“

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### **Dedication**

I have a hand with which to hold  
The tiniest of things in place  
To see an eye with glints of gold  
A claw, antenna, hair or face

The other hand holds pin and needle  
Callus, blisters, stains of glue  
To transfer what is fragile, feeble  
On paper to be seen and true

And after hours – looking, turning  
I cannot shake what I have seen  
Engraved into my eyes by burning  
Lights she looks at me, my queen

The marks it leaves upon my shell  
To be what I have come to be  
A prick of pin, a stain, a smell  
All changing, all transforming me

To my companions on this path  
Be they with six legs, four or two  
It takes brave madness if you love  
The small and strange – this is for you!

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## Contents

1. Introduction .....	1
1.1. Camponotine castes – phenotypes, functions and curiosities .....	1
1.2. Phenotypic integration and modularity – the “building blocks” of life .....	3
1.3. Aims and scope .....	4
2. Included manuscripts and publications .....	6
2.1. Published results .....	6
2.2. Submitted manuscript .....	8
Study 1 .....	9
Study 2 .....	20
Study 3 .....	38
Study 4 .....	46
Study 5 .....	90
3. Discussion .....	134
3.1. Novel insights and consequences.....	134
3.1.1. Which measurements and proportions of external characters are suitable to characterize castes of the camponotine genera <i>Camponotus</i> and <i>Colobopsis</i> ?... 134	
3.1.2. Which character-sets can be interpreted as phenotypically integrated modules? .....	136
3.1.3. How does the influence of mermithid parasitism during ontogenetic development affect morphological proportions and patterns of phenotypic integration in the host ant, and are these changes comparable across host taxa? .....	138

3.2. Open questions and avenues for further research .....	139
3.2.1. Minor workers: Size-variation as an indication of intra-caste polyethism? .....	139
3.2.2. Evolutionary origin of the major worker caste .....	140
3.2.3. Origin and plasticity of mermithogenic phenotypes .....	141
3.2.4. Ecology and evolution of the COCY group .....	142
3.2.5. Adaptive value of integrated modules .....	143
References .....	145
Summary .....	152
Zusammenfassung .....	154

## 1. Introduction

### 1.1. Camponotine castes – phenotypes, functions and curiosities

The ant tribe Camponotini currently includes 1,883 valid species within eight genera (Ward et al. 2016, Bolton 2018). Among them are some of the most striking and peculiar ants known to science, such as the often bizarrely hairy genus *Echinopla* (e.g., Zettel & Laciny 2015), the thorn-bearing *Polyrhachis* (e.g., Dorow 1995), or one of the largest ants, *Dinomyrmex gigas* (Pfeiffer & Linsenmair 2000). In the past, researchers have placed particular focus on the most speciose of the camponotine genera, *Camponotus*, not lastly because of the plethora of different types of worker caste differentiation and diverse lifestyles represented among its more than 1,000 members (Wilson 1953, Wheeler 1986). Using morphometric data, previous studies were able to characterize different forms of worker caste polymorphism in various species of *Camponotus*, indicating a gradual transition from monophasic allometry to complete dimorphism of the worker caste, which has arisen independently multiple times (e.g., Wilson 1953, Busher et al. 1985, Espadaler 1990, Pfeiffer & Linsenmair 2000).

A special case of worker caste dimorphism is known from the former subgenus of *Camponotus*, *Colobopsis* (re-established as a separate genus by Ward et al. 2016): Here, the specialized major workers or “soldiers” bear plug-shaped heads used to close nest-entrances. This phenomenon of animals using parts of their bodies to obstruct their nests is termed “phragmosis”. Several cases of independent evolution of diverse phragmotic structures have also been described in at least five other genera and subgenera of ants (Hölldobler & Wilson 1990). *Colobopsis truncata* is the only member of this genus native to Austria and represents its type species (Mayr 1861). Originally a common Mediterranean species, it is now quite abundant throughout central Europe, where its large, often polydomous colonies can be found in dead wood and hollow twigs (Buschinger 2002, Seifert 2007, Marko et al. 2009).

An especially enigmatic group within the genus *Colobopsis* inhabits the tropical rainforests of Southeast-Asia: Species of the arboreal *Colobopsis cylindrica* (COCY) complex are known for their extraordinary defensive behaviour. While the phragmotic major workers represent a rather passive line of defence by closing the nest-entrances with their enlarged heads, minor workers have evolved a more active, even suicidal strategy: In territorial combat with enemy arthropods, they can actively rupture their integument and eject the sticky and toxic contents of their hypertrophied mandibular gland reservoirs, subsequently killing or immobilizing their rivals (Maschwitz & Maschwitz 1974, Jones et al. 2004, Davidson et al. 2007, Davidson et al. 2012, Davidson et al. 2016). This has earned them the vernacular name “exploding ants”. The ecology

and evolution of this clade of over a dozen described and undescribed species is the subject of the WWTF project “Voluntary self-sacrifice in exploding ants: a mechanism to defend coevolved microbiomes?” which is thematically tightly linked to the present dissertation.

Both, the colonies of *C. truncata* and of COCY ants, typically consist of three distinct female castes, as described for *Colobopsis*, i.e. minor workers, gynes, and phragmotic major workers (often referred to as “soldiers”). The alate gynes mate with the hitherto poorly studied and short-lived males and found the colony. Interestingly, both in *C. truncata* and all known species of COCY ants, gynes also possess truncated heads very similar to those of major workers – probably in order to close the nest entrance during claustral colony-foundation (Buschinger 2002). At 80-90% of individuals, minor workers make up the vast majority of all known colonies (Hölldobler & Wilson 1990, Hasegawa 1997, Pfeiffer & Linsenmair 2000) and engage in a variety of tasks, such as foraging, nest maintenance, and brood care – the exceptionally explosive defence-mechanism of the COCY group being a notable addition. The major workers are characterized by enlarged, plug-shaped heads used to close nest-entrances from within, and also responsible for storing nutrients and liquids for the colony in their role as “repletes” (Walker & Stamps 1989).

In the dissertation at hand, the hyperdiverse genus *Camponotus* is represented by *Camponotus singularis*, a large species widespread throughout Southeast-Asia (Smith 1858, Bharti et al. 2016), and the newly described *Camponotus (Myrmamblys) inquilinus* (Zettel et al. 2018, in press) from a clade of mimetic ants closely associated with the COCY group. Though vastly different in size, morphology and lifestyle, both taxa exhibit a clear worker dimorphism: Soldiers and gynes of *C. singularis* possess greatly enlarged heads and mandibles, while the major workers and gynes of *C. inquilinus* show a tendency towards phragmosis reminiscent of *Colobopsis*. In both cases, the minor workers are much smaller, more abundant and do not exhibit specialized head morphologies. These two taxa are therefore used to draw comparisons to the examined members of genus *Colobopsis* to assess commonalities and trends in caste-specific morphology throughout the tribe Camponotini.

In addition to the expected caste-composition, aberrant specimens exhibiting mosaic- or intercaste phenotypes are known from most subfamilies of ants (Molet et al. 2012). Especially the propensity of parasitic nematodes of the family Mermithidae to induce such altered morphologies in their ant-hosts has long been a subject of scientific study (e.g., Crawley & Baylis 1921, Wheeler 1928, Kaiser 1986, Poinar 2012). These parasitic roundworms are transmitted to their hosts as infective larval stages within a paratenic host (e.g., oligochaetes),

which serves as a protein source for the ants' larvae (Kaiser 1986). The host and parasite subsequently undergo a synchronized maturation, often resulting in striking morphological changes of the host across a wide range of possible phenotypes. While earlier researchers presumed the different types parasitogenic morphologies to be the result of either gynes or workers becoming infested with the worms, Csősz & Majoros (2009) determined an origin of all mermithogenic phenotypes from queen-presumptive larvae. Upon completing its development, the parasite induces suicidal behaviour in the infected individual (as described in the genus *Colobopsis* by Maeyama et al. 1994). Within the present dissertation, the aberrations in morphology of several mermithogynes of the COCY group are described and compared to healthy conspecific gynes, a mermithogyne of *Lasius niger*, and data from previous publications. Such aberrant phenotypes produced under parasitic influence are especially rewarding study subjects for questions within the framework of ecological evolutionary developmental biology (EcoEvoDevo, see Toth & Rehan 2017). The apparent ontogenetic perturbations may shed light on developmental plasticity and robustness during development, as well as the modular nature of these phenotypes. Intercaste- and mosaic phenotypes have therefore been proposed as models to study the developmental pathways facilitating the evolution of novel, specialized castes (Molet et al. 2012, Londe et al. 2015).

## **1.2. Phenotypic integration and modularity – the “building blocks” of life**

Proposed in 1958 by Olson & Miller as “morphological integration”, the concept of phenotypic integration states that certain characters of an organism are linked in ontogenetic development and / or function and thus form interdependent character complexes or “modules”. These groups of characters are defined by strong intrinsic correlation and covariation, and relative independence of other such groups, thereby dividing organisms into distinct parts. In recent years, this concept of modularity has gained particular traction in the fields of evolutionary developmental biology and systems biology (for reviews see Pigliucci 2003, Wagner et al. 2007, Klingenberg 2008, and Armbruster et al. 2014).

In previous studies on vertebrates and plants, phenotypically integrated modules have been found to correspond to structures associated with common roles in particular functions, such as reproduction or foraging (e.g., Berg 1960, Nemeschkal et al. 1992, Conner & Via 1993, Cheverud 1996, Nemeschkal 1999, Van den Elzen & Nemeschkal 2007, Mitteröcker et al. 2012). It thus becomes evident that integration of characters into modules can be linked to a variety of factors, such as physical proximity, common underpinnings by developmental control genes, as well as behavioural or ecological aspects of the organism's life history. All these

factors may potentially lead to natural selection acting on integrated character sets as a whole (Wagner et al. 2007, Armbruster et al. 2014). Due to this broad variety of observed phenomena summarized under the term phenotypic integration and the different modes of calculating “integration indices” used by different authors, Armbruster et al. (2014) caution against simple comparisons across studies and taxa.

Phenotypic integration is hitherto poorly studied in insects, though a comparative study showed holometabolous insects to display overall high levels of phenotypic integration of the body-plan (Conner et al. 2014). Social Hymenoptera have been almost completely overlooked by past researchers, except for a small number of conceptual studies (Molet et al. 2012, Londe et al. 2015) or publications focusing on single phenotypes or species (Wagner 1984, 1990, Miyazaki et al. 2010, Yang & Abouheif 2011, Okada et al. 2013).

The rather sparse body of research on ants is especially surprising, as the often complex caste-systems largely depend on phenotypic plasticity and modularity of the phenotype (West-Eberhard 2003, Molet et al. 2012, 2014, Londe et al. 2015). This allows the development of diverse, specialized phenotypes from genetically similar individuals through variable biotic and abiotic influences, making ants perfect subjects for studies in the framework of EcoEvoDevo (e.g. Abouheif et al. 2014). The resulting morphologically and functionally specialized castes are the foundation for division of labour and hypothesized to be of crucial importance for their enormous evolutionary success and ecological dominance (Peeters & Ito 2015). In fact, authors have argued that the division of labour within a colony of social insects makes the castes themselves comparable to other “functionally specialized modules” – such as different cells within one organism or the different limbs of arthropods (Rueffler et al. 2012).

### **1.3. Aims and Scope**

Using two camponotine genera with pronounced caste dimorphism – *Camponotus* and *Colobopsis* – as examples, the present dissertation represents an attempt to investigate the contributions of individual morphological characters and integrated character-sets towards caste-specific phenotypes and their functions within the colony. This may contribute to our understanding of the adaptive value and functionality of morphological structures, or even uncover similar patterns across distantly related taxa, which may have been shaped by common evolutionary constraints.

Implementing an integrative, multidisciplinary approach, the included publications focus on multivariate statistical analyses of morphometric data, supported by assessments of behavioural

ecology, DNA-barcoding, microCT imaging, and comparison to an outgroup taxon (*Lasius niger*). The publications contained within the present dissertation collectively aim to answer the following research questions:

- 1) Which measurements and proportions of external characters are suitable to characterize castes of the camponotine genera *Camponotus* and *Colobopsis*?
- 2) Which character-sets can be interpreted as phenotypically integrated modules?
  - a) How do such modules differ between castes?
  - b) Can the observed patterns be interpreted in relation to functional ecology and evolution of castes or division of labour within the colonies of the investigated species?
- 3) How does the influence of mermithid parasitism during ontogenetic development affect morphological proportions and patterns of phenotypic integration in the host ant, and are these changes comparable across host taxa?

## 2. Included manuscripts and publications

### 2.1. Published results

#### Study 1)

Laciny A., Zettel H. & Druzhinina I. (2016) **Workers, soldiers, and gynes – morphometric characterization and description of the female castes of *Camponotus singularis* (Smith, 1858) (Hymenoptera, Formicidae)**. Deutsche Entomologische Zeitschrift 63(2): 183-193.

Contribution: 60%

Significance: *Camponotus singularis* is presented as a model species for morphometric caste characterisation in the case of clear worker caste dimorphism. An optimal set of measurements is established and used to identify allometric patterns characteristic for each female caste. Trends in the morphology of dimorphic worker castes are described: a wide size range of minor workers, a major worker (soldier) caste specialized for defence and characterized by an enlarged head and shortened appendages, and a combination of novel, gyne-like, and worker-like traits in such specialized phenotypes. These results lay the groundwork for studies on other camponotine species (studies 2, 4, 5).

#### Study 2)

Laciny A., Zettel H., Metscher B., Kamariah A.S., Kopchinskiy A., Pretzer C. & Druzhinina I.S. (2017) **Morphological variation and mermithism in female castes of *Colobopsis* sp. nrSA, a Bornean "exploding ant" of the *Colobopsis cylindrica* group (Hymenoptera: Formicidae)**. Myrmecological News 24: 91-106.

Contribution: 50%

Significance: The caste-composition of an artificial nest occupied by the “exploding ant” *Colobopsis* sp. nrSA is described in detail, including the discovery of intercaste and mermithogenic phenotypes. All female castes and aberrant specimens are analysed using a morphometric approach supported by DNA barcoding and microCT imaging. Considerable variation in minor worker size, as well as characteristic morphological proportions for each female caste are described, supporting previously described patterns in the Camponotini (study 1). The presence of mermithogynes gives insight into phenotypic plasticity and modularity during ontogenetic development, as well as potential larval food sources (see also study 3).

### Study 3)

Laciny A. (2017) **Evidence of mermithism in a gyne of *Lasius niger* (Linnaeus, 1758) (Hymenoptera: Formicidae) from Burgenland, Austria.** Zeitschrift der Arbeitsgemeinschaft Österreichischer Entomologen 69: 131-138.

Contribution: 100%

Significance: This paper presents the first published record of mermithid parasitism on *Lasius niger* for the Austrian province of Burgenland, Austria. The aberrant anatomy of the parasitized specimen is described, illustrated and compared to previously described mermithogynes in other taxa, especially *Colobopsis* (study 2). The observed morphological aberrations (reduced gyne-specific characters and elongated appendages) show striking similarity to those observed in previous studies, suggesting mechanisms of developmental perturbation common across ant genera and subfamilies.

### Study 4)

Laciny A, Zettel H, Kopchinskiy A, Pretzer C, Pal A, Abu Salim K, Rahimi MJ, Hoenigsberger M, Lim L, Jaitrong W, Druzhinina IS (2018) ***Colobopsis explodens* sp.n., model species for studies on “exploding ants” (Hymenoptera, Formicidae), with biological notes and first illustrations of males of the *Colobopsis cylindrica* group.** ZooKeys 751: 1-40.

Contribution: 60%

Using a multidisciplinary, integrative approach, *Colobopsis explodens* is described as new to science and established as a model species for further studies on the “exploding ant” group. The characteristic morphology of castes is described, including the first illustrations and morphometric characterizations of males of the group. The morphological patterns and proportions of the female castes agree with those previously described in other Camponotini (studies 1, 2). First notes on the species’ biology and the analysis of multiple hitherto unknown modes of behaviour allow the first interpretations of caste-specific morphology in the light of behavioural ecology, functionality and division of labour.

## 2.2. Submitted manuscript

### Study 5)

Laciny A., Nemeschkal H.L., Zettel H., Metscher B., Druzhinina I.S. **Caste-specific morphological modularity in the ant tribe Camponotini (Hymenoptera, Formicidae).** Manuscript submitted to BMC Zoology 21.9.2018. submission id: BZOO-D-18-00018

Contribution: 80%

Significance: In this first study of phenotypic integration and modularity in the tribe Camponotini, the species- and caste-specific interdependences between morphological characters are analysed. Results show integrated character complexes, which differ between taxonomic groups and castes (e.g. strongly correlated extremities in workers, tight integration of mesosoma and eyes in gynes). These patterns can largely be interpreted as functional, caste-specific modules related to behavioural ecology and task allocation within ant colonies. Thereby, this study unites and supports many aspects of caste-specific morphology, functional ecology, and parasite-mediated aberration of proportions presented in previous publications (studies 1, 2, 4).

# Workers, soldiers, and gynes – morphometric characterization and description of the female castes of *Camponotus singularis* (Smith, 1858) (Hymenoptera, Formicidae)

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## Abstract

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## Key Words

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morphometry

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Species of *Camponotus* Mayr, 1861 show a high variation of worker caste numbers, from a monomorphic worker caste to strong polymorphism along a continuous range of worker sizes or true worker dimorphism. *Camponotus singularis* (Smith, 1858) is used as a model for a *Camponotus* species with two very distinct worker castes (minors and majors) which are chiefly defined by morphometric data. We investigated shifts in proportions of *C. singularis* female castes in order to identify major allometric patterns useful for characterizing caste differences in this species as well as in other Camponotini. We describe the main morphological traits which are characteristic for the respective morphs. The major worker, or “soldier”, shows many characteristics which deviate from the minor worker, but also from the alate gyne. Its traits are presumably modified for its function in nest defence. Morphometric data clearly set Bornean specimens of *Camponotus singularis* (described as var. *rufomaculatus* Donisthorpe, 1941) apart from specimens with other proveniences, suggesting that this island population is a distinct species.

## Introduction

Within the eight recent genera of the tribe Camponotini (Blaimer et al. 2015, Ward et al. 2016), the worker caste is developed in a variety of forms, comprising species with monomorphic (e.g., *Polyrhachis* Smith, 1857; Dorow 1995), continuously polymorphic (e.g., some species of *Camponotus* Mayr, 1861; Wilson 1953, Busher et al. 1985) and dimorphic workers (e.g., *Colobopsis* Mayr, 1861). Even the most speciose genus, *Camponotus*, exhibits all known types of worker polymorphism: from species with monophasic allometry to those with true worker dimorphism (Wilson 1953).

During our ongoing revision of the *Colobopsis cylindrica* group, which includes a morphometric analysis of species, a review of previous publications revealed multiple mentions of poly- or dimorphism in workers of Cam-

ponotini (e.g., Wilson 1953, Baroni Urbani 1974, Busher et al. 1985), but a comparatively small number of more recent studies focusing on morphometry (e.g., Diniz-Filho et al. 1994, Rakotonirina et al. 2016).

We herein present morphometric data on the Asian species *Camponotus singularis* (Smith, 1858), a large-bodied species with a wide distribution from Nepal to Borneo and Java (e.g., Smith 1858, Chapman and Capco 1951, Thapa 2000, Jaitrong and Nabhitabata 2005, Guénard and Dunn 2012, Bharti et al. 2016). We have chosen this taxon, because it serves as an example of a species with a clear worker dimorphism, most apparently characterized by the size and shape of the head, and without the occurrence of intermediate phenotypes. We describe the differentiating characters of the two worker castes (minors, majors) and compare them to the corresponding characters of the gynes (see Figs 1–6). Molet



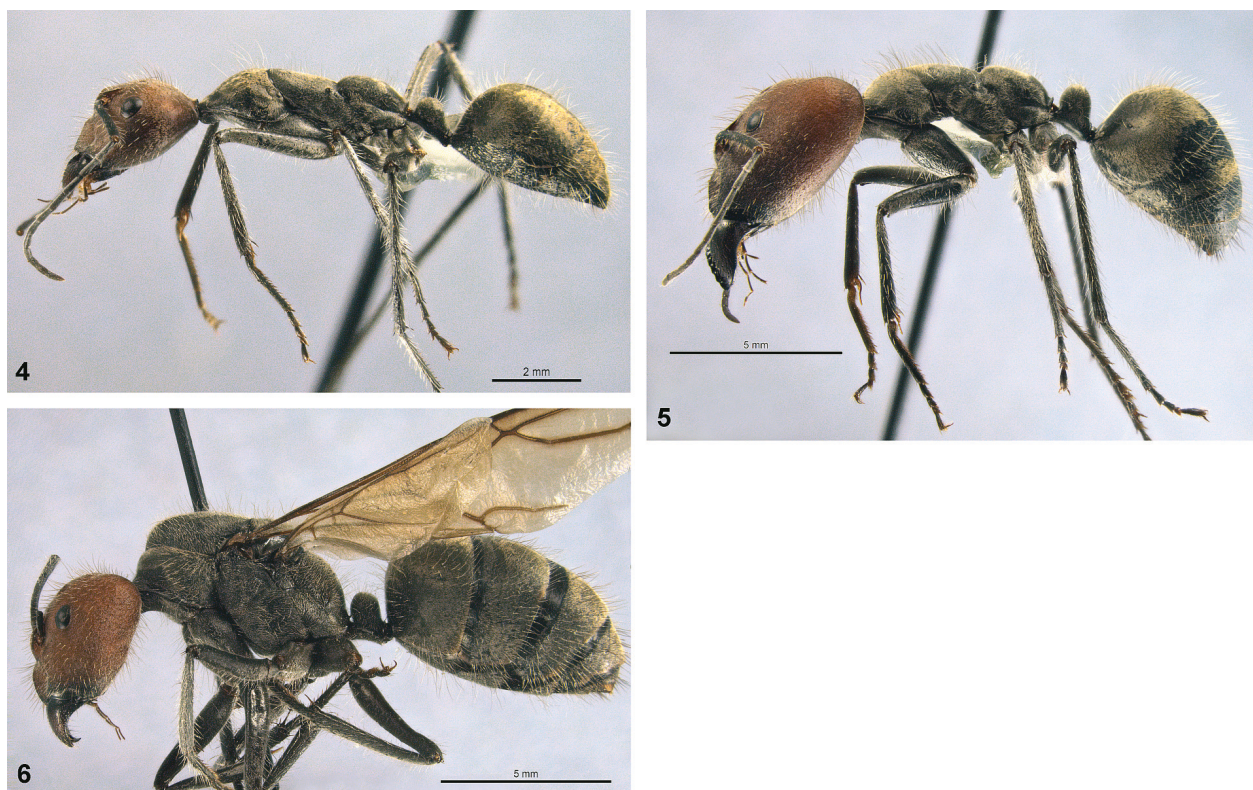
**Figures 1–3.** *Camponotus singularis*, head, frontal view, of (1) minor worker, (2) major worker, and (3) gyne. Notably different are head shape, development of posterior margin, and position of eyes. The minor has much longer scapes and maxillary palpi than the major.

et al. (2012, 2014) interpret specialized castes as mosaics of pre-existing phenotypes with novel or “recycled” developmental pathways. Building on this evolutionary model, we aim to characterize major and minor workers as two distinct subcastes based on their morphological proportions. Although morphometric differences in Camponotini may vary from clade to clade, within this study we attempt to identify representative caste-specific allometric patterns which may not only be applicable to the dimorphism of *C. singularis* but also serve to characterize caste-polymorphism in other camponotine ants.

## Material and methods

### Specimen depositories

BMNH	Natural History Museum, London, United Kingdom
CAS	California Academy of Sciences, San Francisco, USA
CZW	Coll. H. Zettel, Vienna, Austria
MCSN	Museo Civico di Storia Naturale Giacomo Doria (main collection and Carlo Emery’s collection), Genova, Italy



**Figures 4–6.** *Camponotus singularis*, lateral view, of (4) minor worker, (5) major worker, and (6) gyne. Notable are differences between the worker subcastes regarding head size, mesosoma height, and petiole shape. Mesosoma and gaster of the gyne are strongly enlarged for flight and reproduction.

NHMW	Natural History Museum Vienna, Austria
SKYC	Sk. Yamane Collection, Korimoto, Japan
THNHM	Thailand Natural History Museum, Phatumthani, Thailand
VNMN	Vietnam National Museum of Nature, Hanoi, Vietnam

### Material examined

We measured specimens from most parts of the distribution area of *C. singularis*, except from Nepal and India. In total five gynes, ten major workers and 85 minor workers of *C. singularis*, as well as five minor workers and one gyne of *C. singularis* var. *rufomaculatus* Donisthorpe, 1941 were examined. During data processing we noticed that specimens from Borneo differed clearly from specimens collected in other localities. Consequently, we removed Bornean specimens from our in-depth analysis of morphs, but present some data on how to differentiate this population.

*Camponotus singularis*, typical form: **Myanmar:** 1 minor worker (BMNH), Mandalay Region, Pyin U Lwin, ca. 900 m a.s.l., V.1899, coll. Bingham [*Camponotus camelinus* Smith, Upper Burma Maymya, 3000 ft, 5.1899, Bingham coll.]; 1 major worker, 1 minor worker (MCSN), Kayin State, mountains east of Toungoo [Carin Cheba], 900–1100 m a.s.l., V.1888, leg. L. Fea; 1 minor worker (MCSN), Kayin State, Kawkaet, I–II.1887, leg. L. Fea; 1 major worker, 2 minor workers (MCSN, NHMW), Kayin

State, [“Tenasserim”], Thagata, IV.1887, leg. L. Fea; 1 minor worker (MCSN), Tanintharyi Region, [“Tenasserim”], Malewoon, VII–VIII.1887, leg. L. Fea. – **Thailand:** 1 minor worker (BMNH), Chiang Mai [Chiengmai], 19.I.1936, Lot 317/94; 1 minor worker (NHMW), Thailand, Satun Province, Thale Ban National Park, 10–16. III.1993, leg. M. Madl; 1 minor worker (BMNH), Pattani Province, Bukit Besar near Amphoe Nong Chik (for interpretation see Woodley 2012), ca. 900 m a.s.l., 1903, leg. Annandale & Robinson [Bulsit [sic!]] Besar, Siam: Malay States, No. 12, 3000’, Annandale & Robinson, Siamese Malay States 1903–127, *Camponotus camelinus* Sm. Donisthorpe det.]; 1 minor worker (THNHM), Chiang Rai Province, Mae Pha Laung District, 20.VI.2002, leg. W. Jaitrong; 1 minor worker (THNHM), Pattalung Province, Khao Pu – Khao Ya National Park, Reang Tong Waterfall, evergreen forest, 28.IX.2007, leg. P. Kosonpanyapiwat; 1 minor worker (THNHM), Ranong Province, Khlong Naka Wildlife Sanctuary, evergreen forest, 12.VIII.2009, leg. W. Jaitrong. – **Laos:** 6 minor workers (NHMW), Luang Nam Tha Province, 5–10 km SW of Muang Sing, 600 m a.s.l., 11.VI.1996, leg. H. Schillhammer (#23); 1 major worker (NHMW), Luang Nam Tha Province, ca. 20 km SE of Muang Sing, 950 m a.s.l., 12–13.VI.1996, leg. H. Schillhammer (#25); 1 major worker (NHMW), Luang Nam Tha Province, ca. 10 km E of Muang Sing, 600 m a.s.l., 19.VI.1996, leg. H. Schillhammer (#32); 1 major worker, 40 minor workers (CZW), Hua Phan Province, Ban Saleui, Phou Pan (Mt.), 1300–1900 m a.s.l., 20°12’N 104°01’E,

7–29.IV.2014 (various dates), leg. C. Holzschuh. – **Vietnam:** 1 minor worker (VNMN), Lao Cai Province, Van Ban, ca. 150 m a.s.l., 4.VI.2005, leg. B.T. Viet (#Code I). – **West Malaysia:** 1 minor worker (CZW), West Malaysia, Kelantan, 60 km NE Tanah Rata, Tanah Kerajaan, 1000 m a.s.l., 12–30.IV.2007, leg. P. Cechovsky; 3 minor workers (CZW), West Malaysia, Kelantan, 30 km NW Gua Musang, Ulu Lalat, Kpg. Sungai Om, 800–1000 m a.s.l., 21.VI.–14.VII.2010, leg. P. Cechovsky; 2 minor workers (CZW), West Malaysia, Perak, 25 km NE Ipoh, Banjaran Titi Wangsa mts., Korbu (Mt.), 1200 m a.s.l., 11.–16. I.1999, leg. P. Cechovsky; 1 major worker, 8 minor workers (CZW), West Malaysia, Perak, 30 km SE Ipoh, Cameron Highland, Ringlet, 900 m a.s.l., 25.IV.–5.VI.2001, leg. P. Cechovsky; 1 minor worker (CZW), West Malaysia, Perak, 40 km SE Ipoh, Banjaran Titi Wangsa mts., Ringlet, 900 m a.s.l., 25.III.–3.IV.2002, leg. P. Cechovsky. – **Indonesia:** 2 minor workers (NHMW), Sumatra (#877-2); 1 minor worker (NHMW), Sumatra, 1875, leg. Plason; holotype minor worker (BMNH), Java (# 55, 39), “*F. singularis* Sm., Type”, CASENT0903554; 5 alate gynes (NHMW), Java, leg. Adensamer; 1 major worker, 1 minor worker (NHMW), Java, leg. Vollenhov; 1 minor worker (NHMW), Java, 1874, leg. Plason; 2 minor workers (MCSN), Java, Kota Bogor [Buitenzorg], 1875, leg. G.B. Ferrari; 1 minor worker (MCSN), Java, Kota Bogor [Buitenzorg], IV.1876, leg. Beccari; 1 major worker (SKYC), Java, Kota Bogor, 3–5.XI.1985, leg. Sk. Yamane; 1 minor worker (SKYC), Java, G. Sarak, near Kota Bogor, 8.III.1997, leg. Sk. Yamane; 1 minor worker (CAS), Java, Kota Bogor, Botanical Gardens, 16.XII.1978, leg. E. S. Ross. – **Unknown localities:** 1 major worker, 1 minor worker (NHMW), coll. Felder; 1 major worker (NHMW), unknown collector.

*Camponotus singularis* var. *rufomaculatus*: **East Malaysia (Borneo):** 2 minor workers (MCSN), Sarawak, 1865–1866, leg. G. Doria; 1 minor worker (SKYC), Sabah, Crocker Range, Inobong (forest), 500–600 m a.s.l., 26.II.2008, leg. Sk. Yamane; 1 dealate gyne (SKYC), Sabah, Sipitang, Muaya Forest Reserve, 700 m a.s.l., 28.VIII.2012, leg. Sk. Yamane; 1 minor worker (SKYC), Sarawak, Kubah National Park, 5.XII.1993, leg. Sk. Yamane; 1 minor worker (SKYC), Sarawak, Engkari, Ubah Ribu, 23.IV.1994, leg. K. Het.

Examined specimens were either pinned or dry mounted on card squares or triangles. Examination and measurements of specimens were carried out with a Nikon SMZ1500 binocular microscope at magnifications of up to 256×.

### Measurements and indices

TL	Total length. The added lengths of head (including mandibles), mesosoma, petiole, and gaster.
HW	Head width. Maximum width of head in full-face view, excluding eyes if laterally protruding (few minor workers).
HL	Head length. Maximum length of head in full-face view, excluding mandibles, measured parallel to midline from anterior-most point of cly-

peus to midpoint of occipital margin (in minor workers) or to midpoint of an imaginary line connecting the apices of posterior projections (major workers and some gynes).

HS	Head size. $(HW+HL) / 2$ .
EL	Eye length. Maximum diameter of compound eye.
SL	Scape length. Maximum length of antennal scape in dorsal view excluding basal neck and condyle.
ML	Mesosoma length. Measured laterally from anterior surface of pronotum proper (excluding collar) to posterior extension of propodeal lobes.
PH	Petiole height. Maximum height of the petiole in lateral view, measured from ventral-most point of petiolar sternum to dorsal apex.
PL	Petiole length. Maximum length of petiole in lateral view, measured from inflexion point of anterior constriction to posterior margin.
NH	Node height. Height of petiolar node, measured laterally, from the intersection point of the axes of maximum height and length to dorsal apex.
FeL	Femur length. Maximum length of hind femur, measured from base to apex.
PS5	Length of maxillary palp segment 5, measured from base to apex.
PS6	Length of maxillary palp segment 6, measured from base to apex.
CI	Cephalic index. $HW / HL \times 100$ .
SI	Scape index. $SL / HW \times 100$ .
EI	Eye Index. $EL / HW \times 100$ .
PI	Petiole Index. $PH / PL \times 100$ .
FeI	Femur Index. $FeL / HW \times 100$ .
PSI	Palp Segment Index. $(PS5+PS6) / HS \times 100$ .

All measurements are in millimetres and separated by caste. Due to the condition of some specimens, not all measurements were taken from all animals. Measurements of minor workers include the holotype (plotted separately in Figs 9, 12, 14). The total number of specimens measured is given for each morph (n), deviating numbers for individual characters are written in parentheses. The raw data set of all measurements is available as an electronic supplement (Suppl. material 1).

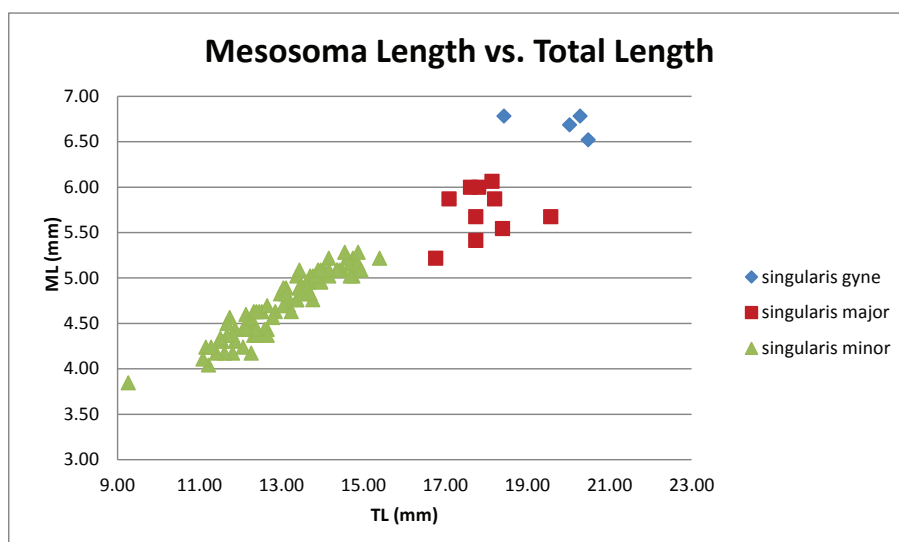
Digital photos were taken with a Leica DFC camera attached to a Leica MZ16 binocular microscope with the help of Leica Application Suite V3, and stacked with Zere-neStacker 64-bit. Processing of images was performed with Adobe Photoshop 7.0.

## Results

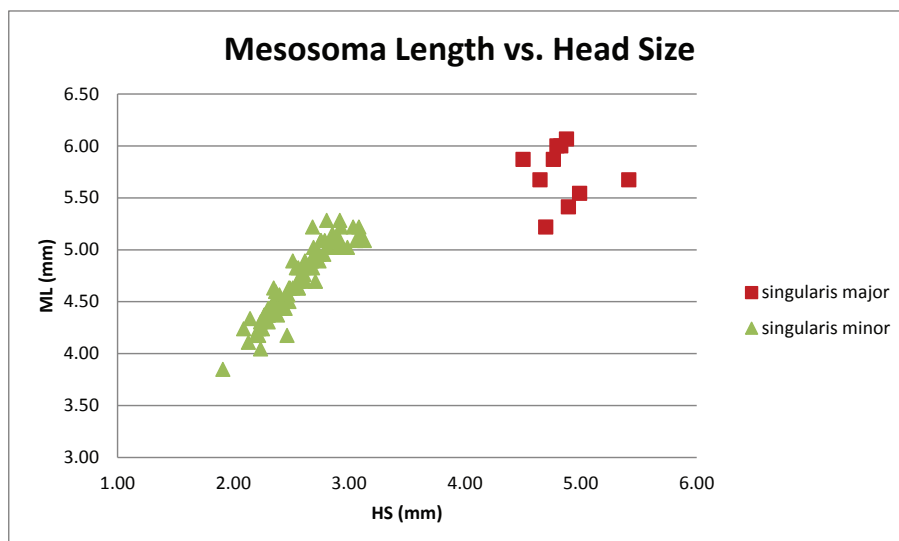
### Dimorphism of workers of *Camponotus singularis* (typical form):

#### Body size:

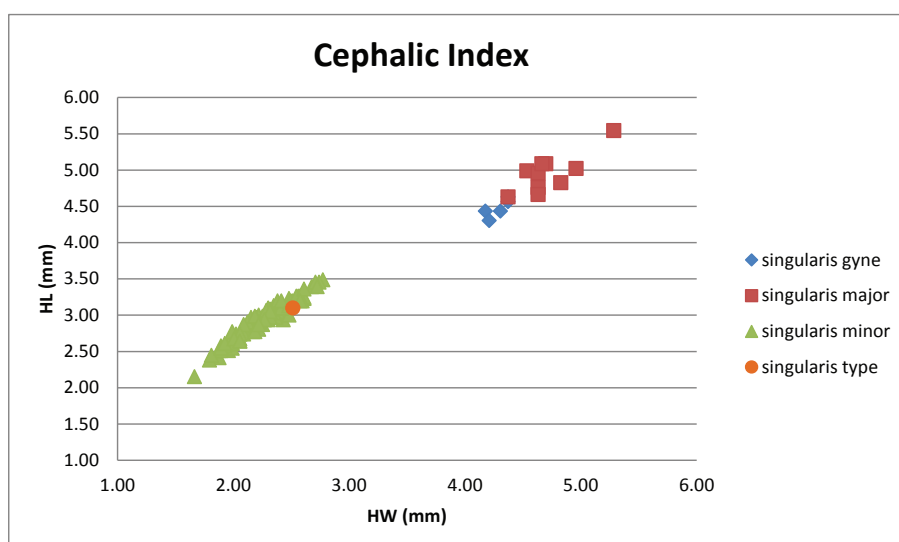
Measurements: alate gynes (n = 5): TL 18.42–20.48 (4); ML 6.52–6.78; major workers (n = 10): TL 16.76–19.57; ML 5.22–6.07; minor workers (n = 85): TL 9.26–15.39; ML 3.85–5.28 (84).



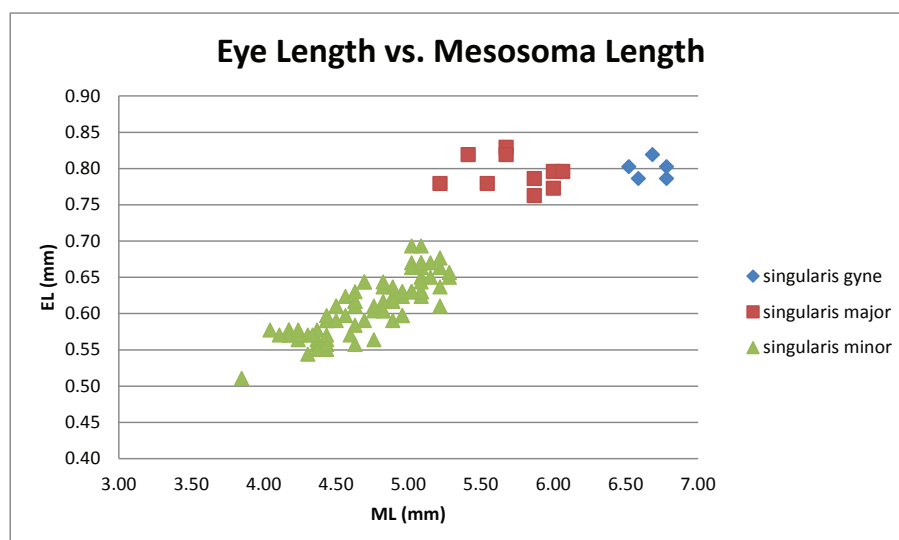
**Figure 7.** Distribution of mesosoma length (ML) in relation to total length (TL) in gynes, majors and minors of *Camponotus singularis*.



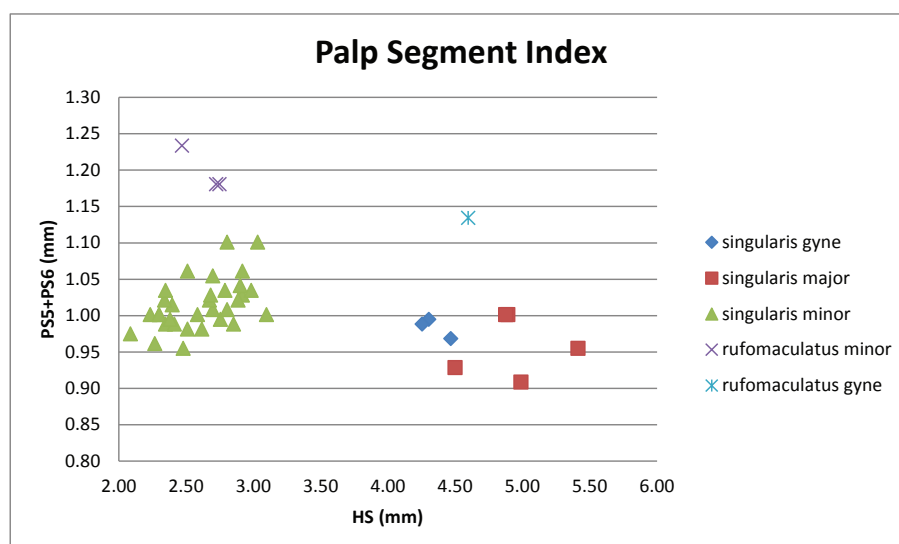
**Figure 8.** Distribution of mesosoma length (ML) in relation to head size (HS) in majors and minors of *Camponotus singularis*.



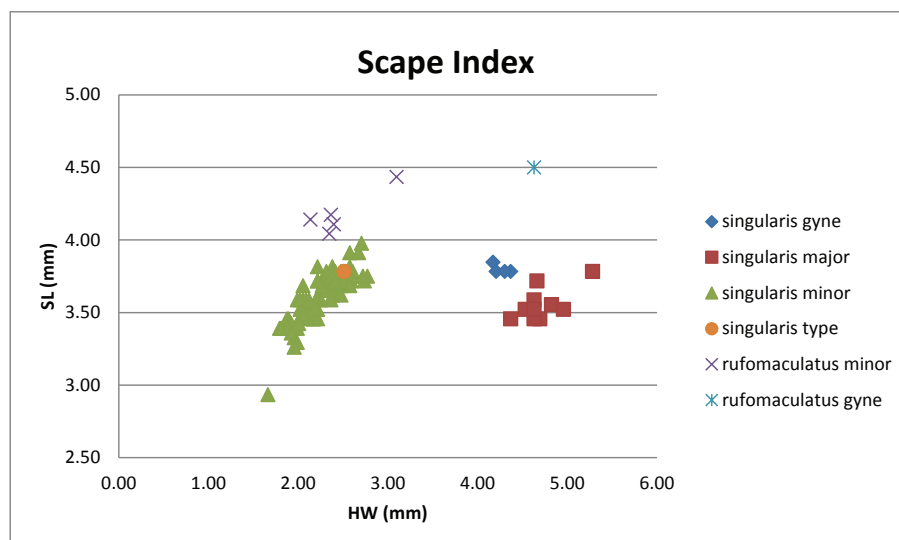
**Figure 9.** Distribution cephalic index (CI) in gynes, majors and minors of *Camponotus singularis*, holotype minor worker plotted separately.



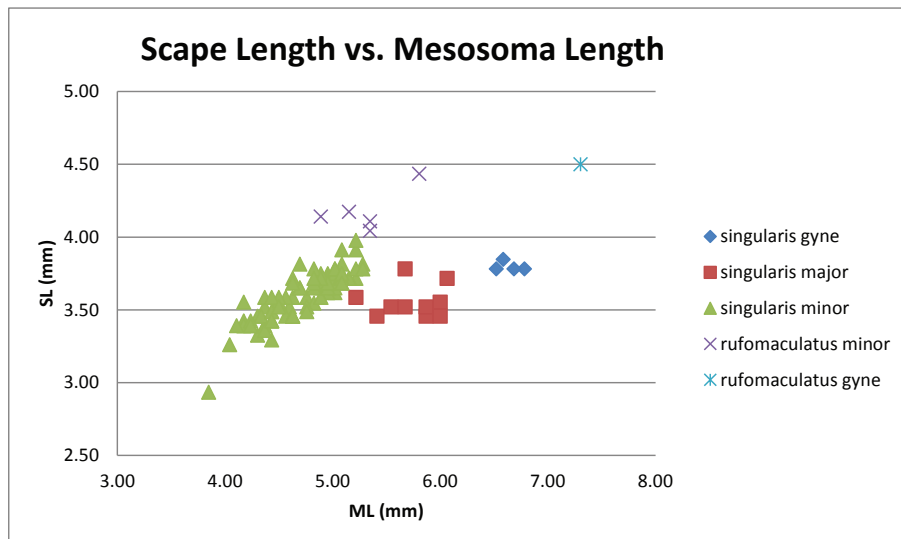
**Figure 10.** Distribution of eye length (EL) in relation to mesosoma length (ML) in gynes, majors and minors of *Camponotus singularis*.



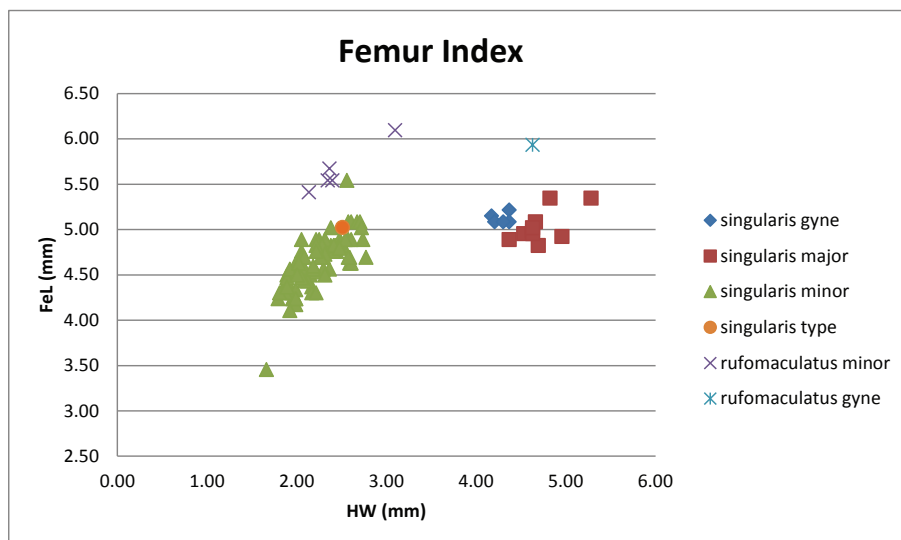
**Figure 11.** Distribution of maxillary palp segment index (PSI) in gynes, majors and minors of *Camponotus singularis*, as well as minors and gyne of var. *rufomaculatus*.



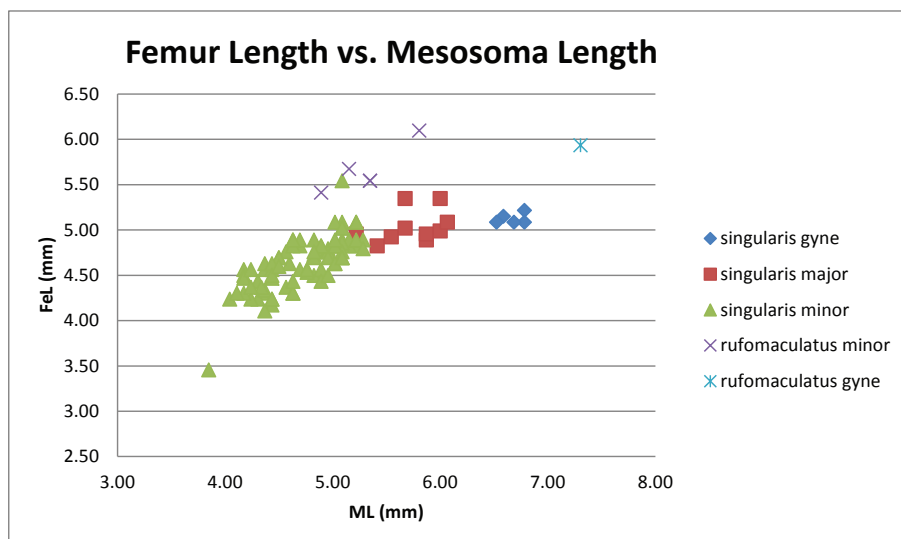
**Figure 12.** Distribution of scape index (SI) in gynes, majors and minors of *Camponotus singularis* (holotype minor worker plotted separately), as well as minors and gyne of var. *rufomaculatus*.



**Figure 13.** Distribution of scape length (SL) in relation to mesosoma length (ML) in gynes, majors and minors of *Camponotus singularis*, as well as minors and gyne of var. *rufomaculatus*.



**Figure 14.** Distribution of femur index (FeI) in gynes, majors and minors of *Camponotus singularis* (holotype minor worker plotted separately), as well as minors and gyne of var. *rufomaculatus*.



**Figure 15.** Distribution of femur length (FeL) in relation to mesosoma length (ML) in gynes, majors and minors of *Camponotus singularis*, as well as minors and gyne of var. *rufomaculatus*.

We used two measurements to describe body size, total length (TL) and mesosoma length (ML). Although traditionally used in myrmecology to facilitate rough comparisons of castes and species, total length strongly depends on the condition of the specimen (e.g., dilation or shrinking of the gaster from storage in alcohol or the drying process) so that TL can be relatively inaccurate. In *C. singularis* TL and ML are directly proportional in all female castes (Fig. 7). We therefore use ML as the main measurement for overall body size. There are two distinct subcastes of workers, minors and majors, which strongly differ in size range. Size variation is considerable in minors, whereas majors and gynes are more uniform in size (Fig. 7).

#### Head size and head shape:

Measurements: alate gynes (n = 5): HW 4.17–4.37; HL 4.30–4.63; HS 4.26–4.50; CI 94–98; major workers (n = 10): HW 4.37–5.28; HL 4.63–5.54; HS 4.50–5.41; CI 91–100; minor workers (n = 85): HW 1.66–2.77; HL 2.15–3.49; HS 1.91–3.13; CI 72–83.

HW and HL were measured. In relation to body size, head size (HS) is strongly disproportional in the two worker castes (Fig. 8). Major workers have a notably large head. The heads of gynes are also large, but on average less wide compared to major workers (Fig. 9).

The three castes differ considerably in head shape (Figs 1–3). In minor workers the ovate head is rounded posterior of the eyes, with a short, protruding collar; the narrow margin is dorsomedially depressed, and dorso-laterally slightly upcurved. In major workers the head is heart-shaped and posteriorly deeply concave. In gynes the head is slightly narrower than in majors and posteriorly almost straight. Furthermore, in both majors and gynes no collar or occipital margin is developed.

#### Eye size and eye position:

Measurements: alate gynes (n = 5): EL 0.79–0.82; EI 27–28; major workers (n = 10): EL 0.76–0.83; EI 23–27; minor workers (n = 85): EL 0.51–0.69; EI 36–46.

Eye size in minor workers is strongly correlated with body size (ML) (Fig. 10), and eyes appear smaller in majors since these workers have disproportionally large heads. In comparison to HW, the eyes of gynes are larger than those of major workers (EI 27–28 vs. 23–27).

In minor workers the eyes are dorsolaterally located, close to the lateral outline of the head in frontal view (Fig. 1). In contrast, the eyes of majors and gynes are shifted dorsally and placed approximately equidistant from the sides of the head and the frontal carinae (Figs 2, 3).

#### Ocelli:

Minor workers do not possess ocelli or other structures in their place. In one major worker from Myanmar we observed three reduced ocelli, whereas many other majors have small depressions or scars at these positions.

#### Clypeus shape:

The clypeus is similarly shaped in major and minor workers, whereas the medial protrusion is slightly longer in gynes (Figs 1–3). In major workers the lateral emarginations of the foremargin are wider than in minors to accommodate the thick bases of the mandibles.

#### Mandibles:

The mandibles of majors are much stouter than those of minors, especially in the basal half. The mandibles of gynes are of intermediate shape.

#### Maxillary palpi:

Measurements: alate gynes (n = 3): PS5 0.50–0.52; PS6 0.46–0.47; PSI 22–23; major workers (n = 5): PS5 0.49–0.54; PS6 0.42–0.48; PSI 18–21; minor workers (n = 32): PS5 0.52–0.60; PS6 0.42–0.50; PSI 32–47.

The maxillary palpi of minors are much longer in relation to HS than those of majors and gynes (PSI 18–23 in majors and gynes vs. 32–47 in minors; Fig. 11; see also Figs 1, 2). As palpi are very often obscured or damaged in mounted specimens, examination of this character was only possible in a small number of animals.

#### Antenna:

Measurements: alate gynes (n = 4): SL 3.78–3.85; SI 87–92; major workers (n = 10): SL 3.46–3.78; SI 71–80; minor workers (n = 83): SL 2.93–3.98; SI 135–189.

The antennae of majors and gynes are relatively shorter and thicker than those of minors. The scape index (SI) differs considerably (Fig. 12), but in relation to body size (ML), scape length is only slightly smaller in majors than in minors (Fig. 13).

#### Mesosoma shape:

The mesosoma of majors is slightly more robust (wider and higher) than that of minors, though no morphometric data were recorded except ML. Gynes always possess a well-developed flight apparatus, and their mesosoma morphology is strongly modified accordingly.

#### Leg length:

Measurements: alate gynes (n = 5): FeL 5.09–5.22; FeI 116–123; major workers (n = 10): FeL 4.83–5.35; FeI 101–114; minor workers (n = 85): FeL 4.46–5.45; FeI 169–238.

We measured the length of the hind femur (FeL) as an indicator for leg length. Whereas the femur index (FeI) is highly dissimilar in minors and majors (Fig. 14), majors have only slightly shortened legs compared to mesosoma length (Fig. 15). Gynes also possess shortened femora in comparison to minor workers, though the difference is not as pronounced as in majors (Fig. 14).

#### Petiole shape and petiole index:

Measurements: alate gynes (n = 5): PH 1.48–1.63; PL 1.04–1.17; NH 1.00–1.17; PI 134–144; major workers (n = 5): PH 1.13–1.48; PL 1.04–1.09 (4); NH 0.76–0.89 (4); PI 104–136 (4); minor workers (n = 49): PH 0.59–0.87 (38); PL 0.67–1.02 (35); NH 0.30–0.61 (46); PI 66–95 (31).

PI is highest in gynes, intermediate in majors and lowest in minors, reflecting the differences in dorsoventral height of the petiole and development of the petiolar node. While gynes and majors possess a petiole that is higher than long (PI > 100), with a well-developed node, the petiole of minors is dorsally rounded and longer than high (PI < 100). Due to the mounting method applied in some specimens, petiolar characters were partially obscured and therefore could not be measured in part of the material.

**Gaster:**

No evident differences were observed when comparing the gasters of minor and major workers. Due to the presence of reproductive organs, gynes possess a larger gaster relative to the rest of the body, but without apparent structural differences compared to the other castes.

**Deviating characters of *Camponotus singularis* var. *rufomaculatus* from Borneo**

The specimens from Borneo consistently differ from the rest of the examined material by the length of their appendages (maxillary palpi, antennae, and legs; Figs 11–15), as well as reddish colour of the dorsal mesosoma in some specimens, and darker setae on the head. Examination of photographs of the types of var. *rufomaculatus* (AntWeb: syntype major worker CAsENT0903555, BMNH; syntype minor worker CAsENT0903556, BMNH) suggests that this name is applicable to the Bornean population.

**Maxillary palpi:**

The examined gyne and minors of *C. s.* var. *rufomaculatus* possess relatively longer palpi than all the other examined specimens (PSI 43–50 in minors, 25 in gyne, see Fig. 11).

**Antenna:**

All examined *C. s.* var. *rufomaculatus* specimens differ from the rest of the material by considerably longer scapes relative to HW and ML (SI 143–194 in minors, 97 in gyne, see Figs 12, 13).

**Leg length:**

*Camponotus singularis* var. *rufomaculatus* differs from the typical form by relatively longer legs (FeL 197–253 in minors, 128 in gyne, see Figs 14, 15).

**Discussion****Worker dimorphism and evolution of the major subcaste**

The results of our morphometric analyses show that *C. singularis* possesses a true worker dimorphism sensu Wilson (1953) with distinct major and minor worker subcastes and without expression of intermediate morphology. As per the material represented in collections, majors have been observed to be much rarer compared to minors (85 minors and 10 majors examined). Although no complete nest series were available for analysis, the observed relatively low number of majors compared to minors agrees well with the results of previous studies on other Camponotini: Walker and Stamps (1986) as well as Hasegawa (1997) examined two species of *Colobopsis* and found optimal caste ratios of soldiers to lie at around 20% of the total nest-population.

Perhaps the most striking difference between the two worker subcastes is in the shape of the head, which is narrow and ovate with a well-developed collar in minors, but greatly enlarged, heart-shaped and without collar or margin in majors (Figs 1, 2). Though differing in the depth of the posterior concavity, the head-shape of majors is

strongly reminiscent of that of conspecific gynes (Fig. 3) with several major workers also possessing small ocelli or remnants thereof.

When comparing mesosomal architecture between castes, both major and minor workers possess a well-developed prothorax which is slightly enlarged in majors, whereas the mesothorax is greatly hypertrophied in gynes (Fig. 2). Keller et al. (2014) observed this morphological trade-off in multiple subfamilies of ants. They interpret a large prothorax as optimization for foraging and defence in workers, additionally providing attachments for muscles in large-headed majors, whereas the first mesosomal tergite is reduced in gynes to allow for the development of the flight apparatus. Additionally, majors and minors differ by the relative lengths of their appendages with major workers having consistently shortened legs, antennae and maxillary palpi when compared to conspecific minors (Figs 11–15). We could not find comparable data on Camponotini in the literature, but similar results were recently obtained in a study on *Colobopsis* sp. (Laciny et al., in prep., see below for details). In their study on leafcutter ants (*Atta colombica* Guérin-Méneville, 1844) and army ants (*Eciton hamatum* (Fabricius, 1782)), Feener et al. (1988) also found that legs became shorter with increasing body mass in workers of *Atta* and soldiers of *Eciton*. The authors attributed this result to the mechanics of foraging in the examined species. Shortened appendages relative to HS as well as to ML were also observed in the examined gynes of *C. singularis*, though to a slightly lesser extent than in majors. These observations of morphological similarities between majors and gynes may indicate the evolution of the developmental programme for major workers in this species by partial “recycling” of growth parameters for gyne-development (Molet et al. 2014). As elaborated in their 2014 study on *Cataglyphis bombycina* (Roger, 1859), Molet et al. propose that the evolution of specialized castes may be facilitated by reusing and recombining parts of developmental programmes already established in pre-existing castes. The resulting phenotypes are therefore often characterized by a mosaic of novel and recycled traits that set them apart as a distinct caste. Our results clearly show that major workers of *C. singularis* are not simply isometrically scaled-up versions of minor workers, but differ from all other castes by shifts in morphological proportions resulting from differing growth rules; they may therefore be more accurately termed “soldiers” sensu Molet et al. (2012, 2014).

**Comparison to other Camponotini**

A recent study focusing on morphological variation in a species of the *Colobopsis cylindrica* (COCY) group (Laciny et al., in prep.) found considerable size-variation within the examined minor workers and relatively uniform sizes of major workers (phragmotic in *Colobopsis*) and gynes. This trend is in accordance with studies on other camponotines (e.g., Lee 1938, Busher et al. 1985, Espadaler et al. 1990) reporting wide size ranges in work-

ers that are often correlated with size-specific division of labour. A similar result was obtained in the present study: In *C. singularis* the largest minors surpass their smallest conspecifics by up to 50% regarding head width as well as mesosoma length, while majors and gynes show far less variability (Figs 7–9). Another parallel between the observations in COCY ants and *C. singularis* is the shortening of appendages relative to body size in the major worker subcaste: Majors of both groups possess shorter legs, antennae and maxillary palpi than conspecific minors. However, while in *C. singularis* this holds true for majors as well as gynes, in the COCY group only major workers have shortened appendages, whereas the proportions of gynes were more similar to those of minor workers. The caste-specific architecture of the mesosoma, with a prothorax that is enlarged in workers – particularly in large-headed majors – and reduced relative to the mesothorax in alate gynes, as observed in *C. singularis* and in species of the COCY group, conforms to the morphology of other studied ant species (Keller et al. 2014).

### Polyethism and the role of soldiers

The results of this study show a clear worker dimorphism and a conspicuously broad size range of minors, which is apparent in intraspecific as well as intracolony comparison. It is therefore a valid assumption that *C. singularis* colonies have evolved some form of polyethism or division of labour amongst their members. However, nothing is known about colony composition and task allocation in this species as of now. Studies on other formicine ants have yielded results that suggest a tendency towards division of labour even within the (minor) worker caste: Lee (1938) described such a trend in *Camponotus japonicus* var. *aterimus* Emery, 1895, Busher et al. (1985) observed division of labour in *Camponotus sericeiventris* (Guérin-Méneville, 1838), and Higashi (1974) reported similar results for *Formica yessensis* Wheeler, 1913. In contrast, in the moderately polymorphic dolichoderine ant *Liometopum microcephalum* (Panzer, 1798) differences in task performance could not be detected (Petráková and Schlaghamerský 2014).

The high nutritional investment necessary for producing a distinct soldier subcaste suggests that these animals must serve a function greatly beneficial to colony survival and fitness (Tian and Zhou 2014). In many of the previously studied ant species the tasks of major workers or specialized soldiers do not only include nest defence, but also storage of food and liquid within their bodies: This was found to be true for the phragmotomic major workers of *Colobopsis nipponica* (Wheeler, 1928) (Hasegawa 1993), large workers of the polymorphic *Camponotus foreli* Emery, 1881 (Espadaler et al. 1990) and the highly specialized soldiers of *Cataglyphis bombycina* (Molet et al. 2014). Whether this is also the case in *C. singularis* still remains to be studied. In majors of *C. singularis* the greatly enlarged, heart-shaped head is correlated with the presence of strongly developed mandibular adductor muscles, most probably linked to a function in colony defence. The defensive be-

haviour of soldiers may also contribute to the development of shortened cephalic appendages (antennae, maxillary palpi), so as to reduce the danger of injury in combat. Mysore et al. (2009, 2010) reported major workers of *Camponotus compressus* (Fabricius, 1787) and *Camponotus sericeus* (Fabricius, 1798) to have shorter antennae with reduced sensilla and corresponding neural structures in comparison to conspecific minors. The authors link this to the majors' specialization in nest defence, which makes differentiation of chemical cues less essential than for foraging minor workers. Corresponding to the observed shortened antennae of majors, the situation may be similar in *C. singularis*. However, this can only be a tentative assumption at this point and requires further investigation.

Altogether the results obtained within this study and their comparison to trends observed in the COCY clade (Laciny et al., in prep.) and other previously studied species (e.g., Lee 1938, Wilson 1953, Espadaler et al. 1990, Mysore et al. 2009, 2010, Molet et al. 2012) lead us to propose *C. singularis* as a useful model to characterize major morphometric trends in camponotine caste dimorphism: a wide size range of minor workers, a major worker or soldier subcaste specialized for defence and characterized by an enlarged head and shortened appendages, and a mosaic of novel, gyne-like, and worker-like traits expressed in such specialized phenotypes.

### Differing characters of var. *rufomaculatus*

Examined specimens from Borneo clearly differ from specimens from other localities by proportions of appendages, colour pattern and setae. Donisthorpe (1941) originally described var. *rufomaculatus* as a colour variation of *Camponotus singularis*. New data suggest that this name might be applied to a distinct species. Taxonomic implications and details on this issue will be the subject of a separate publication after having examined the types of var. *rufomaculatus* (Zettel et al. in prep.). Faunistic data suggest that this form may be endemic to the island of Borneo, as no matching specimens from other proveniences have been reported so far.

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### Supplementary material 1

**Table S1: Measurements (mm) of all examined specimens of *Camponotus singularis* (n=100) and *C. s.* var. *rufomaculatus* (n=6).**

Authors: Alice Laciny, Herbert Zettel, Irina Druzhinina

Data type: specimens data

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# Morphological variation and mermithism in female castes of *Colobopsis* sp. nrSA, a Bornean "exploding ant" of the *Colobopsis cylindrica* group (Hymenoptera: Formicidae)

Alice LACINY, Herbert ZETTEL, Brian METSCHER, Abu Salim KAMARIAH, Alexey KOPCHINSKIY, Carina PRETZER & Irina S. DRUZHININA



## Abstract

*Colobopsis* sp. nrSA is an ant species of the Southeast Asian *Colobopsis cylindrica* group (formerly in the genus *Camponotus*), which is currently being studied in the course of a long-term project focusing on the ecology and evolution of its members. We herein describe the morphological diversity of a colony fragment in an artificial nest established at the Kuala Belalong Field Studies Centre, Brunei Darussalam. The caste composition of the nest fragment was analysed and species conformity was proved by DNA barcoding. We describe the morphological traits in both worker subcastes, gynes, an intercaste individual, and aberrations of gyne morphology caused by mermithid parasitism with a focus on morphometry. Mermithogynes were examined by micro-CT imaging to confirm the presence of parasitic nematodes. The high morphological diversity of *Colobopsis* sp. nrSA in comparison with more distantly related species of *Colobopsis* is discussed in the light of the recently revised phylogeny of Camponotini, and ecological as well as evolutionary questions are addressed.

**Key words:** Formicinae, Camponotini, *Colobopsis*, *Camponotus*, morphology, castes, phragmosis, Mermithidae, soldier, polymorphism, phylogeny.

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## Introduction

Phragmosis, i.e., blocking nest entrances with a body part, is found in various ant genera (e.g., HÖLDOBLER & WILSON 1990, FISCHER & al. 2015), but constitutes a key character for *Colobopsis* ants. *Colobopsis* was described as a genus by Gustav MAYR (1861) but later on frequently treated as a subgenus of *Camponotus* MAYR, 1861. Most recently published molecular data suggest that *Colobopsis* is a distinct clade of the Camponotini and not even the sister taxon of *Camponotus* (BLAIMER & al. 2015, WARD & al. 2016). According to WARD & al. (2016), *Colobopsis* comprises 94 valid species (including one fossil) and is commonly defined by a worker dimorphism: Minor workers are clearly distinguished from major workers with phragmotic heads (e.g., MCARTHUR 2012), which in some species, e.g., in the type species *Colobopsis truncata* (SPINOLA, 1808), bear a well-defined anterior shield composed of clypeus and genae, whereas in other species the head

can be less strongly modified. Gynes are also phragmotic and resemble the major worker phenotype in many characters. Advanced forms of phragmotic heads (vertically truncated, with sharply limited shields) have likely evolved independently at least twice in *Colobopsis*, in the *C. truncata* group and in the *C. saundersi* complex of the *C. cylindrica* group (H. Zettel & A. Laciny, unpubl.). However, besides size and head structures, little has been published on the morphological differences of *Colobopsis* worker morphs (e.g., HASEGAWA 1993).

The *Colobopsis cylindrica* group (COCY) was established by MCARTHUR (2012), although some species were subsequently excluded to keep the clade monophyletic. The species group can be subdivided into several distinct complexes by morphological traits (e.g., microsculpture of abdominal tergites) and molecular data (COOK 2008; I.S. Druzhinina, A. Kopchinskiy, A. Laciny, C. Pretzer &

H. Zettel, unpubl.). Although the two distinct phenotypes of minor and major workers also occur in COCY species, our preliminary morphometric studies revealed a surprisingly high variability in body size of the minor worker caste, subsequently leading to the study at hand.

Using a unique multidisciplinary approach of morphometry, DNA barcoding, and X-ray microtomography (micro-CT), this study aims to provide the first detailed characterizations of all female castes of the hitherto undescribed Bornean COCY species *Colobopsis* sp. nrSA ("near *saundersi*", DAVIDSON & al. 2007). We analyse the caste composition of a nest fragment and confirm the species identity and presence of parasites in presumed mermithogynes. Our results shed light on the morphological consequences of mermithid parasitism and contribute our perspective on unsolved questions of nutritional biology and the evolution of the soldier caste. Furthermore, we interpret our findings taking into account the newly revised phylogeny of the Camponotini (WARD & al. 2016).

### Female polymorphism in Camponotini

A conspicuous difference between ants and other social Hymenoptera (wasps and bees) is the extent of morphological divergence between reproductive females, called gynes or queens, and non-reproductive female helpers, called workers (PEETERS & ITO 2015). Whereas ant males differ from diploid females by a haploid genotype, female differentiation is largely phenotypic, although genetic and epigenetic factors have been found to play a role in the caste determination of several species (ANDERSON & al. 2008). The emergence of the wingless worker phenotype is hypothesized to have been a major facilitator for the evolution of novel behavioural adaptations as well as the striking intraspecific divergence in size often observed between gynes and workers (PEETERS & ITO 2015). Further differentiation of specialised worker castes is found in many ant genera and polymorphism of gynes (e.g., ergatoid or brachypterous gynes) is even more common (PEETERS 2012).

Within the tribe Camponotini there is a strong variation in the number of distinct helper castes. The scattered distribution of worker mono-, di- and polymorphism in a tree based on 959 ultraconserved element loci (from BLAIMER & al. 2015) indicates high plasticity (Fig. 1). The conservative character of a monomorphic worker caste is retained in *Polyrhachis* SMITH, 1857, *Calomyrmex* MAYR, 1861 and *Echinopla* SMITH, 1857 (e.g., DOROW 1995, ZETTEL & LACINY 2015). In the Australian genus *Opisthopsis* DALLA TORRE, 1893 workers are "monomorphic or slightly dimorphic" (WHEELER 1918). In the genera *Camponotus*, *Colobopsis*, and *Dinomyrmex* ASHMEAD, 1905, which were treated as one genus *Camponotus* until recently (WARD & al. 2016), the presence of more or less distinct minor and major worker subcastes is most common (BRADY & al. 2000, MCARTHUR 2007, LACINY & al. 2016), but within the speciose genus *Camponotus* many exceptions are known. For example, *Camponotus ligniperda* (LATREILLE, 1802) (LEUTERT 1962) and *C. sericeiventris* (GUÉRIN-MÉNEVILLE, 1838) (BUSER & al. 1985) are strongly polymorphic along a continuous series with a wide range of worker sizes, the subgenus *Dendromyrmex* EMERY, 1895 is reported as monomorphic (BRADY & al. 2000), and in the subgenus *Forelophilus* KUTTER, 1931 (formerly a separate genus) some intermediates between

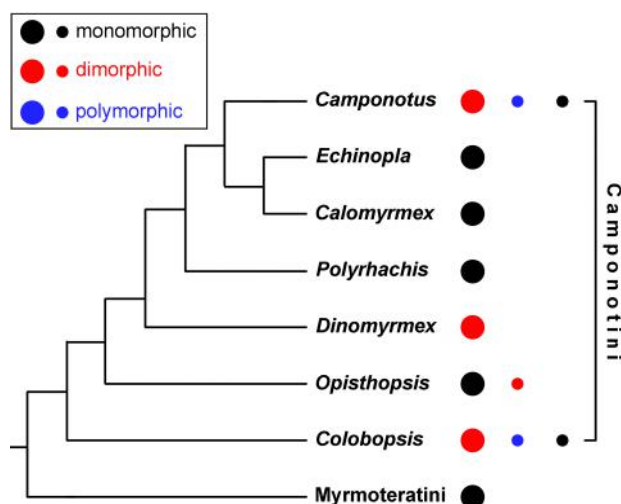


Fig. 1: Worker caste diversity in Camponotini applied to a tree by BLAIMER & al. (2015) based on 959 ultraconserved element loci. Large symbols indicate the dominant situation in the genus, small symbols deviations.

minor and major workers have occasionally been observed (ZETTEL & ZIMMERMANN 2007). The situation in *Colobopsis* is discussed below.

### The soldier question

A special case of ant polymorphism is the evolution of "soldiers", large specimens that often possess some similarities with gynes and have important functions in nest defence. Whether the soldier caste is derived from gynes or workers has been controversially discussed in the past (BARONI URBANI & PASSERA 1996, WARD 1997). More recently MOLET & al. (2012) presented a conceptual model based on modularity in morphology and development: The authors hypothesize that the basis for novel phenotypes with distinct functions may lie in the occurrence of rare mosaic castes or intercastes, stabilized by colonial buffering. According to this model, soldiers are not merely scaled-up versions of conspecific minor workers but are defined by possessing novel traits and proportions due to differing or partially "recycled" developmental pathways (MOLET & al. 2012, 2014, LONDE & al. 2015). If this is true for the studied clade, majors of COCY species may also be referred to as "soldiers", but will be termed "phragmotic major workers" in the course of this study, to better illustrate their function within the colony.

### Morphological changes caused by mermithid parasitism

One fascinating phenomenon that has long captivated the attention of researchers is the infestation of ants by parasitic mermithid nematodes, and the accompanying aberrant morphologies brought about in the hosts. Such parasitized individuals are known from several subfamilies of ants (WHEELER 1928, CSÖSZ 2012) and have also been observed to infest a *Colobopsis* species of New Guinea (MAEYAMA & al. 1994). Infested individuals often bear morphological features that are intermediate between castes, resulting in so-called intercaste phenotypes (WHEELER 1928). Observable morphological changes include smaller overall size, elongated legs, enlarged gaster (due to distension by the



Fig. 2: (a) Construction of artificial nest as used for this study. (b) Nest #27 at the Kuala Belalong Field Studies Centre, Brunei Darussalam.

nematode), reduced size of head, deviations in pilosity and sculpture, as well as reduction of wings, thoracic sclerites, ovaries, and ocelli in specimens with otherwise gyne-like morphology (WHEELER 1928, KAISER 1986, CZECHOWSKI & al. 2007, O'GRADY & BREEN 2011, POINAR 2012). These parasitogenic phenotypes have even led to taxonomic confusion in the past (CZECHOWSKI & al. 2007, CSÖSZ 2012, BOROWIEC & SALATA 2015), as morphologically aberrant individuals were erroneously described as separate, parasitic species (e.g., the holotype of *Myrmica symbiotica* (MENOZZI, 1925), actually a mermithized individual of *M. scabrinodis* NYLANDER, 1846; CSÖSZ 2012). Since the phenotypes produced by mermithid infestation can lie anywhere on a range from almost normal worker morphology ("mermithergates") to gyne-like specimens with only slightly reduced features ("mermithogynes") (CSÖSZ & MAJOROS 2009), many authors have stated that all castes are potential hosts to parasitic nematodes (e.g., PASSERA 1975, POINAR 2012). However, studies on the genera *Lasius* FABRICIUS, 1804 (KAISER 1986, O'GRADY & BREEN 2011) and *Myrmica* LATREILLE, 1804 (CSÖSZ & MAJOROS 2009) revealed that all potential mermithogenic phenotypes develop from larvae destined to be sexuals, i.e., gynes or males, and showed no infestation of workers.

A noteworthy consideration put forth by MOLET & al. (2012) is that such mermithogenic intercaste specimens are the results of – presumably hormonal – perturbations during development preventing the complete switch between worker and gyne phenotypes. Studying these animals therefore provides valuable insight into the modular nature of caste development, as some traits are modified under parasitic influence, but not others. Furthermore, if the propensity to produce such aberrant mermithogenic phenotypes results from heritable phenotypic plasticity and modularity, these same developmental processes could also play a role in the evolution of novel castes (MOLET & al. 2012, LONDE & al. 2015).

## Material and methods

### Sampling

Field work was carried out at the Kuala Belalong Field Studies Center (KBFSC) of the Universiti Brunei Darussalam located in the Ulu Temburong National Park, Brunei Darussalam (4° 32' 35.5" N, 115° 09' 09.3" E), in the course

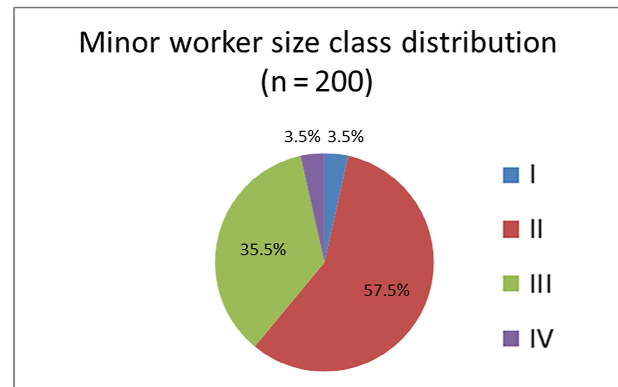


Fig. 3: Distribution of head width classes in a sample of 200 minor workers of *Colobopsis* sp. nrSA. Specimens were sorted into four size classes by head width, each group ranging 0.125 mm. Class-ranges (mm): I (< 1.375), II (1.375 - < 1.50), III (1.50 - < 1.625), IV ( $\geq$  1.625).

of an ongoing long-term investigation of ecology and evolution of the *C. cylindrica* species complex. The specimens were collected from an artificial nest (#27) constructed as described in DAVIDSON & al. (2009). The nest is illustrated in Figure 2a. Briefly, a cavity approximately 15 mm in diameter was drilled into the center of a 100 cm tall and 6 cm wide square wooden stake. Within the top third of the stake an entrance hole was drilled at a right angle to the center cavity using a 4 mm drill-bit. The entire construction was covered with waterproof green acrylic paint. In May 2014, nest #27 was installed standing vertically at the base of a *Shorea* sp. tree in the vicinity of a large colony of *Colobopsis* sp. nrSA. The artificial nest was connected to the stem of a small adjacent tree using a thin rope (Fig. 2b).

A year later, on April 16, 2015, nest #27 was visually inspected in situ. As *Colobopsis cylindrica* ants are strictly diurnal, sampling was performed at night assuming maximum nest occupation. The entrance hole was blocked with a wooden stick; the nest was tightly packed into plastic wrap and transferred to the laboratory at KBFSC. In the laboratory it was first cooled in the refrigerator (+10 °C) for 45 minutes and subsequently transversely cut into 5 cm sections. All nest occupants were taken out with sterile forceps and stored in 70% ethanol or RNAlater® Solution (ThermoFisher Scientific, MA, USA) in several 2 ml microcentrifuge tubes.

### Specimens and depositories

The imagines of nest #27 were sorted according to morph and counted. A subsample of specimens to be used for morphometry was selected in the following way: all phragmotic major workers (6 specimens); the only intercaste individual (1 specimen), all mermithogynes (2 specimens); 10 alate gynes (selected randomly from a pool of 43 specimens), and 42 minor workers selected as follows: 200 minor workers were taken randomly from a pool of ca. 550 specimens and roughly sorted to four size classes by head width (Fig. 3) at magnifications of  $25.6\times$  with a Nikon SMZ1500 binocular microscope (each group ranging two micrometer units = 0.125 mm). Out of these, 42 minor workers were selected for mounting and measurements. We hereby included a representative number of individu-

Tab. 1: Primers used in this study.

Gene	Name	Sequence 5' - 3'	Length	GC content [%] 40 - 60	Fragment length [bp]	Annealing temp. [°C]	Reference
COI	LCO1490-F	GGTCAACAAATCATAAAGATATTGG	25	32	709	45	CHEN & al. (2013)
	HCO2198-R	TAAACTTCAGGGTGACCAAAAAATCA	26	35			
COII	J2791-F	ATACCHCGDCGATAYTCAGA	20	40 - 55	858	51	CHEN & al. (2013)
	H3665-R	CCACARATTCWGAACATTG	20	35 - 40			

als from each of the four classes (corresponding to their approximate proportions in the larger subsample) as well as the overall largest and smallest specimens. In total, 61 female specimens were dry-mounted on triangular glueboards, individually numbered, and used to take measurements at magnifications from  $25.6\times$  up to  $256\times$ , depending on the respective character measured. Results present minimum and maximum values for each morph. The data set of measurements (Tab. S1) is available as an electronic supplement on the journal's web page.

Specimens are preliminarily kept in the Natural History Museum Vienna, but after a formal description of the species will be shared between the Brunei Museum, the Natural History Museum Vienna, and other institutions.

### Measurements and indices

The following measurements and indices were obtained from 61 specimens for morphometric characterization of female phenotypes.

CI	Cephalic index. $HW / HL \times 100$
EI	Eye Index. $EL / HW \times 100$
EL	Eye length. Maximum diameter of compound eye, measured in lateral view.
FeI	Femur Index. $FeL / HW \times 100$
FeL	Femur length. Maximum length of metafemur, measured from base to apex.
FWL	Forewing length. Length of forewing, measured from tegula to distal tip.
HaL	Hair length. Length of the longest standing hair on first gastral tergite, measured from base to apex.
HL	Head length. Maximum length of head in full-face view, excluding mandibles, measured from anterior-most point of clypeus to posterior-most point of head vertex, parallel to midline.
HW	Head width. Maximum width of head in full-face view (including eyes if protruding; only in gyness).
ML	Mesosoma length. Measured laterally from anterior surface of pronotum proper (excluding collar) to posterior extension of propodeal lobes.
MSW	Mesoscutum width. Maximum diameter of mesoscutum, measured dorsally.
OcD	Ocellar distance. Minimum distance between lateral ocelli, measured between median borders.
OcW	Ocellus width. Maximum diameter of median ocellus.
OED	Ocellar eye distance. Minimum distance between lateral ocellus and outer border of compound eye.
OI	Ocellar Index: $OED / OcD \times 100$
PH	Petiole height. Maximum height of the petiole in lateral view, measured from ventral-most point of petiolar sternum to dorsal apex.
PI	Petiole Index. $PH / PL \times 100$
PL	Petiole length. Maximum length of petiole in lateral view, measured from inflexion point of anterior

constriction to posterior margin, perpendicular to axis of maximum height.

SI	Scape index. $SL / HW \times 100$
SL	Scape length. Maximum length of antennal scape in dorsal view excluding basal neck and condyle.
SW	Scape width. Maximum width of antennal scape, measured dorsally.
SWI	Scape width index. $SW / SL \times 100$
TL	Total length. The added lengths of head (excluding mandibles), mesosoma, petiole, and gaster.

### Photographs

Stacked digital images were taken with a Leica DFC camera attached to a Leica MZ16 binocular microscope with the help of Leica Application Suite V3, stacked with Zerene-Stacker 64-bit, and processed with Adobe Photoshop 7.0.

### Micro-CT

Two morphologically aberrant specimens (specimen numbers 1137 and 1138) as well as an alate gyne (no. 1139) and the intercaste individual (no. 1385) were analysed using micro-CT to screen for parasites. Pinned specimens were mounted dry without contrast staining (METSCHER 2009, SOMBKE & al. 2015). For some, the glue-board was removed from the pin and held in a clamp during scans; for others the pin itself was clamped in place for scanning.

X-ray microtomographic images were made with a high-resolution micro-CT system (Xradia, MicroXCT-200, Zeiss X-Ray Microscopy, Pleasanton, CA) with a tungsten microfocus X-ray source and variable secondary optical magnification. These scans were made with an anode voltage setting of 60 - 75 kV at 5 W, with an exposure time of 2 - 3 seconds for projection images every 0.25 or 0.20 °. Tomographic reconstructions were exported as image stacks with isotropic voxel sizes of 4.7 - 5.0  $\mu\text{m}$ .

### DNA extraction, PCR amplification and sequencing

For DNA barcoding genomic DNA was extracted from legs using Qiagen's QIAamp DNA Micro kit (Qiagen, Venlo, Netherlands) using the tissue protocol. Three legs of one major worker, one alate gyne and one micropterous mermithogyne, respectively, 12 legs of two minor workers and five legs of the intercaste specimen were placed separately in 1.5 ml microcentrifuge tubes and frozen with liquid nitrogen. The frozen legs were ground into small pieces with disposable pestles and an appendant pestle motor (Kimble, Vineland, NJ, USA). Subsequent steps were performed according to manufacturer's instructions with the following modifications: sample lysis for 20 hours, final elution step with 23  $\mu\text{l}$  elution buffer. DNA concentration and 260 / 280 nm ratio to assess the purity of the extracted DNA were measured with NanoDrop ND-1000 Spectrophotometer

(Software Version ND-1000 V.3.8.1, Thermo Fisher Scientific, MA, USA).

Fragments of cytochrome c oxidase subunit I and II (COI, COII) were amplified. Primer sequences and temperature protocols are given in Table 1.

Final concentrations for PCRs were 1X GoTaq Flexi Buffer (Promega, Madison, Wisconsin, USA), 0.016 mM dNTPs, 3 mM MgCl<sub>2</sub> (Promega), forward and reverse primer (Microsynth, Balgach, Switzerland) (final concentrations: COI: 0.3 µM, COII: 0.35 µM), 0.7 Units GoTaqG2 Flexi Polymerase (Promega) and 5 - 12 ng sample (diluted with HPLC water, ROTH), in a final volume of 50 µl. PCR was performed with a Biometra T3 Thermocycler (Biometra, Göttingen, Germany) with the following conditions: 2 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at primer specific annealing temperature (45 °C for COI, 51 °C for COII) and 90 sec at 72 °C and finally 7 min at 72 °C. PCR products were separated by 1.5% agarose gel electrophoresis. PCR products were purified using mi-PCR Purification Kit (Metabion, Planegg, Germany) and one direction sequencing was performed at Microsynth.

Sequences are deposited in the NCBI GenBank under KU975365 and KU975366 for COI and COII, respectively. The following COI and COII sequences were accessed via GenBank and used for comparative alignments (Fig. S1): EF634187 "*Camponotus cylindricus* s.l. nrSA" EF634197 "*Camponotus cylindricus* s.l. SCY" EF634203 "*Camponotus cylindricus* s.l. OG" EF634199 "*Camponotus cylindricus* s.l. RHOG" EF634201 "*Camponotus cylindricus* s.l. YG" EF634193 "*Camponotus cylindricus* s.l. CL"

### Statistical analysis of measurements

Eleven measurements, taken from major workers, minor workers, and gynes, were compared using Kruskal-Wallis rank tests. All comparisons that yielded significant differences between sample medians were followed by a pairwise Mann-Whitney test with Bonferroni correction (modified after MOLET & al. 2014). Each test was adjusted for three multiple comparisons ( $N_p = 3$ ), thereby lowering the limit for significance to  $p = 0.05/3 = 0.0167$ . Values shown in Table 3 are  $p' = p \times N_p$ , therefore marked as significant if  $p' < 0.05$ .

In addition, coefficients of variation were computed for the two main measures of body size (HW, ML) in majors, minors and gynes. Lower and upper limits for 95% confidence intervals were obtained via Bootstrap (10,000 resamplings). In the case of non- or only very marginally overlapping confidence intervals, coefficients of variation are interpreted as significantly different at or above the 95% level (H.L. Nemeschkal, pers. comm.; see also PAYTON & al. 2003). All calculations were performed in Past 3 (HAMMER & al. 2001). The intercaste individual and the two mermithogynes were excluded from all analyses due to insufficient sample size.

## Results

### Sampling

The artificial nest contained approximately 550 minor workers, six phragmotic major workers, one intercaste specimen, 43 alate gynes, two micropterous mermithogynes, as well as brood (eggs, larvae, pupae) of *Colobopsis* sp. nrSA. In addition to the colony members, a small number of myr-

mecophile or parasitic arthropods were detected as well: three symbiotic crickets of the genus *Camponophilus* INGRISCH, 1995 and one "interloper" ant – a mimetic, presumably socially parasitic ant of an undescribed species of *Camponotus* frequently observed within samples of Bornean *Colobopsis* colonies (DAVIDSON & al. 2016). The examined sample of 200 minor workers ranged from 1.37 mm to 1.67 mm in head width. Specimens belonging to the largest and smallest size classes were rare (7 individuals in classes I and IV, i.e., 3.5% respectively) compared to the two intermediate classes (class II: 115 individuals, i.e., 57.5%; class III: 71 individuals, i.e., 35.5%; see Fig. 3).

### Species identification

Within the COCY group, the studied species belongs to a complex including *Colobopsis saundersi* (EMERY, 1889) and *C. badia* (SMITH, 1857). Based on our comparisons to type specimens and high-quality photographs thereof (ANTWEB 2016) it is most probably undescribed. Species identity of the specimens previously studied by D. Davidson and the material used in the present study was confirmed by comparison of morphology as well as COI and COII sequences deposited in NCBI GenBank (Fig. S1). We currently refrain from presenting a formal description of the species, as it would be preferable to describe it in the course of a revision of the *C. saundersi* complex.

### Micro-CT

X-ray microtomographic images of both examined micropterous gynes clearly showed parasitic mermithid nematodes coiled up within the gaster (Fig. 4). The diameter of the nematodes was measured to be roughly 0.25 mm; their length was estimated to be around 40 mm. Images of the alate gyne and the intercaste specimen showed only the remnants of internal organs and no trace of parasites, thus indicating that they were not infested by mermithid nematodes.

### Molecular diagnostics

The concentration of extracted DNA and DNA purity are given in Table 2. Molecular verification of genetic identity between minor and major workers, alate gynes, the intercaste specimen, and micropterous mermithogynes was confirmed based on the analysis of COI and COII sequences (see Fig. S1). The results of the molecular analyses also place *Colobopsis* sp. nrSA within the *saundersi* complex of the *C. cylindrica* group, which is in accordance with morphological results.

### Description of phenotypes

**Minor worker** (Figs. 5a, b, 7a): Measurements ( $n = 42$ ): TL 5.55 - 7.21; HW 1.37 - 1.67; HL 1.54 - 1.93; EL 0.37 - 0.44; SL 1.37 - 1.52; SW 0.13 - 0.16; ML 1.89 - 2.35; HaL 0.10 - 0.19; PH 0.47 - 0.58; PL 0.37 - 0.46; FeL 2.05 - 2.33. Indices: CI 86 - 92; SI 90 - 102; SWI 9 - 11; EI 26 - 29; PI 115 - 132; FeI 136 - 155.

Structures: Head subovate, longer than wide; sides anteriorly convex; microstructure consisting of very fine, isodiametric or transverse meshes; intermixed puncturation very fine and inconspicuous on face, larger but shallow laterally and ventrally. Eyes small, flat, positioned dorso-laterally, in smallest workers almost reaching outline of head. Ocelli lacking, in some larger specimens their posi-

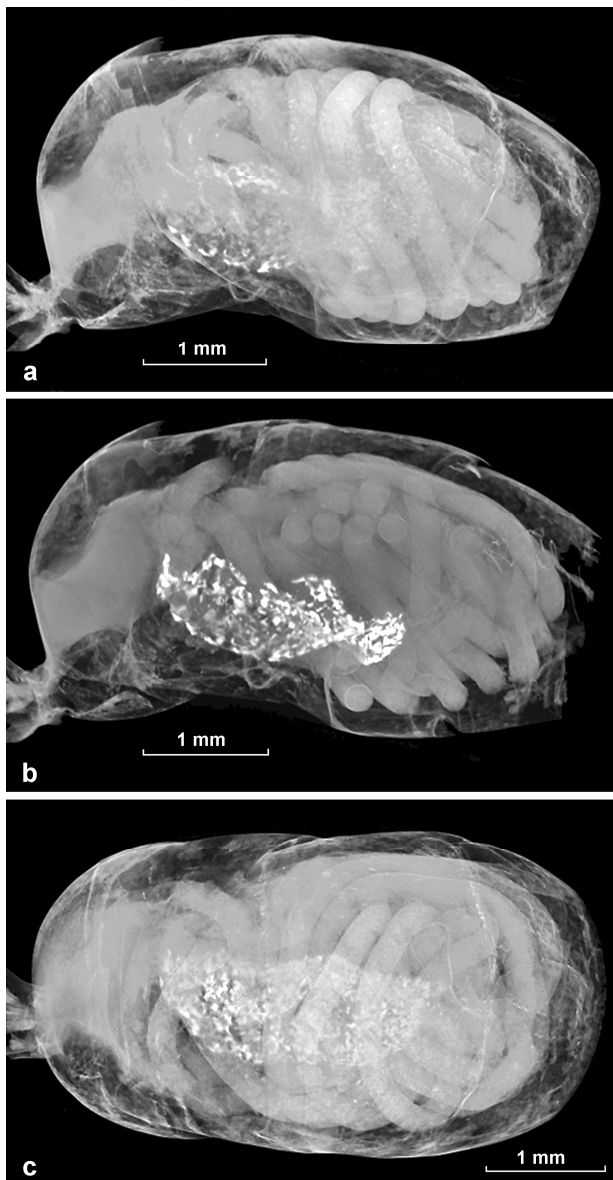


Fig. 4: X-ray microtomographic images illustrating the gaster of the mermithogyne #1137 in (a) lateral, (b) sagittal cutaway, and (c) dorsal aspect.

tion indicated by shallow impressions. Frons with very fine impressed midline; frontal carinae slightly converging anteriorly, not elevated, with small distance to each other. Clypeus with median carina that, especially in small specimens, does not reach anterior margin; faint longitudinal rugae present only in a few large specimens. Mandibles smooth, with rather dense puncturation; masticatory margin with five teeth. Antennal scape moderately flattened, slightly widened towards apex, with fine punctures. Antennal segment 3 about one fifth shorter than each 4 and 5, and about two fifths shorter than 2. – Mesosoma slender, moderately low. Microreticulation isodiametric or slightly transverse, dorsally denser than laterally. Metanotum distinctly delimited from mesonotum and propodeum by shallow grooves. Dorsal and posterior outline of propodeum meeting at an obtuse angle, dorsal face very shallowly, posterior face distinctly concave. – Legs slender. – Petiole with isodiametric reticulation; node in lateral aspect moderately

Tab. 2: Concentration [ng /  $\mu$ l] and 260 / 280 ratio of extracted DNA.

Preservation before extraction	TUCIM No. (Sample ID)	ng / $\mu$ l	260 / 280
Ethanol	5696 (362) minor worker	24.2	1.8
	5700 (359) major worker	9.3	2.3
	5698 (360) mermithogyne	50.1	2.1
	5699 (361) alate gyne	23.7	2.1
RNAlater® solution	6060 (479) intercaste specimen	10.5	1.6

high, its short anterior and its posterior faces parallel, its apex obliquely truncated, forming a distinct angle posteriorly; in dorsal aspect narrow, especially in small specimens a crest indistinct; a medial depression slightly indicated in large specimens. – Gaster: dorsal areas of tergites 1 - 3 with extremely fine, dense, transverse microreticulation, slightly shiny; on lateral areas of tergites 1 - 3, tergite 4, and sternites the reticulation wider, therefore meshes appearing not so strongly transverse, and the integument shinier.

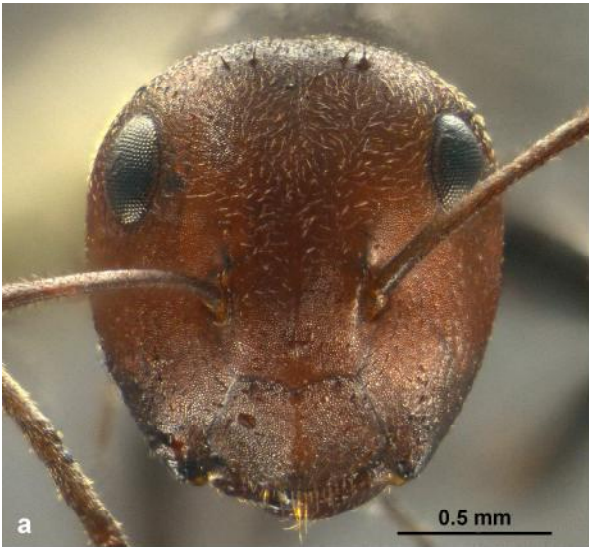
Colour: Body black. Head red, in some specimens more or less infuscated at vertex and anterior margins. Gaster tergites and sternites with very narrow hyaline margins. Mandibles dark brown to blackish. On antenna, scape and first funicular segment reddish brown, following antennomeres more or less brownish infuscated. Legs black.

Pilosity: Dorsum of head with very short, inconspicuous, appressed and subdecumbent setae; a few very long, standing setae on frons near declivity to vertex, medial of frontal carinae, and on clypeus sides. Mesosoma and petiole with fine and short, whitish, velvety pilosity; long, standing, slightly undulated setae restricted to pronotum; declivity of propodeum and node of petiole with few short standing setae. Gaster tergites with three types of setae: a dense layer of short appressed setae, short decumbent setae, and very long black standing setae.

Notes: Minors of *Colobopsis* sp. nrSA can be identified as belonging to the *C. saundersi* complex by the distribution of head and mesosoma pilosity, the microstructures of the dorsal areas of gaster tergites 1 - 3, and a large SWI. Within this complex *Colobopsis* sp. nrSA strongly differs from hitherto described species by colour: other known species of the complex are rather uniformly reddish-brown, while *Colobopsis* sp. nrSA is characterized by a red head and a black body.

**Intercaste** (Figs. 5c, 6a, 7b): Measurements (n = 1): TL 8.61; HW 1.83; HL 2.22; EL 0.50; SL 1.54; SW 0.18; ML 2.61; HaL 0.17; PH 0.63; PL 0.51; FeL 2.32. Indices: CI 82; SI 85; SWI 11; EI 27; PI 123; FeI 127.

Fig. 5: Heads, full face view, of female castes of *Colobopsis* sp. nrSA. (a) Small minor worker; (b) large minor worker; (c) intercaste specimen; (d) phragmotic major worker; (e) mermithogyne; (f) alate gyne.



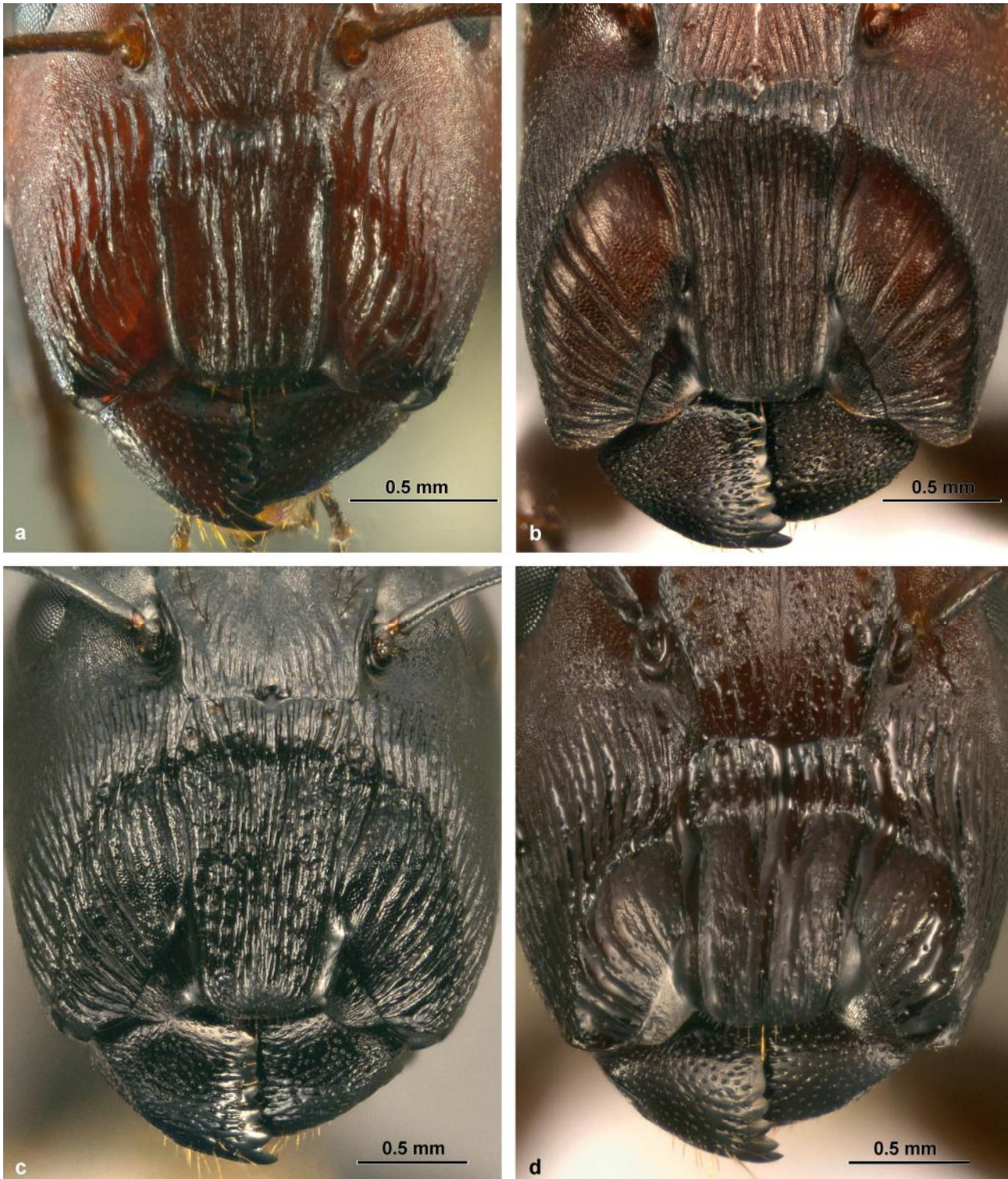


Fig. 6: Head shields, perpendicular view, in specimens of *Colobopsis* sp. nrSA. (a) Intercaste specimen; (b) phragmotic major worker; (c) mermithogyne; (d) alate gyne.

Structures: Head large, subcylindrical, anteriorly truncated. On posterior areas of face puncturation slightly stronger than in minor worker. Eyes distant from vertex. Ocelli lacking, position of anterior ocellus indicated by shallow groove. Anterior part of head forming a declivous shield, but in contrast to major workers the shield is not sharply limited by a crest. Clypeus with coarse longitudinal stria-

tion. This striae medially extended onto frons and genae till level of antennal grooves, laterally on genae shorter and weaker. Mandible with distinct ventrolateral ridge; its lateral face rugose-striate, punctures of dorsal-anterior face as in minors. – Structures of mesosoma, legs, petiole, and gaster as in minor worker.

Colour: as in minor worker.

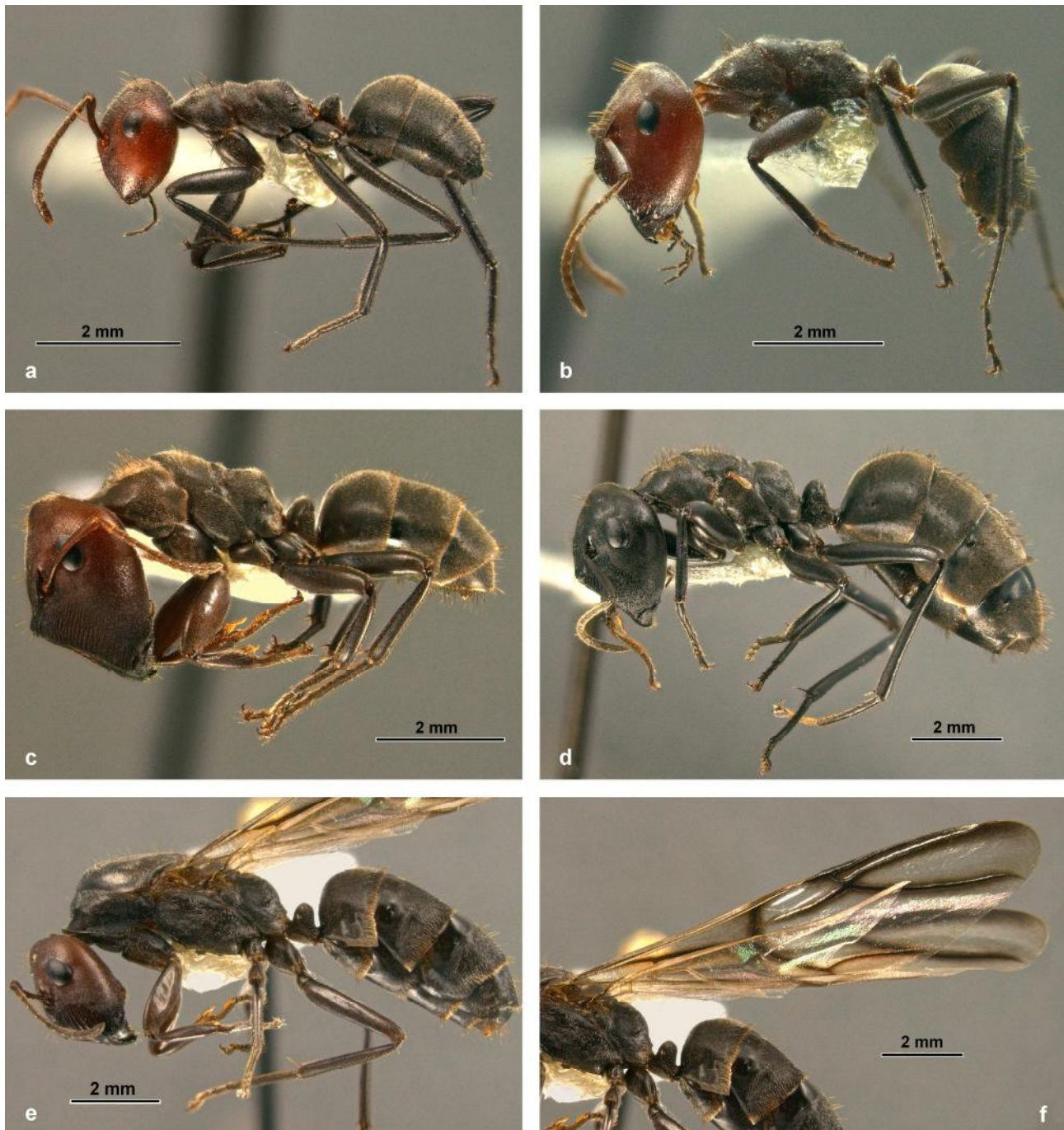


Fig. 7: Body, lateral view, of female castes of *Colobopsis* sp. nrSA. (a) Minor worker; (b) intercaste specimen; (c) phragmotic major worker; (d) mermithogyne; (e) alate gyne; (f) wings of alate gyne.

Pilosity: as in minor worker, except long setae on clypeus sides restricted to the posterior, horizontal part.

Notes: Based on the rarity of this morph and its co-occurrence with alate gynes in the same nest, this individual is regarded as an anomalous intercaste (in the sense of MOLET & al. 2012; see also PEETERS 1991, HEINZE 1998). It differs from both minor workers and phragmotic major workers in the intermediate structures of clypeus and mandibles. The possibility of this aberrant morphology being the result of mermithism was ruled out by a micro-CT scan of the animal's gaster which showed no parasite.

**Phragmotic major worker** (Figs. 5d, 6b, 7c): Measurements (n = 6): TL 8.25 - 9.42; HW 1.91 - 2.07; HL

2.58 - 2.77; EL 0.53 - 0.60; SL 1.28 - 1.38; SW 0.18 - 0.22; ML 2.80 - 3.26; HaL 0.15 - 0.23; PH 0.71 - 0.79; PL 0.45 - 0.53; FeL 1.73 - 1.91. Indices: CI 72 - 75; SI 66 - 69; SWI 14 - 16; EI 28 - 30; PI 151 - 153; FeI 90 - 95.

Structures: Head large, subcylindrical, anteriorly truncated. On posterior areas of face puncturation slightly stronger than in minor worker. Eyes distant from vertex. Ocelli lacking, position of anterior ocellus indicated by shallow groove. Anterior part of head forming a large shield that is limited by a sharp and elevated crest so that the shield surface is concave. Clypeus with coarse longitudinal striation. This striation medially extended onto entire frons and onto genae up to level of antennal grooves, laterally on genae

similarly long and strong. Mandible with sharp and high ventrolateral ridge; its lateral face rugose-striate, dorsal-anterior side with fine striation in addition to coarse puncturation; dentition on masticatory margin obsolete. Antenna shorter than in all other morphs. – Mesosoma stouter and higher than in minors, especially mesothorax expanded; in lateral aspect dorsal and posterior face of propodeum forming a less obtuse angle than in minor workers, dorsally without concavity. – Legs much shorter and stouter than in minors and intercaste individual. – Petiole wider than in minors, its node more rounded. – Structures of gaster similar as in minor worker.

Colour: Chiefly as in minor worker, but head darker, posteriorly chestnut brown, anteriorly at head shield strongly infuscated, often blackish.

Pilosity: as in minor worker, except long setae on clypeus sides restricted to the horizontal part behind clypeus shield; mesonotum with standing setae which are only slightly shorter than those on pronotum.

Notes: The head shield with a sharp, elevated crest is typical for majors of the *Colobopsis saundersi* complex.

**Alate gyne** (Figs. 5f, 6d, 7e, f): Measurements (n = 10): TL 12.03 - 13.57; HW 1.93 - 2.00; HL 2.51 - 2.64; EL 0.66 - 0.70; SL 1.52 - 1.61; SW 0.22 - 0.23; ML 4.83 - 5.09; HaL 0.21 - 0.30; PH 0.83 - 0.92; PL 0.63 - 0.67; FeL 2.51 - 2.64; OcW 0.18 - 0.19; OED 0.42 - 0.46; OcD 0.53 - 0.60; FWL 11.61 - 12.59; MSW 1.89 - 2.28. Indices: CI 75 - 78; SI 77 - 82; SWI 14 - 15; EI 34 - 36; PI 130 - 147; FeI 127 - 134; OI 71 - 81.

Structures: Head large, subcylindrical, anteriorly truncated, similar as in major worker with the following exceptions: Eyes slightly larger and slightly breaking outline of head in full-face view. Ocelli fully developed. Head shield sharply limited, but slightly smaller, distinctly narrower than head width. Striation of clypeus, frons and genae similar as in major. Mandible with sharp ventrolateral ridge; its lateral face rugose-striate, but lower than in major; dorsal-anterior face punctured; dentition on masticatory margin present, but weak. – Mesosoma large, structures as typical for alate ants; propodeum large and evenly convex in lateral view. Microstructures dorsally consisting of a very fine puncturation, with intermixed larger punctures, laterally finely reticulated. – Legs stout, but not as short as in major. – Forewing venation strongly reduced, as in most *Campoponini*; M-Cu absent; Mf2 + interstitial. – Petiole distinctly wider than in workers; node rounded in lateral aspect, its apex slightly impressed medially. – Gaster tergites 1 - 4 and sternites 1 - 4 with extremely fine and dense microstructures consisting of strongly transverse meshes; only sides of tergites with wide meshes and shiny; tergite 5 with dense isodiametric reticulation.

Colour: Body chiefly black. Head chestnut brown; anteriorly, especially margins of head shield infuscated. Pronotum and mesonotum with very narrow yellow margins. In specimens that are not yet fully hardened mesosoma with larger yellow parts, and head rather orange red. Gaster tergites medially with very narrow hyaline margins, broadened laterally; sternites with relatively broad posterior margins. Mandibles black. Antenna and legs chiefly black. Wings hyaline, but forewing cells strongly infuscated along veins. On forewing vein Sc + R (except yellow apex) and pterostigma black; other veins brown to yellowish. On hind wing all veins pale yellow.

Pilosity: Short pilosity and distribution of long setae on head, petiole, and gaster similar as in major worker, but that of mesosoma different; pronotum without long setae. Medial part of mesonotum (between parapsidal furrows) and scutellum with numerous long erect setae; lateral part of mesonotum only with 1 - 2 long setae in front of tegulae.

Notes: The head shield with a sharp, elevated crest is typical for gynes of the *Colobopsis saundersi* complex.

**Mermithogyne** (Figs. 4a-c, 5e, 6c, 7d): Measurements (n = 2): TL 12.36, 12.03; HW 2.22; HL 2.80, 2.84; EL 0.69, 0.66; SL 1.98; SW 0.20, 0.23; ML 3.68, 3.46; HaL 0.33, 0.34; PH 1.04, 1.06; PL 0.64, 0.63; FeL 3.23, 3.16. Indices: CI 79, 78; SI 89; SWI 10, 12; EI 31, 30; PI 162, 168; FeI 146, 143.

Structures: Head large, subcylindrical, anteriorly truncated, intermediate between major and gyne. Eyes distant from vertex and lateral outline of head. Ocelli lacking, their position indicated by small pits. Anterior part of head forming a large, clearly limited shield similar as in alate gyne; but its edge hardly elevated and not crested, so that the shield surface is only weakly concave. Striation of clypeus, frons and genae similar as in alate gyne, slightly finer and denser. Mandible as in alate gyne. Antennal scape conspicuously elongated, less widened towards apex than in alate gynes. – Mesosoma structures similar to majors, but in one specimen with a small lobe-shaped forewing remnant, and in the other specimen with a small groove containing a minute laminate structure (presumable wing remnant) at this position. – Legs similar as in alate gyne, but distinctly elongated. – Petiole similar as in alate gyne, but dorso-ventrally higher and with distinct median impression of the node. – Structures of gaster similar as in alate gyne; gaster enlarged due to presence of parasite.

Colour: Almost entirely black, including appendages. Wing remnants pale hyaline. Gaster tergites medially with very narrow hyaline margins, broadened laterally; sternites with moderately broad posterior margins.

Pilosity: similar to major worker.

Notes: Mermithogynes differ strongly from all other phenotypes by colour, pilosity of mesosoma, structures of petiole, and minute wing remnants. The head morphology, with incompletely crested head shield margins, differs strongly from gynes and phragmotic major workers, and resembles that of majors of more basic representatives of the *Colobopsis cylindrica* group, e.g., *C. leonardi* (EMERY, 1889). The elongated appendages differ distinctly from those of major workers and gynes and rather resemble the morphological proportions of conspecific minor workers. Among their nest mates the examined mermithogynes appear to belong to a completely different species. However, species identity is clearly confirmed by molecular analyses (see Fig. S1).

### Comparison of morphological characters in female castes of *Colobopsis* sp. nrSA

When comparing the morphometric data acquired from the different castes of the studied sample, one of the most striking results is the considerable size range of the minor workers in comparison to the other castes. When measuring "size" as total body length, the largest minor workers are up to 50% larger than their smallest nestmates, while gynes and major workers respectively are rather uniformly

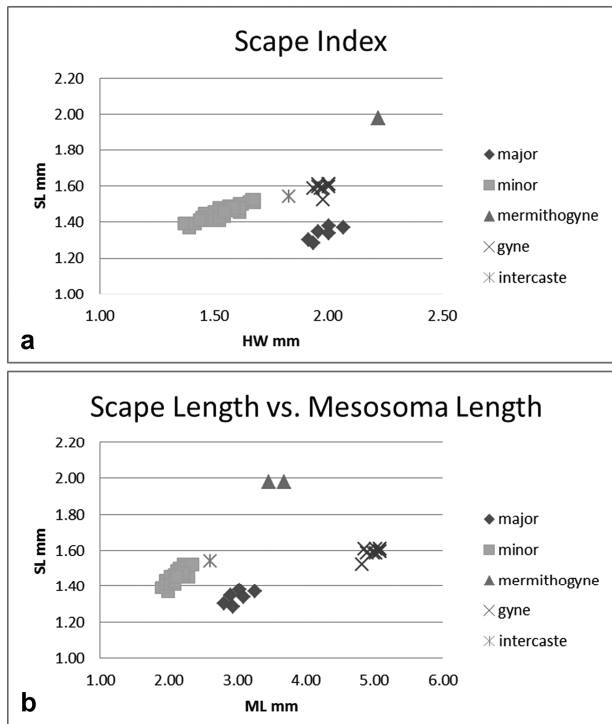


Fig. 8: Variation of scape length in female castes of *Colobopsis* sp. nrSA. (a) In relation to head width; (b) in relation to mesosoma length.

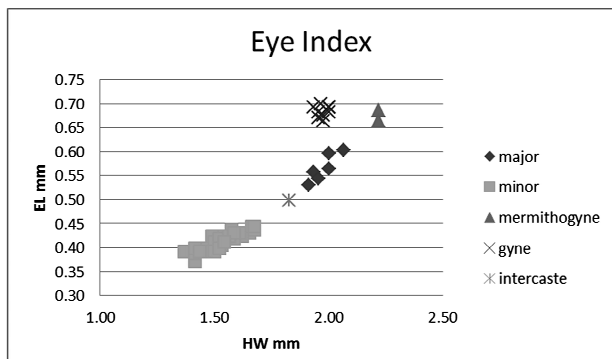


Fig. 9: Variation of eye length in female castes of *Colobopsis* sp. nrSA in relation to head width.

sized. Although often used and quite helpful for overall comparability of castes and species, total body length is a rather unreliable indicator of exact size due to fluctuations caused by storage in ethanol and the drying process. Considering head width, the largest minors surpass the smallest ones by ca. 20% (Fig. 8a, 10a). Although head width is the most commonly used indicator of size – especially in monomorphic ants – one must bear in mind that the major workers of many di- and polymorphic ant species possess greatly enlarged heads in relation to the rest of their bodies, which may lead to problems in comparisons between castes. We therefore prefer to use mesosoma length (often termed Weber's length) as reference to total body size for workers – in this respect the largest minor workers are ca. 25% larger than the smallest specimens (Figs. 8b, 10b). When comparing the largest and smallest of the minor workers it also becomes evident that certain morphological proportions shift with increasing size: FeI,

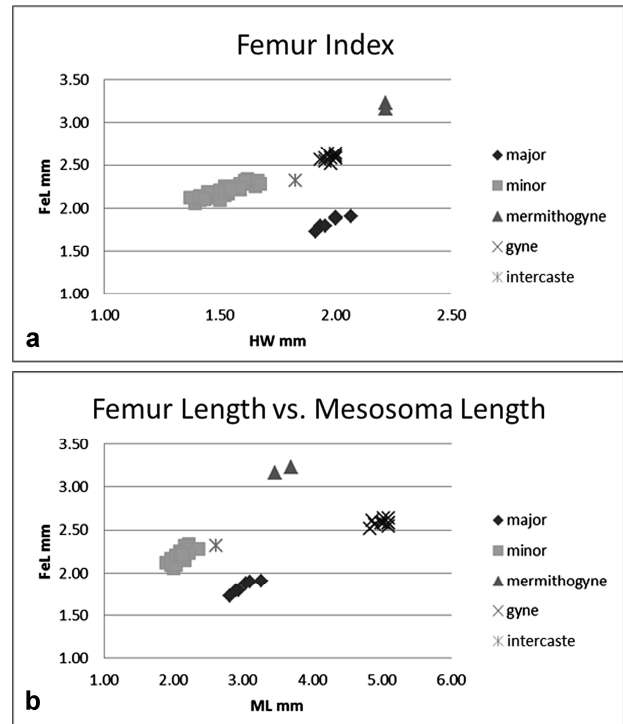


Fig. 10: Variation of metafemur length in female castes of *Colobopsis* sp. nrSA. (a) In relation to head width; (b) in relation to mesosoma length.

SI and EI all tend to be lower in bigger workers (Tab. S1). In workers the size of the mesosoma is of course reduced compared to that of gynes, due to the loss of the flight-apparatus (compare Figs. 7a, c, and e; see also Figs. 8b, 10b).

Gynes of *Colobopsis* sp. nrSA naturally differ from all other castes by their larger overall size, the presence of wings and ocelli, but also by their enlarged eyes (EI 34 - 36 vs. 26 - 31 in all other castes; Fig. 9).

When focusing on phragmotic major workers, perhaps their most striking feature – apart from their truncated heads – is the shape of their appendages. Antennae, legs and palpi are all shortened in comparison to the other castes, and particularly scapes and femora are greatly thickened relative to their length. These differences in proportions become evident in relation to head width (SI, FeI) as well as mesosoma length (which avoids shifts in allometry due to enlarged heads of majors) (Figs. 8b, 10b). In comparison, minor workers, queens and the intercaste specimen are quite similar regarding SI and FeI, while the mermithogynes possess disproportionately longer legs and scapes (Figs. 8a, 10a).

**Results of statistical analysis:** All eleven measurements compared between majors, minors and gynes in the Kruskal-Wallis rank test yielded significant differences in the sample medians (all  $p < 0.001$ ). The results of the pairwise Mann-Whitney tests are given in Table 3. Majors and minors as well as gynes and minors differed significantly in all compared measurements. Majors and gynes differed significantly in all characters except for the two measurements of the head (HW, HL). These results reflect the overall morphological caste differences also observed in our morphometric analysis. The lack of significant differences in HW and HL of majors and gynes corresponds to the observed similarity of head-morphology in these castes.

Tab. 3: Results of the pairwise Mann-Whitney tests with Bonferroni correction comparing 11 measurements of major workers, minor workers, and gynes of *Colobopsis* sp. nrSA: Values shown are  $p' = p \times N_p$ , adjusted for three multiple comparisons ( $N_p = 3$ ), rounded to three decimal places. Levels of significance:  $p' > 0.05$  = not significant (n.s.),  $p' < 0.05$  = \*,  $p' < 0.1$  = \*\*,  $p' < 0.001$  = \*\*\*.

		Major	Minor	Gyne
EL	major (n = 6)		0.002 **	0.004 **
	minor (n = 42)	0.002 **		< 0.001 ***
	gyne (n = 10)	0.004 **	< 0.001 ***	
FeL	major (n = 6)		< 0.001 ***	0.004 **
	minor (n = 42)	< 0.001 ***		< 0.001 ***
	gyne (n = 10)	0.004 **	< 0.001 ***	
HaL	major (n = 6)		0.001 **	0.011 *
	minor (n = 42)	0.001 **		< 0.001 ***
	gyne (n = 10)	0.011 *	< 0.001 ***	
HL	major (n = 6)		< 0.001 ***	> 0.05 n.s.
	minor (n = 42)	< 0.001 ***		< 0.001 ***
	gyne (n = 10)	> 0.05 n.s.	< 0.001 ***	
HW	major (n = 6)		< 0.001 ***	> 0.05 n.s.
	minor (n = 42)	< 0.001 ***		< 0.001 ***
	gyne (n = 10)	> 0.05 n.s.	< 0.001 ***	
ML	major (n = 6)		< 0.001 ***	0.004 **
	minor (n = 42)	< 0.001 ***		< 0.001 ***
	gyne (n = 10)	0.004 **	< 0.001 ***	
PH	major (n = 3)		0.014 *	0.037 *
	minor (n = 31)	0.014 *		< 0.001 ***
	gyne (n = 10)	0.037 *	< 0.001 ***	
PL	major (n = 4)		0.005 **	0.015 *
	minor (n = 35)	0.005 **		< 0.001 ***
	gyne (n = 10)	0.015 *	< 0.001 ***	
SL	major (n = 6)		< 0.001 ***	0.004 **
	minor (n = 42)	< 0.001 ***		< 0.001 ***
	gyne (n = 10)	0.004 **	< 0.001 ***	
SW	major (n = 6)		< 0.001 ***	0.004 **
	minor (n = 42)	< 0.001 ***		< 0.001 ***
	gyne (n = 10)	0.004 **	< 0.001 ***	
TL	major (n = 6)		< 0.001 ***	0.004 **
	minor (n = 42)	< 0.001 ***		< 0.001 ***
	gyne (n = 10)	0.004 **	< 0.001 ***	

Coefficients of variation for HW were highest in minor workers, intermediate in majors and lowest in gynes. Confidence intervals indicate significant differences between the three groups. For ML, coefficients of variation were similarly high in majors and minors and again significantly lower in gynes (Fig. 11). These analyses should be repeated at a later point, when a larger sample of phragmotic major workers is available, to eliminate any effects of small sample sizes and allow for cross-species comparisons.

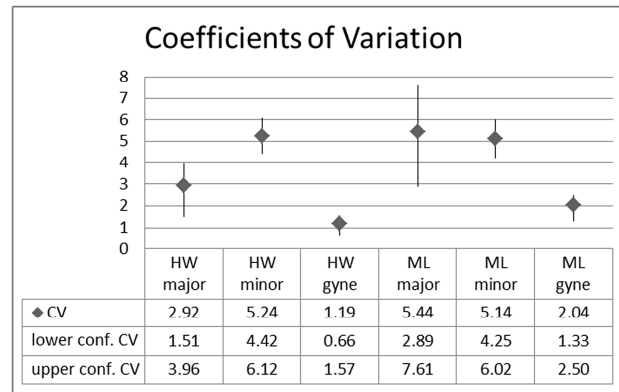


Fig. 11: Coefficients of variation and their lower and upper limits for 95% confidence intervals, for two main measures of size (HW, ML) in majors, minors, and gynes of *Colobopsis* sp. nrSA.

## Discussion

**Nest composition:** It is concluded from the absence of a dealate gyne (queen) and the exposure time of the artificial nest (12 months) that the collected individuals are not an entire, newly founded colony, but a subsample of the mature colony also occupying nesting sites in the neighbouring trees. As an exchange of individuals from different nest-sites was observed among colony fragments of several species of the COCY group (A. Kopchinskiy, unpubl.) we assume that the caste composition of the artificial nest may be similar to that of an entire mature colony. However, studies on *Dinomyrmex gigas* (LATREILLE, 1802), another polydomous camponotine ant native to Southeast Asia, have shown significant differences in the percentages of major workers between central and outer nests (PFEIFFER & LINSSENMAIR 2001). Therefore it cannot be entirely ruled out that the examined artificial nest was preferentially used by certain castes or for specific tasks (e.g., raising of brood) in comparison to other nesting sites. This question will be addressed in more detail in the course of further observations of COCY colonies.

**Female polymorphism in the *Colobopsis cylindrica* group:** The worker polymorphism in *Colobopsis* is very incompletely known, but a well-studied example is the European species *Colobopsis truncata*, the type species of *Colobopsis*: Its worker caste is composed of two distinct subcastes – minor workers and phragmotic majors (e.g., KUTTER 1977, SEIFERT 2007); the minor workers vary only slightly in size, at least within the same colony (A. Lacy & H. Zettel, unpubl.). Such dimorphism seems to be very common in *Colobopsis*, and was reported for many species (e.g., MCARTHUR 2012). In Southeast Asia we have observed dimorphism in some species of McArthur's *C. vitreus* group. However, other species from Southeast Asia are reported to have no phragmotic workers, and *Colobopsis macarangae* (DUMPERT, 1996) apparently lacks a major worker caste altogether (DUMPERT 1996). Also some Fijian species have reduced phragmosis (SARNAT & ECONOMO 2012).

Compared with these examples, the morphological variation of *Colobopsis* sp. nrSA is extremely high, even when mermithogynes and the single intercaste individual are not considered. The size range of minor workers (within the

same colony) is considerable (Fig. 11) and many morphological characters, such as relative size of compound eyes and length of appendages, change in relation to size (Figs. 5a, b; Tab. S1). This variability of minor workers has been observed in all species of the *Colobopsis cylindrica* group (H. Zettel & A. Laciny, unpubl.) and a similarly broad size range was reported for workers of *Camponotus singularis* (SMITH, 1858) (LACINY & al. 2016) and *Cataglyphis bombycina* (ROGER, 1859) in the tribe Formicini (MOLET & al. 2014). It is unknown whether this phenomenon is related to a division of tasks within the minor worker caste as it has been described for other ants (e.g., LEE 1938, HIGASHI 1974, ESPADALER & al. 1990). TSCHINKEL (1988) reported an increase in worker size and size-variability for older, larger colonies of *Solenopsis invicta* BUREN, 1972. The differentiation of morphology and tasks could therefore be related to the large size of colonies of *Colobopsis* sp. nrSA and related species (several thousand individuals; FEDERLE & al. 1998) compared to the small worker numbers in *C. truncata* (at most a few hundred individuals; SEIFERT 2007). As only a colony fragment was analysed within this study, however, our data do not allow us to safely interpret size-variation in minor workers as evidence for differential task allocation. The considerable intraspecific variability (see Fig. 11) of minor workers of the *Colobopsis cylindrica* complex largely obscures species-specific differentiation which poses a great obstacle for identification and morphology-based taxonomy, especially because most samples lack major workers or gynes.

The phragmotic head characters observed in gynes and major workers of *Colobopsis* sp. nrSA (and some closely related species like *C. saundersi*) have evolved from less differentiated forms of species that are phylogenetically more basal within the *C. cylindrica* group – e.g., *C. cylindrica* (FABRICIUS, 1798) and *C. leonardi* (COOK 2008; I.S. Druzhinina, A. Kopchinskiy, A. Laciny, C. Pretzer & H. Zettel, unpubl.). Gynes and majors of these species do not possess such sharply delimited head shields and more closely resemble the intercaste of *Colobopsis* sp. nrSA in this character.

**Traits of minor workers and gynes in the phragmotic major worker:** Phragmotic major workers are often regarded as a special form of "soldiers", a caste that has been controversially discussed in the past as derived either from gynes (BARONI URBANI & PASSERA 1996) or from "normal" workers (WARD 1997). Traits of both workers and gynes are expressed in majors of *Colobopsis* sp. nrSA, but they also possess specialized morphological features present in neither of the other female castes, such as distinctly shortened and thickened appendages. Figures 5 - 7 show that while the overall appearance of the body is more similar to minor workers, the truncated head and distinctive shield bear close resemblance to gynes (see also Tab. 3). The slightly enlarged mesothorax of major workers is also reminiscent of gyne-morphology, though much less prominent. However, the well-developed pronotum of major workers is more similar to minors and doubtlessly correlated with the presence of strong neck muscles to support the enlarged head. Such task-dependent morphological trade-offs between the mesothorax (hypertrophied for flight in alate queens) and the pronotum (enlarged in workers for optimized foraging and defence) have been observed across

all major subfamilies of ants (KELLER & al. 2014). The overall larger body size in majors of *Colobopsis* has also been linked to their function as repletes – i.e., animals which store liquid and nutrients – within the colony (HASEGAWA 1993).

Our morphometric data (Figs. 8, 10) show a pronounced thickening and shortening of all appendages in relation to head width for majors, resulting in considerably lower SI and FeI, and higher SWI compared to all other castes. Similar results were obtained in a recent study in *Camponotus singularis*, where shortened appendages (antennae, legs, maxillary palpi) were observed in majors and gynes (LACINY & al. 2016). In their 1988 study, FEENER & al. found that shorter legs correlated with increased body mass in workers of *Atta colombica* GUÉRIN-MÉNEVILLE, 1844 and soldiers of *Eciton hamatum* (FABRICIUS, 1782). Similar to the major workers of various species of *Camponotus* (e.g., PFEIFFER & LINSSENMAIR 2001, MYSORE & al. 2010, LACINY & al. 2016), majors of more basal COCY species are known to engage in combat outside the nest (COOK 2008). The strongly modified phragmotic majors of the *C. saundersi* complex, however, rarely leave the nest and have never been observed to fight. In these species, foraging and combat are presumably performed exclusively by the minor workers which kill enemy arthropods by autothysis (i.e., the eponymous "exploding" behaviour; COOK 2008, DAVIDSON & al. 2016). We therefore interpret the shortened and thickened appendages of phragmotic COCY majors as adaptations to their tasks within the nest: short, flat scapes can be kept close to the head for optimal fit into nest-entrances and short, thick legs are certainly most suitable to support a heavy body with an enlarged head. In addition, the shorter antennae of *Camponotus compressus* (FABRICIUS, 1787) majors specialized in defence have been linked to a reduced number of chemosensory sensilla compared to foraging minors (MYSORE & al. 2010). Together with the observed combinations of gyne- and worker-like characteristics in head and mesosoma morphology of majors, our results strongly support the conceptual model of MOLET & al. (2012, 2014) that views such specialized castes as developmental mosaics of gyne and worker growth programmes.

**Morphological aberrations in mermithogynes:** The two micropterous specimens found within the studied artificial nest were presumed to be mermithogynes based on their apparently altered morphology which largely corresponds to descriptions of mermithized gynes by other authors (WHEELER 1928, KAISER 1986, CZECHOWSKI & al. 2007, O'GRADY & BREEN 2011, POINAR 2012). Species conformity with nestmates as well as the presence of parasitic nematodes was confirmed with DNA barcoding and micro-CT (Figs. 4, S1). Size and appearance of the parasites correspond well to descriptions of mermithid nematodes found in *Lasius* sp. (O'GRADY & BREEN 2011). As reported for other genera, we recorded elongated appendages, altered colour, a distended gaster, and reduced ocelli (see Figs. 7d, 8, 10). Although wings and thoracic sclerites were also reduced, mesosomal volume was still visibly higher than in workers (see Fig. 7), which can be interpreted as a gyne-like character (KELLER & al. 2014). Contrary to other publications (e.g., CZECHOWSKI & al. 2007, O'GRADY & BREEN 2011) we did not observe overall smaller body or head sizes in mermithogynes, which

were roughly the same size as uninfected gynes and possessed even wider heads (Figs. 8a, 10a).

The hypothesis that mermithid nematodes may infect only sexuals (CSÖSZ & MAJOROS 2009, O'GRADY & BREEN 2011) is corroborated by the presence of small wing remnants and gyne-like mesosomal architecture which would not be developed in workers. The exact physiological effects by which the parasite alters host morphology have not been studied to our knowledge. However, based on the conspicuous reductions of the aforementioned gyne-specific characters and the amount of space taken up by the nematode where ovarian structures would be in healthy individuals, hormonal suppression during development may be a crucial factor (O'GRADY & BREEN 2011, MOLET & al. 2012).

#### **Mermithid parasitism as hint to larval food-source?**

Although the exploding ants of the *Colobopsis cylindrica* group have been the object of several ecological studies (DAVIDSON & al. 2007, 2009, 2016; COOK 2008), the nutrition of adult ants within this group and their brood still remains an open question. The fact that COCY ants have only rarely been observed to prey or feed on animal carcasses (HASHIMOTO & al. 1997), but rather seem to feed on microbes including fungal spores gathered from leaf surfaces, is corroborated by isotopic evidence (DAVIDSON & al. 2016). This leaves the question of larval nutrition to be answered.

In other ant genera associated with mermithid parasitism, it is well known that infection of the host occurs in the larval stage, when the nematodes' intermediate hosts – often oligochaetes – are fed to the ant's larvae as high-protein food-sources (KAISER 1986). Such an indirect life-cycle is typical for nematodes infecting terrestrial insects and in all studied cases of ants the infection is linked to oral uptake of the nematode's infective stages by the host's larvae (POINAR 2012).

As mermithid infection of adult ants of *Colobopsis* sp. nrSA was confirmed within this study, the question arises whether perhaps hitherto unobserved predation (e.g., of oligochaetes) does occur in this species as a means of supplying developing larvae with protein. Observations that may help to support this hypothesis are recently documented instances of predation (H. Zettel & W. Jaitrong, unpubl.) as well as rare occurrences of nematodes within buccal pellets in other species of the COCY group (DAVIDSON & al. 2016). Naturally, without access to more infected specimens and lacking the evidence of observed predation in the studied species, this can only be a tentative assumption at this point, albeit one that may help to uncover the mystery of larval nutrition in this clade within further studies.

**Resurrection of *Colobopsis* as a genus (WARD & al. 2016):** Based on the molecular study by BLAIMER & al. (2015), WARD & al. (2016) resurrected *Colobopsis* as a separate genus. The morphological differentiation between *Colobopsis* and *Camponotus* attempted in the same paper is based exclusively on head morphology of minors and is not without exceptions. The classification of species that are only known by minor workers – where phragmosis was not yet observed – remains doubtful. Based on observations first reported by WHEELER (1904), WARD & al. (2016) describe an important biological differentiation: pupae of *Colobopsis* are naked, whereas those of *Camponotus* are

enclosed in cocoons. It is unknown how many species have been studied in this respect since the first description of this trait. We ourselves have observed naked pupae in several species of the *Colobopsis cylindrica* group (Fig. S2).

In the study conducted by BLAIMER & al. (2015) the taxon sample is quite small (four species of *Colobopsis*, nine of *Camponotus* in the old classification; both type species not included). It might therefore be a question for future studies whether the diverse genus *Colobopsis*, which now comprises 94 species (WARD & al. 2016), is truly monophyletic or not. In the light of morphological characters only, the consideration might be allowed that a certain head morphology (wide head with distant frontal carinae and pronounced genae) may have served as a pre-adaptation for the evolution of phragmosis. BLAIMER & al. (2015) studied *C. saundersi* which is a close relative of *Colobopsis* sp. nrSA (I.S. Druzhinina, C. Pretzer, unpublished molecular data). That the monophyletic clade *Colobopsis* of BLAIMER & al. (2015) and WARD & al. (2016) corresponds to the taxon *Colobopsis* MAYR, 1861 depends on the assumption that the Mexican species "*C. BCA01*" is not only morphologically similar, but also closely related to the type species *C. truncata* from Europe. In addition, the assumed independent evolution of distinctive head shields in the *C. truncata* group and in the *C. saundersi* complex of the *C. cylindrica* group (H. Zettel & A. Laciny, unpubl.) further complicates the question of monophyly. These matters should be investigated in the course of future studies.

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## Digital supplementary material to

LACINY, A., ZETTEL, H., METSCHER, B., KAMARIAH, A.S., KOPCHINSKIY, A., PRETZER, C. & DRUZHININA, I.S. 2017: Morphological variation and mermithism in female castes of *Colobopsis* sp. nrSA, a Bornean "exploding ant" of the *Colobopsis cylindrica* group (Hymenoptera: Formicidae). – Myrmecological News 24: 91-106.

Fig. S1: Alignment of COI and COII sequences of minor and major workers, one alate gyne, the intercaste specimen, and one micropterous mermithogyne of *Colobopsis* sp. nrSA from nest #27. NCBI GenBank Accession numbers for the most similar reference sequences (retrieved on July 25<sup>th</sup> 2016) are given in the alignment. Vertical bar indicates the sequences obtained in this study while the arrow indicates the most similar sequence in the public database deposited as "*Camponotus cylindricus* s.l. nrSA" by Davidson, Cook & al.

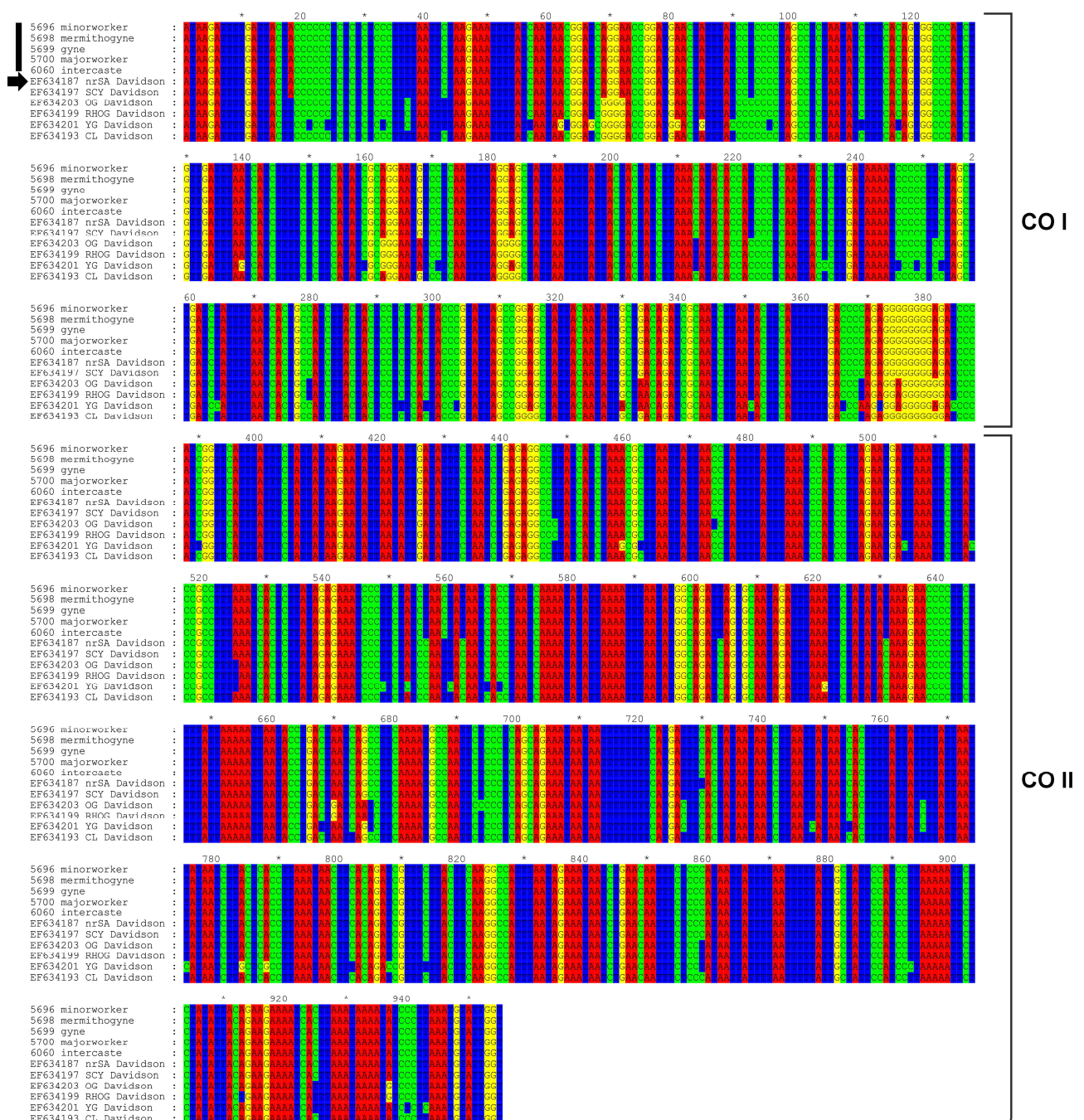


Fig. S2: View into artificial nest #27 after cutting, showing minor workers and naked pupae, which are referenced as characteristic for *Colobopsis*.



## Evidence of mermithism in a gyne of *Lasius niger* (LINNAEUS, 1758) (Hymenoptera: Formicidae) from Burgenland, Austria

Alice LACINY

### Abstract

This paper presents a new record of mermithid infestation in a gyne of the ant species *Lasius niger* (LINNAEUS, 1758) from Burgenland, Austria. The aberrant anatomy of the parasitized specimen is described and illustrated. The taxonomic placement of host and parasite is corroborated by molecular methods. In addition, comparisons of morphometric characters to those of healthy individuals and to a previously published dataset are conducted. The observed morphological aberrations of the mermithogyne include reduced gyne-specific characters and elongated appendages. These results, as well as caste identity, parasitic load and ecological conditions are in accordance with the current state of knowledge on comparable host-parasite systems.

**Key words:** Hymenoptera, Formicidae, *Lasius*, Mermithidae, parasitism, morphometry.

### Zusammenfassung

In dieser Arbeit wird ein neuer Beleg einer mit einem parasitischen Fadenwurm (Mermithidae) befallenen Gyne der Ameisenart *Lasius niger* (LINNAEUS, 1758) aus dem Burgenland vorgestellt. Die abweichende Anatomie des parasitierten Exemplars wird beschrieben und illustriert. Die taxonomische Stellung von Wirt und Parasit wird durch molekulare Methoden gestützt. Zusätzlich werden morphometrische Merkmale mit jenen gesunder Individuen sowie mit einem zuvor publizierten Datensatz verglichen. Die Mermithogyne zeichnet sich morphologisch u. a. durch reduzierte Gynenmerkmale und verlängerte Extremitäten aus. Diese Ergebnisse, sowie Kastenzugehörigkeit, Parasitenlast und ökologische Gegebenheiten decken sich mit dem aktuellen Wissensstand über vergleichbare Wirt-Parasiten-Systeme.

### Introduction

The earliest published scientific record of ants in general – and *Lasius* FABRICIUS, 1804 in particular – infested by mermithid nematodes dates back to GOULD (1747), who noted long white worms in “large and small ant-flies” (i.e., female and male sexuals). Later CRAWLEY & BAYLIS (1921) only reported mermithid infections of *Lasius* gyenes.

To date, seven genera of the family Mermithidae are known to parasitize ants, with three species of the genus *Pheromermis* POINAR, LANE & THOMAS, 1967 described from *Lasius* spp. (POINAR 2012). In all studied cases, infection takes place when an intermediate host containing the juvenile nematodes is fed to the larvae of the host ant as a protein source (KAISER 1986, POINAR 2012). Intermediate hosts of *Pheromermis* spp. are oligochaete worms (KAISER 1986) or aquatic insects, such as caddisflies (POINAR 1981). To complete their life cycle, mermithid parasites alter their hosts' behavior, eventually leading to the infested ants

becoming restless, seeking a body of water or damp earth to release the mature nematode and subsequently dying (CRAWLEY & BAYLIS 1921, KAISER 1986, MAEYAMA et al. 1994).

Mermithid infestations are usually accompanied by morphological changes in the hosts, possibly brought about by hormonal perturbations during development (O'GRADY & BREEN 2011). Such aberrant morphologies of parasitized individuals are known from several subfamilies of ants (WHEELER 1928, CSŐSZ 2012, MAEYAMA et al. 1994, LACINY et al. 2017). Parasitized specimens are often morphologically intermediate between castes, resulting in so-called intercaste phenotypes (WHEELER 1928). Characteristic changes include reduced body size, elongated extremities, enlarged gaster (due to distension by the nematode), reduced size of the head, deviations in color, pilosity and sculpture, as well as reduction of all sexual characters, such as wings, thoracic sclerites, ovaries, and ocelli (WHEELER 1928, KAISER 1986, CZECHOWSKI et al. 2007, O'GRADY & BREEN 2011, POINAR 2012, LACINY et al. 2017). Phenotypes produced by mermithid infestation can vary considerably, lying anywhere on a range from almost normal worker morphology ("mermithergates") to gyne-like specimens with only slightly reduced features ("mermithogynes") (CSŐSZ & MAJOROS 2009). Studies on *Lasius* (KAISER 1986, O'GRADY & BREEN 2011) and *Myrmica* LATREILLE, 1804 (CSŐSZ & MAJOROS 2009) have revealed that all known mermithogenic phenotypes develop from larvae destined to be sexuals, i.e., gynes or males, and no confirmed infestation of workers has been documented for these taxa.

### Material and methods

**Sampling conditions:** During an excursion to Neusiedl am See (Burgenland) on July 18<sup>th</sup> 2017, a living alate gyne with an enlarged gaster and short wings was found crawling on a log, seemingly unable to fly (M. Madl, pers. comm.). It was caught alive and kept under laboratory conditions for the purpose of breeding experiments: The specimen was placed in a glass petri dish containing a small paper tube for shelter and a glass vial with cotton wool soaked in sugar-water for nutrition. It survived in captivity for approximately one week and was then found dead with an – also already deceased – nematode protruding ventrolaterally from its ruptured gaster (see Fig. 3).

The animal was dry-mounted on a cardboard point for determination, morphometry and photography. It was identified as a gyne of *Lasius niger* with the help of SEIFERT (2007). To assess morphological aberrations, it was compared to an alate *L. niger* gyne from the NHMW Hymenoptera collection (species identity confirmed with SEIFERT 2007), as well as illustrations of a conspecific specimen provided by ANTWEB.org. Based on previously published records of host-parasite-relations (POINAR 2012), the parasitic nematode was presumed to belong to the genus *Pheromermis* sp., but a more exact determination was not feasible. Three legs from the right side of the mermithogyne as well as the parasitic nematode were subsequently removed for molecular analysis.

**Molecular methods:** For DNA barcoding, genetic material was extracted from three detached legs of the mermithogyne, as well as half of the removed nematode. Fragments of cytochrome c oxidase subunit I and II (*coI*, *coII*) were amplified and sequenced. For detailed methods and primers used, see LACINY et al. (2017). The resulting sequences were compared to previously deposited sequences in NCBI GenBank using BLAST (BENSON et al. 2005). Sequences are deposited in NCBI GenBank under accession numbers MF993322 and MF993323 for *coI* and *coII* of the mermithogyne, respectively, and MF993321 for *coI* of the nematode.

## Measurements and indices:

HW	Head width. Maximum width of head in full-face view (including eyes).
HL	Head length. Maximum length of head in full-face view, excluding mandibles, measured from anteriormost point of clypeus to posterior-most point of head vertex, parallel to midline.
EL	Eye length. Maximum diameter of compound eye, measured in lateral view.
FeL	Femur length. Maximum length of profemur, measured from base to apex.
FWL	Forewing length. Length of forewing, measured from tegula to distal tip.
ML	Mesosoma length (Weber's length). Measured laterally from anterior surface of pronotum proper (excluding collar) to posterior extension of propodeal lobes.
MSW	Mesoscutum width. Maximum diameter of mesoscutum, measured dorsally.
OcW	Ocellus width. Maximum diameter of median ocellus.
PSL	Palp segment length. Combined lengths of maxillary palp segments 5 and 6.
SL	Scape length. Maximum length of antennal scape in dorsal view excluding basal neck and condyle.
TL	Total length. The added lengths of head (excluding mandibles), mesosoma, petiole, and gaster.
NL	Nematode length. Maximum length of longest parasitic nematode found within mermithogynes.
CI	Cephalic index. $HW / HL \times 100$
EI	Eye index. $EL / HW \times 100$
FeI	Femur index. $FeL / HW \times 100$
PSI	Palp segment index. $PSL / HW \times 100$
SI	Scape index. $SL / HW \times 100$
WI	Wing index. $FWL / TL \times 100$

Examination and morphometric analysis of specimens was carried out on a Nikon SMZ1500 binocular microscope with an ocular micrometer, at magnifications of up to 256 $\times$ .

Stacked digital photos were taken with a Leica DFC camera attached to a Leica MZ16 binocular microscope with the help of Leica Application Suite V3, stacked with Zerene-Stacker 64-bit, and processed with Adobe Photoshop 7.0. Diagrams and calculations were made with MS Excel 2016.

## Depositories:

CASC California Academy of Sciences, San Francisco, CA, USA

NHMW Natural History Museum Vienna, Austria

## Material examined:

Specimens examined: 1 mermithogyne (NHMW), Austria, Burgenland, Neusiedl am See, bike path (47.945555°N, 16.833358°E), 118 m a.s.l., 18.VII.2017, leg. M. Madl. 1 alate gyne (NHMW), Spain, Pontevedra env., leg. H. Franz (no collection date).

Illustrations examined: CASENT0178773, 1 alate gyne (CASC), Netherlands, Bergen (52.67694°N, 4.69944°E), 5.VII.2007, leg. P. Boer.

Data examined: Biometric data of 20 mermithogynes and 19 alate gynes, Ireland, Clare Island, (53.8028°N, 9.9545°W), 20.VIII.2005, leg. A. O'Grady & J. Breen (see O'GRADY & BREEN 2011).



Figs. 1–3: *Lasius niger* mermithogyne, habitus. (1) Frontal, (2) dorsal, (3) lateral (note parasitic nematode protruding through gastral wall).

## Results

Measurements (in mm): Mermithogyne: HW 1.61, HL 1.46, EL 0.44, FeL 1.47, FWL 6.66, ML 3.06, MSW 1.86, OcW 0.07, PSL 0.53, SL 1.32, TL 10.59, NL 35; CI 110, EI 27, FeI 91, PSI 33, SI 82, WI 63.

Alate gyne: HW 1.67, HL 1.54, EL 0.54, FeL 1.30, FWL 9.38, ML 3.25, MSW 1.83, OcW 0.13, PSL 0.38, SL 1.30, TL 9.66; CI 108, EI 27, FeI 78, PSI 23, SI 78, WI 97.

Aberrant morphological characters of mermithogyne (Figs. 1–3): In comparison to healthy specimens, the examined mermithogenic phenotype exhibited con-

Tab. 1: Comparison of four biometric characters between alate gynes (AG) and mermithogynes (MG) from three localities (AUT Burgenland, Austria; IRL Clare Island, Ireland; ESP Pontevedra, Spain). IRL data are means of 20 mermithogynes and 19 alate gynes, respectively.

	HW (mm)	FeL1 (mm)	FeI	NL (mm)
<b>MG AUT</b>	1.61	1.47	91	35
<b>MG IRL (mean)</b>	1.40	1.31	93	41
<b>AG ESP</b>	1.67	1.30	78	–
<b>AG IRL (mean)</b>	1.49	1.20	79	–

spicuously elongated legs (FeI 91 vs. 78, see Fig. 4) and maxillary palps (PSI 33 vs. 23, see Fig. 3), and somewhat elongated scapes (SI 82 vs. 78). Gyne-specific sexual characters were reduced in the parasitized specimen: The size of all ocelli was reduced, with the diameter of the median ocellus measuring only 50 % of that in the similarly sized uninfected gyne (Fig. 1). The wings were completely developed regarding their shape and venation, but the forewings were considerably reduced in size, reaching only 63 % of the total body length, while they were almost exactly as long as the body in the measured healthy specimen as well as the gyne imaged by ANTWEB.org (WI 63 vs. 97, see Figs. 2, 3). The length of the mesosoma was somewhat reduced, while its width was unaffected. The total body length was greater than in healthy specimens, which was at least partly due to the distension of the gaster by the parasitic nematode. Characters of head size (HW and HL) were marginally smaller in the mermithized specimen. The proportions of the compound eyes, as well as pubescence, coloration and cuticular sculpture showed no detectable aberrations.

Comparison to sample of O'GRADY & BREEN (2011) (Tab. 1, Fig. 4): The two specimens measured for this study were compared to selected morphometric characters taken from the dataset of Irish specimens treated in O'GRADY & BREEN (2011). Only sexuals (gynes and males) of *L. niger* and *L. flavus* were found to be parasitized. As in the individual described herein, mermithogynes were readily recognizable in the field by their short wings and distended gaster. Although the Irish specimens were consistently somewhat smaller than those measured for this study, similarities in allometry were apparent (Tab. 1, Fig. 4): Regarding the size of the observed parasitic nematodes, the authors reported lengths of 27 – 68 mm for parasites found in *L. niger* gynes. The parasite discovered in the Austrian specimen falls well within this range at 35 mm. As in the specimen from Burgenland, most infested gynes only had one parasitic nematode; an occurrence of multiple parasites was only recorded in two cases. Irish specimens also showed significant differences in femur length and head width between healthy and parasitized individuals, with the mermithogenic phenotypes possessing longer legs (mean FeI 93 vs. 79) and smaller heads (see Tab. 1). The radial cells (a size indicator of the forewing) of mermithogynes were significantly smaller, but the wings showed no aberrant proportions. No effect of parasitism on overall variation of morphological characters was detected.

Molecular analysis: The amplification and sequencing of both *coI* and *coII* was successful for the mermithogyne of *L. niger*. Comparisons to *coI* sequences deposited in NCBI GenBank yielded similarities of 100 % to *L. niger* (GQ503247). Along with the morphological examination, this confirms the species identity of the parasitized specimen.

For the parasitic nematode, only *coI* was successfully amplified. Due to a lack of comparable sequences deposited in GenBank, no conclusive result regarding species identity

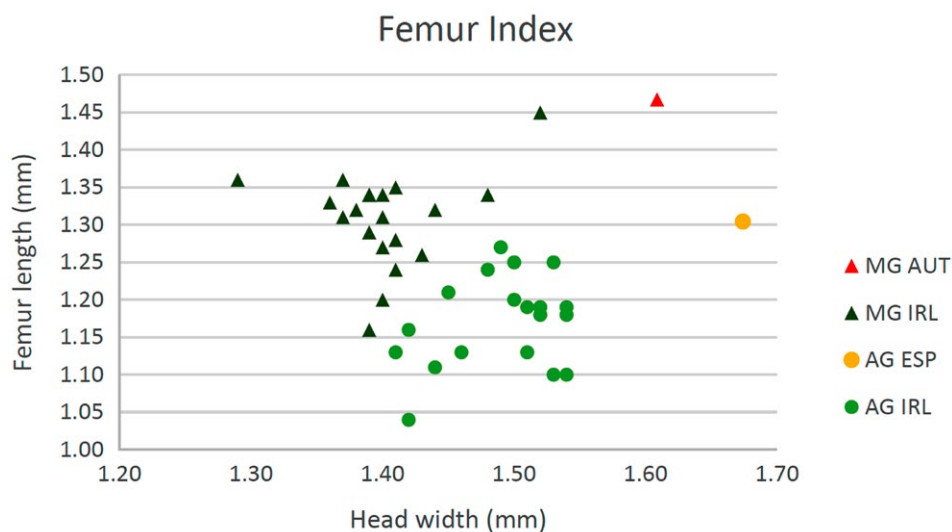


Fig. 4: Compared Femur index of alate gynes (AG) and mermithogynes (MG) from three localities (AUT Burgenland, Austria; IRL Clare Island, Ireland; ESP Pontevedra, Spain).

can be drawn here. The most similar sequences – with 74%, 72%, and 71 % similarity, respectively – were those of other entomopathogenic mermithid nematodes: *Hexamermis agrotis* (EF368011), which is parasitic on noctuid moths (LI & XIONG 2005), as well as *Romanomermis nielsenii* (EF175763) and *Strelkovimermis spiculatus* (DQ520860), both parasitic on mosquitos (FINNEY & MOKRY 1980, POINAR & CAMINO 1986).

## Discussion

This paper represents the first published record of mermithid parasitism on *Lasius* for the Austrian province of Burgenland. Other Austrian records come from KAISER (1986), who reported *L. niger* and *L. flavus* from Styria parasitized by *Pheromermis villosa* KAISER, 1986.

The species identity of the examined mermithogyne is supported by morphological as well as molecular data. The purely morphological identification of parasitogenic phenotypes can prove difficult, as traditional keys rely heavily on characters such as pubescence, sculpture and morphometric indices (e.g., SEIFERT 2007). All these characters can be altered by parasitic influence, which has led to cases of erroneous species descriptions in the past (CZECHOWSKI et al. 2007, CSÖSZ 2012).

The results obtained within this study correspond well with previously published records of mermithogenic phenotypes and parasitic loads in this genus (e.g., CRAWLEY & BAYLIS 1921, KAISER 1986, O'GRADY & BREEN 2011). In all of these cases, parasitized gynes were infested with one to three individual nematodes of one to seven centimeters in length. The described mermithogynes all possessed a distended gaster, and a smaller head and mesosoma. All mermithogynes were brachypterous to some extent, but the wings were always present and fully formed, apart from their size. While more extremely altered mermithogenic phenotypes have been documented for species of *Myrmica* (CZECHOWSKI et al. 2007, CSÖSZ & MAJOROS 2009) and *Colobopsis* (LACINY et al. 2017), I have found

no case of entirely apterous or worker-like *Lasius* mermithogynes (“mermithergates”) in the literature. It is possible that the development of certain host species is more resilient to parasitic stress or that the reason lies in the different species of parasitic nematodes.

A further interesting result was the consistently increased length of extremities in mermithogynes across different genera and subfamilies: Studies have found mermithogynes to possess elongated metafemora (in *Colobopsis* sp., LACINY et al. 2017), profemora (in *Lasius* spp., O’GRADY & BREEN 2011, this study), metatibiae (in *Myrmica* spp., CZECHOWSKI et al. 2007), maxillary palps (in *Colobopsis* sp., LACINY et al. 2017, in *Lasius niger*, this study), and antennal scapes (in *Colobopsis* sp., LACINY et al. 2017, in *Lasius niger*, this study, and in *Myrmica* spp., CZECHOWSKI et al. 2007, CSÓSZ & MAJOROS 2009). The reason for these particular aberrations is currently unknown. Presumably, the developmental perturbation by the parasite causes a shift from gyne-like to more worker-like growth programs (sensu MOLET et al. 2012), resulting in animals that are as large as gynes but more closely resemble the morphological proportions of workers.

Judging by the high incidence of mermithism in sexuals of European *Lasius* species (CRAWLEY & BAYLIS 1921, O’GRADY & BREEN 2011), it seems likely that the intermediate hosts (e.g., oligochaetes) of the parasitic nematodes constitute a significant part of the diet fed to queen- and male-destined larvae. Especially more damp environments seem to favor the development of both intermediate hosts and parasites (KAISER 1986). The sampling locality of the Austrian specimen treated in this study also fits this scheme: The mermithogyne was discovered close to a small channel only 2 km from the shore of Lake Neusiedl (M. Madl, pers. comm.).

The role of mermithogynes in *Lasius* colonies is hitherto unknown. According to previous publications (CRAWLEY & BAYLIS 1921, WHEELER 1928, O’GRADY & BREEN 2011), brachypterous mermithogynes are usually flightless with reduced ovaries and thus cannot take part in mating flights and reproduction. They seem to be generally accepted by their nestmates and have been observed to aid in brood care and beg workers for food (CRAWLEY & BAYLIS 1921). Mermithized specimens will stay with their native colony until compelled by the mature parasite to seek a suitable spot for its release (CRAWLEY & BAYLIS 1921, KAISER 1986, MAEYAMA et al. 1994). Recent models suggest that the survival of intercaste phenotypes may be facilitated by mechanisms of colonial buffering (MOLET et al. 2012). Building on these hypotheses, the study of morphological changes brought about by mermithid parasitism could provide valuable insight into phenotypic plasticity, developmental modularity, and the evolution of novel castes (MOLET et al. 2012, LONDE et al. 2015, LACINY et al. 2017).

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# ***Colobopsis explodens* sp. n., model species for studies on “exploding ants” (Hymenoptera, Formicidae), with biological notes and first illustrations of males of the *Colobopsis cylindrica* group**

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## **Abstract**

A taxonomic description of all castes of *Colobopsis explodens* Laciny & Zettel, **sp. n.** from Borneo, Thailand, and Malaysia is provided, which serves as a model species for biological studies on “exploding ants” in Southeast Asia. The new species is a member of the *Colobopsis cylindrica* (COCY) group and falls into a species complex that has been repeatedly summarized under the name *Colobopsis saundersi* (Emery, 1889) (formerly *Camponotus saundersi*). The COCY species group is known under its vernacular name “exploding ants” for a unique behaviour: during territorial combat, workers of some species sacrifice themselves by

rupturing their gaster and releasing sticky and irritant contents of their hypertrophied mandibular gland reservoirs to kill or repel rivals. This study includes first illustrations and morphometric characterizations of males of the COCY group: *Colobopsis explodens* Laciny & Zettel, **sp. n.** and *Colobopsis badia* (Smith, 1857). Characters of male genitalia and external morphology are compared with other selected taxa of Camponotini. Preliminary notes on the biology of *C. explodens* Laciny & Zettel, **sp. n.** are provided. To fix the species identity of the closely related *C. badia*, a lectotype from Singapore is designated. The following taxonomic changes within the *C. saundersi* complex are proposed: *Colobopsis solenobia* (Menozzi, 1926), **syn. n.** and *Colobopsis trieterica* (Menozzi, 1926), **syn. n.** are synonymized with *Colobopsis corallina* Roger, 1863, a common endemic species of the Philippines. *Colobopsis saginata* Stitz, 1925, **stat. n.**, hitherto a subspecies of *C. badia*, is raised to species level.

### Keywords

autothysis, behavioural ecology, Camponotini, *Colobopsis*, Formicidae, integrative taxonomy, male morphology, molecular biology, morphometry, new species, new status, new synonymy, phylogeny, Southeast Asia, taxonomy

### Introduction

The *Colobopsis cylindrica* (COCY) group likely represents a monophyletic clade containing Southeast Asian ant species with distinctive hypertrophied mandibular gland reservoirs. In territorial combat, minor workers use the sticky and irritant contents of their enlarged mandibular gland reservoirs to kill or repel rival arthropods. In species where this defensive behaviour is more advanced, this happens via the characteristic suicidal “exploding” by voluntary rupture of the gastral integument (autothysis) (Cook 2008). This behaviour was first mentioned by Viehmeyer as early as 1916, and subsequently described in detail by Maschwitz and Maschwitz (1974), as well as Davidson et al. (2012), and Shorter and Rueppell (2012).

The Bornean members of the COCY group have been the subject of various ecological (e.g., Cook 2008, Davidson et al. 2007, 2009, 2016), morphological (Davidson et al. 2012, Laciny et al. 2017) and chemical (Jones et al. 2004, Hoenigsberger et al. in prep.) studies in the past. Based on the results of previous investigations, in 2014 an interdisciplinary research project started to explore the evolution and ecological significance of autothysis in the COCY group. From the surroundings of the Kuala Belalong Field Studies Centre (KBFSC) in Brunei, at least 15 species are known (Davidson et al. 2007), most of which are probably new to science. One species, previously referred to as “yellow goo” (Davidson et al. 2007) or “YG” (Davidson et al. 2016) for the bright yellow colour of its mandibular gland secretion, was found to have a large colony just at the KBFSC. As this abundant species frequently exhibits characteristic autothysis behaviour and can be observed *in situ* and *in vitro*, it became the main object of behavioural and chemical experiments, and a model species for biological studies on “exploding ants”. Preliminary taxonomic and molecular analyses revealed that this morphospecies is in fact an undescribed species. As the revision of the COCY group is still ongoing (I. Druzhinina et al. in prep.), the aim of this paper is to provide a

valid name, *Colobopsis explodens* Laciny & Zettel, sp. n., for subsequent use in the various behavioural, chemical, microbiological, and evolutionary publications currently in preparation. Within this study, we employ the multidisciplinary concept of integrative taxonomy (*sensu* Schlick-Steiner et al. 2010) by combining morphometric, ecological, and molecular data. We provide a taxonomic description of all castes of *Colobopsis explodens* sp. n. including males. Illustrations and morphometric characterizations of males of the COCY group had not been previously published. We compare males of *Colobopsis explodens* sp. n. with the newly illustrated male of *C. badia* (Smith, 1857) to highlight species-specific characters in the complex. Morphological characters of the male, including genitalia, are also compared with other selected taxa of Camponotini. Based on field observations, the first records on the natural history and biology of *Colobopsis explodens* sp. n. are provided.

## Materials and methods

### Sampling-sites and imaging of living ants

The primary field research took place in the lowland dipterocarp rainforest at the Kuala Belalong Field Studies Centre (KBFSC), Temburong District, Brunei Darussalam (4°32'48.2"N, 115°09'27.9"E), where *Colobopsis explodens* sp. n. was sampled during five collecting trips (each of 30 days duration) encompassing different seasons from 2014 to 2016.

The behaviour of *C. explodens* sp. n. was observed at multiple nesting sites on several height-levels, starting from the forest floor and understory up to the canopy and emergent layer. The activity of ants was recorded *in situ* and *in vitro* using a CANON 70D Digital SLR Camera with a CANON EF 100 mm macro lens and a Tamron AF 28–200 mm F/3.8–5.6 XR Di aspherical (IF) macro zoom lens (Suppl. material 2: S2a). For macro and close-up filming the Neewer adjustable LED light with LCD display was used. When necessary, the camera was mounted with the use of a Manfrotto Gorillapod 494RC2 tripod. The movie (Suppl. material 7) was annotated and cut using Corel VideoStudio X10 Software.

Sampling of *Colobopsis badia* in southern Thailand was conducted by H. Zettel and W. Jaitrong in June 2016. The sampling site was located in the Khao Chong Botanical Garden, near the Ton Pliw Waterfall (07°32'34"N, 99°47'33"E); a single male specimen was caught at a light at the Botanical Garden headquarters.

### Host trees and activity assessment

Nesting habits of *C. explodens* sp. n. were observed based on the model colony occupying several trees and an artificial nest (nest #38, Fig. 9) in direct vicinity to the kitchen facility at KBFSC. The artificial nest consisted of a 100 cm tall and 6 cm wide square wooden stake,

with a cavity of approximately 15 mm in diameter drilled into the centre and a 4 mm wide entrance hole in the top third of the stake. The nest was painted with green acrylic paint and fastened to a small tree with rope (for detailed method of construction, see Davidson et al. 2009 and Laciny et al. 2017). The host trees were identified by comparison with type samples preserved in the herbarium of Universiti Brunei Darussalam, Brunei. The main host tree was DNA barcoded (see Suppl. material 6 “accession numbers”).

The activity of *C. explodens* sp. n. occupying artificial nest #38 was observed from 14<sup>th</sup> to 30<sup>th</sup> November of 2015 at different times during the day, for 30 minutes each by counting the ants entering and leaving the nest. Temperature, barometric pressure, and weather conditions were recorded, as well as any observed noteworthy behaviour (see Fig. 9; Suppl. material 6 “activity”).

### DNA Extraction, PCR amplification, and Sanger sequencing

DNA extraction, gene fragment amplification, and sequencing were performed for minor worker ants of five different taxa (*C. explodens* sp. n., *C. badia*, *C. nr. saundersi*, *C. aruensis* Karawajew, 1933, and *C. cylindrica* (Fabricius, 1798)), as well as for mandibular gland reservoir content of *C. explodens* sp. n., one symbiotic cricket (*Camponophilus* sp.) from artificial nest #38, and the host plant of *C. explodens* sp. n. (*Shorea johorensis*).

For DNA barcoding of ant specimens, DNA was extracted from ant legs using Qiagen’s tissue QIAamp DNA Micro kit following the manufacturer’s protocol (Qiagen, Venlo, Netherlands). To obtain sufficient DNA quantity for further processing, the amount of legs used per sample varied. For the DNA extraction of queens, a minimum of three legs of one individual were transferred into one 1.5 ml microcentrifuge tube and frozen with liquid nitrogen. Three legs of one individual was also the minimum amount for males and major workers. For minor workers, all legs from two to four individuals were pooled (see Suppl. material 6 “accession numbers”). The frozen legs were ground into small pieces with disposable pestles attached to a pestle motor (Kimble, Vineland, NJ, USA). Subsequent steps were performed according to manufacturer’s instructions with the following exceptions: sample lysis for 20 hours, final elution step with 25–50 µl elution buffer. To assess the purity of the extraction, DNA concentration and 260/280 nm ratio were measured with a NanoDrop ND-1000 Spectrophotometer (Software Version ND-1000 v.3.8.1, Thermo Fisher Scientific, MA, USA).

For DNA barcoding of symbiotic crickets, DNA was extracted from one whole specimen applying the same procedure as used for ants legs, but with a pretreatment with an enzymatic lysis buffer (Tris·Cl 20 mM, pH 8.0, sodium EDTA 2 mM, Triton X-100 1.2%, add lysozyme to 20 mg/ml for 60 min).

For DNA barcoding of the host plant, 100 mg of a leaf were ground with mortar and pestle under the use of liquid nitrogen and DNA was extracted using Qiagen’s DNeasy Plant Mini Kit according to manufacturer’s instructions.

For the ants, the gene fragments cytochrome C oxidase subunit I and II (COI, COII), cytochrome B (cytB), and carbamoyl-phosphate synthase II (cad) were am-

plified, for the cricket only COI. Additionally, a fragment of 16S rRNA was amplified from the DNA extracted from the mandibular gland content of *C. explodens* sp. n. minor workers, to assess the presence of bacteria. For the plant, the gene fragment maturase K (matK) was amplified. Primer sequences and specific annealing temperatures are given in Tab. 1. Final concentrations for PCR were 1× GoTaq Flexi Buffer (Promega, Madison, Wisconsin, USA), 0.16 mM dNTP's, 3 mM MgCl<sub>2</sub> (Promega), 0.4 μM forward and reverse primer (Microsynth, Balgach, Switzerland), 0.8 Units GoTaqG2 Flexi polymerase (Promega) and 2–50 ng sample (diluted with HPLC water, ROTH), in a final volume of 50 μl. PCR was performed with a Biometra T3 Thermocycler (Biometra, Göttingen, Germany) with the following conditions: 2 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at primer specific annealing temperature, 90 sec at 72 °C and finally 7 min at 72 °C. PCR products were separated by 1.5% agarose gel electrophoresis. PCR products were purified using mi-PCR Purification Kit (Metabion, Planegg, Germany) and one direction sequencing was performed at Microsynth (Austria).

Sequences are deposited in NCBI GenBank. Accession numbers for ant specimens are given in Table 2; see Suppl. material 6 “accession numbers” for additional details.

**Table 1.** Primers used in this study.

Gene	Name	Sequence 5'–3'	Length [bp]	GC content [%]	Fragment Length [bp]	Annealing Temp [°C]	Reference
COI	LCO1490-F	GGTCAACAAATCAT-AAAGATATTGG	25	32	709	45	Chen et al. 2013
	HCO2198-R	TAAACTTCAGGGT-GACCAAAAAATCA	26	35			
COII	J2791-F	ATACCHCGDCGA-TAYTCAGA	20	40–55	858	51	Chen et al. 2013
	H3665-R	CCACARATTTTCW-GAACATTG	20	35–40			
cytB	CB11400-F	TATGTACTACCHT-GAGGDCAAATATC	26	35–42	485	45	Chen et al. 2013
	CB11884-R	ATTACACCNCCTAA-TTTATTAGGRAT	26	27–35			
cad	CD1423EF	AGGTRATACRATCG-GARAGRCCDGA	25	40–60	800	55	Ward et al. 2010
	CD1910R	CCGAGRGRTRCRAC-RTTYTCCATRTTR-CAYAC	32	38–63			
matK	472F	CCCRTYCATCTG-GAAATCTTGGTTC	25	44–52	750	47	Yu et al. 2011
	1248R	GCTRTRATAATGA-GAAAGATTCTGTC	26	31–38			
16S rRNA	fD1	AGAGTTTGATCCTG-GCTCAG	20	50	1500	56	Weisburg et al. 1991
	rP1	ACGGTTACCTTGT-TACGACTT	21	43			

**Table 2.** List of sequence accession numbers in NCBI GenBank. \* Nucleotide sequences from NCBI GenBank.

TUCIM	Other IDs	Organism	ng/ $\mu$ l	COI	COII	cytB	cad
5053		<i>C. explodens</i> sp. n.	14.7	MF993252	MF993269	MF993286	MF993304
5056		<i>C. explodens</i> sp. n.	19.7	MF993253	MF993270	MF993287	MF993305
5080		<i>C. explodens</i> sp. n.	10.4	MF993254	MF993271	MF993288	MF993306
5098		<i>C. explodens</i> sp. n.	8.6	MF993256	MF993273	MF993290	MF993308
5104		<i>C. explodens</i> sp. n.	16.6	MF993257	MF993274	MF993291	MF993309
5148		<i>C. explodens</i> sp. n.	6	MF993258	MF993275	MF993292	MF993310
5185		<i>C. explodens</i> sp. n.	7.3	MF993259	MF993276	MF993293	–
5205		<i>C. explodens</i> sp. n.	29.8	MF993260	MF993277	MF993294	MF993311
6600		<i>C. explodens</i> sp. n.	8	–	MF993284	–	–
5855		<i>C. explodens</i> sp. n.	16.9	MF993262	MF993278	MF993297	MF993314
5856		<i>C. explodens</i> sp. n.	28.2	MF993263	MF993279	MF993298	MF993315
5942		<i>C. explodens</i> sp. n.	34.3	MF993264	MF993280	MF993299	MF993316
5943		<i>C. explodens</i> sp. n.	142.1	MF993265	MF993281	MF993300	–
	YG*	<i>C. explodens</i> sp. n.	n.a.	EF634201		–	–
6461		<i>C. badia</i>	21.3	MF993266	MF993282	MF993301	MF993317
6463		<i>C. badia</i>	5.4	MF993267	MF993283	MF993302	MF993318
6601		<i>C. badia</i>	17.91	MF993268	MF993285	MF993303	MF993319
5698		<i>C. nr. saundersi</i>	50.1	KU975365.1	KU975366.1	MF993296	MF993313
	CH*	<i>C. cf. cylindrica</i>	n.a.	EF634198		–	–
5086		<i>C. cylindrica</i>	26	MF993255	MF993272	MF993289	MF993307
5300	CAMP004	<i>C. aruensis</i>	169.1	MF993261	–	MF993295	MF993312
	Cflor36*	<i>Camponotus floridanus</i>	n.a.	AY334397	–	–	–

Sequences of non-ant material are deposited under MG582639 for COI of myrmecophilous crickets (*Camponophilus* sp.), MF993320 for matK of *Shorea johorensis* and MF996752 for 16S rRNA of cf. *Blochmannia* (Enterobacteriales).

Phylogenetic analysis

GapStreeze v. 2.1.0 (<https://www.hiv.lanl.gov/content/sequence/GAPSTREEZE/gap.html>) was used for COI gene alignment with 95 % gap tolerance in order to retain only the conserved region. The individual gene alignments were subjected to best substitution model selection using the BIC criterion in SMS (Lefort et al. 2017). Consecutively, HKY85, HKY85+I, HKY85+G, and GTR+G were chosen as best substitution models for genes cad, cytB, COI, and COII respectively. The concatenated alignment was partitioned for each locus using MrBayes v. 3.2.5 (Ronquist et al. 2012), and the respective substitution models were assigned to each partition. The substitution and branch length estimates were allowed to vary independently between each partition. Priors for an exponential distribution with mean 1 to all branch lengths and to all shape parameters were assigned for all four partitions. Metropolis-coupled Markov

chain Monte Carlo (MCMCMC) sampling was performed using MrBayes v. 3.0B4 (Ronquist et al. 2012) with two simultaneous runs of four incrementally heated chains that performed 1 million generations. Bayesian posterior probabilities (PP) were obtained from the 50 % majority rule consensus of trees sampled every 100 generations after removing the first 25 % of trees using the “burnin” command. According to the protocol of Leache and Reeder (2002), PP values higher than 0.94 were considered significant. The phylogenetic trees were visualized in FigTree v. 1.4.3 (Rambaut 2016) and then annotated using vector graphic software.

## Morphological methods

All specimens used for morphometry were card-mounted, individually numbered, and measured at magnifications from 25.6× up to 256× with a Nikon SMZ1500 binocular microscope. Genital structures of two male specimens were dissected and mounted separately. Results represent minimum and maximum values for each morph; in cases where a character could not be measured in all individuals, the number of measured specimens is given in parentheses. The complete dataset of measurements is provided in Suppl. material 6 “measurements”.

## Measurements and indices (\* = only gynes and males)

<b>EL</b>	Eye length. Maximum diameter of compound eye, measured in lateral view.
<b>FeL</b>	Femur length. Maximum length of metafemur, measured from base to apex.
<b>FWL*</b>	Forewing length. Length of forewing, measured from tegula to distal tip.
<b>HaL</b>	Hair length. Length of the longest standing hair on first gastral tergite, measured from base to apex.
<b>HL</b>	Head length. Maximum length of head in full-face view, excluding mandibles, measured from anteriormost point of clypeus to posterior-most point of head vertex, parallel to midline.
<b>HS</b>	Head size. $(HW + HL) / 2$ .
<b>HW</b>	Head width. Maximum width of head in full-face view (including eyes if protruding; only in gynes).
<b>ML</b>	Mesosoma length. Measured laterally from anterior surface of pronotum proper (excluding collar) to posterior extension of propodeal lobes.
<b>MSW*</b>	Mesoscutum width. Maximum diameter of mesoscutum, measured dorsally.
<b>NH</b>	Node height. Height of petiolar node, measured laterally, from the intersection point of the axes of maximum height and length to dorsal apex
<b>OcD*</b>	Ocellar distance. Minimum distance between lateral ocelli, measured between median borders.
<b>OcW*</b>	Ocellus width. Maximum diameter of median ocellus.
<b>OED*</b>	Ocellar eye distance. Minimum distance between lateral ocellus and outer border of compound eye.

<b>PH</b>	Petiole height. Maximum height of petiole in lateral view, measured from ventral-most point of petiolar sternum to dorsal apex.
<b>PL</b>	Petiole length. Maximum length of petiole in lateral view, measured from inflexion point of anterior constriction to posterior margin, perpendicular to axis of maximum height.
<b>PS5</b>	Length of maxillary palp segment V, measured from base to apex.
<b>PS6</b>	Length of maxillary palp segment VI, measured from base to apex.
<b>SL</b>	Scape length. Maximum length of antennal scape in dorsal view excluding basal neck and condyle.
<b>SW</b>	Scape width. Maximum width of antennal scape, measured dorsally.
<b>TL</b>	Total length. The added lengths of head (excluding mandibles), mesosoma, petiole, and gaster.
<b>2r*</b>	Maximum length of 2 <sup>nd</sup> radial crossvein (see Figs 5e, 6b).
<b>4Rs+M*</b>	Length of 4 <sup>th</sup> radial sector fused with median (see Figs 5e, 6b).
<b>CI</b>	Cephalic index. $HW / HL \times 100$
<b>EI</b>	Eye Index. $EL / HW \times 100$
<b>FeI</b>	Femur Index. $FeL / HW \times 100$
<b>OI*</b>	Ocellar Index: $OED / OcD \times 100$
<b>PI</b>	Petiole Index. $PH / PL \times 100$
<b>PSI</b>	Palp Segment Index. $(PS5 + PS6) / HS \times 100$
<b>SI</b>	Scape index. $SL / HW \times 100$
<b>SWI</b>	Scape width index. $SW / SL \times 100$
<b>WVI*</b>	Wing Vein Index. $4RsM / 2r \times 100$

Digital stacked images of most specimens (Figs 2–6) were acquired with a Leica DFC camera attached to a Leica MZ16 binocular microscope with Leica Application Suite v3 and stacked with Zerene-Stacker 64-bit. Images of labels were taken with a Nikon D60 camera with an AF-S Micro Nikkor 105 mm objective and an EM-140 DG macro ring flash. Photographs of genital structures of males (Figs 7, 10c–f) as well as of the male *C. badia* specimen (Fig. 10 a, b) were created with the help of Leica Application Suite v3.8, using a Leica DFC450 camera attached to a Leica Z16APO optics carrier. All images were processed with Adobe Photoshop 7.0.

## Material examined

Type material of *C. explodens*: **Holotype** (minor worker): Brunei, Temburong, Kuala Belalong Field Studies Centre, 04°33'N, 115°09'E, 60 m a.s.l., 10.XI.–5.XII.2015, leg. A. Laciny & A. Kopchinskiy (“YG Vienna Colony”, specimen number COCY 01565).

**Paratypes** (59 minor workers, 8 major workers, 16 gynes, and 6 males dry mounted; > 500 imagines stored in 96 % ethanol): 19 minor workers, 2 major workers, 12 alate gynes, 4 dealate gynes, 6 males (including allotype) (all dry mounted), as well as 8 males, 2 alate gynes, ca. 500 minor workers (in alcohol) from the same nest sam-

ple as holotype; 1 major worker, same locality and collector as holotype, 17.IV.2015, “YG 373 main natural nest”; 1 major worker, same data as holotype (“YG doorkeeper #19”); 2 major workers, same data as holotype except 20.IV.2015, leg. A. Kopchinskiy (“cf. YG 39 (351) artificial nest”); 8 minor workers, 2 major workers, same locality as holotype, 2002, leg. D.W. Davidson (“YG KB02-108”); 4 minor workers, same locality and collector as previous, no collection date, “YG 2025”; 5 minor workers, same locality and collector as previous, I.2012, “YG T-trail (202)”); 5 minor workers, same locality and collector as previous, 15.V.2014, “YG-2 (73)”); 7 minor workers, same locality and collector as previous, 15.V.2014, “YG-2 (49)”); 2 minor workers, same data as previous except Batu Apoi Forest Reserve, N04°32', E115°10', 200 m a.s.l., 25.XI.2004, (“CAYG A-370”); 15 minor workers (on 5 pins), same data as previous, except N04°55', E115°19', 60 m, 3.VII.2002, (“YG KB02-108 voucher”); 4 minor workers, Thailand, Chumphon Province, Krom Luang Chumphon W.S, 3.II.2002, leg. W. Jaitrong (“WJT02-TH-0116”); 5 minor workers, West Malaysia, Kelantan, 60 km NE Tanah Rata, Tanah Kerajaan, 1000 m a.s.l., 12.–30.IV.2007, leg. P. Cechovský.

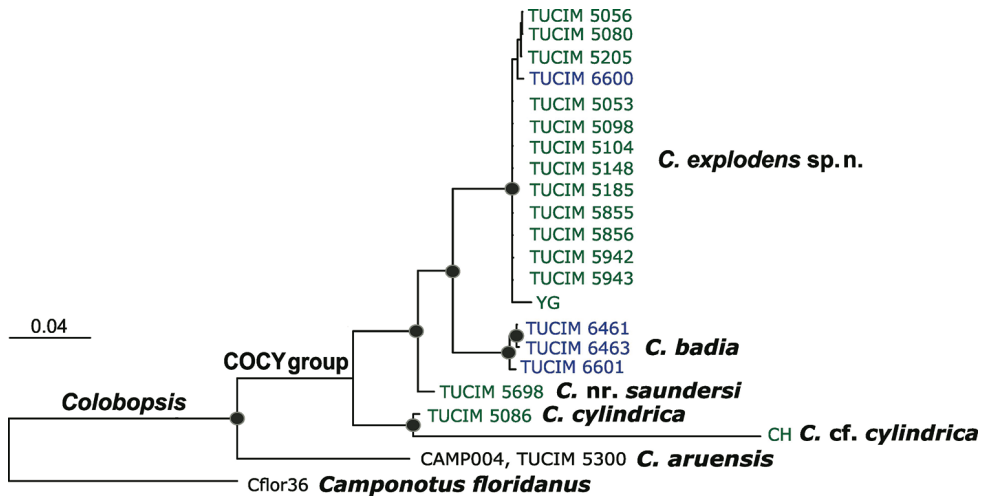
Additional material: 3 pupae (Suppl. material 5) and 6 myrmecophilous crickets (*Camponophilus* sp., det. S. Ingrisch), from the same nest sample as the holotype.

For unique identification numbers of all 90 dry mounted specimens (60 minor workers, 8 major workers, 16 gynes, and 6 males), as well as information on caste and colony affiliation, see Suppl. material 6 “measurements”.

The holotype and a portion of the paratypes will be deposited at the Brunei Museum; additional paratypes will be housed in the Universiti Brunei Darussalam, the Natural History Museum Vienna, the University of California (Davis, USA), the Natural History Museum of Los Angeles County (Los Angeles, USA), the Thailand Natural History Museum (Technopolis, Thailand), and the collection of H. Zettel (Vienna, Austria).

## Molecular results

The topology of the phylogram based on the concatenated alignment of 2757 bp was concordant with topologies of COI and COII and not contradicted by the topology of cytB. The phylogram based on cad was statistically unresolved (data not shown). The obtained Bayesian consensus tree (Fig. 1) shows conspecificity of the newly obtained *C. explodens* sp. n. specimens from Brunei and Thailand (TUCIM 6600) with a sequence previously deposited under “*Colobopsis cylindrica* s.l. YG”. While there is some intraspecific variation within the analysed *C. explodens* sp. n. specimens, they form a clade distinctly separate from the closely related *C. badia*. The male of *C. badia* (TUCIM 6463) is clearly grouped with its conspecific workers from a nearby locality, thus confirming species identity. The herein examined representatives of the *C. saundersi* subclade, *C. explodens* sp. n., *C. badia* and the undescribed *C. nrSA* (see Laciny et al. 2017), are clearly distinct from other members of the COCY group (e.g., *C. cylindrica*) and selected outgroup taxa of *Colobopsis* and *Camponotus*.



**Figure 1.** Bayesian consensus phylogram of *C. explodens* sp. n. and related taxa based on the concatenated alignments (2757 bp) of the three mitochondrial (COI, COII, cytB) and one nuclear (cad) phylogenetic markers. Nodes with posterior probabilities above 0.94 are marked with black dots. Specimens from Borneo are shown in green, from Thailand in blue. TUCIM 6463 corresponds to a male specimen.

## Taxonomic results

### *Colobopsis explodens* Laciny & Zettel, sp. n.

<http://zoobank.org/DB4767B0-C745-4843-BE3F-B17DBCEB3A96>

Figs 2–9; Suppl. materials 1–7

*Camponotus* (*Colobopsis*) sp. Yellow Goo: Davidson et al. 2007: 470.

*Camponotus* (*Colobopsis*) sp. YG: Cook 2008. Davidson et al. 2012: 488.

*Colobopsis* sp. YG: Davidson et al. 2016: 518. Laciny et al. 2017: 95.

**Etymology.** Present participle of Latin *explodere*, referring to the “exploding”-like autolysis behaviour.

### Description of phenotypes.

**Minor worker** (Figs 2, 4b–d; Suppl. material 1: S1a).

Measurements of holotype minor worker: TL 6.78; HW 1.48; HL 1.67; HS 1.58; PS5 0.23; PS6 0.25; EL 0.42; SL 1.33; SW 0.14; ML 2.05; HaL 0.15; PH 0.55; PL 0.47; NH 0.33; FeL 2.05. Indices: CI 88; SI 90; SWI 11; EI 29; PI 116; FeI 139; PSI 30.

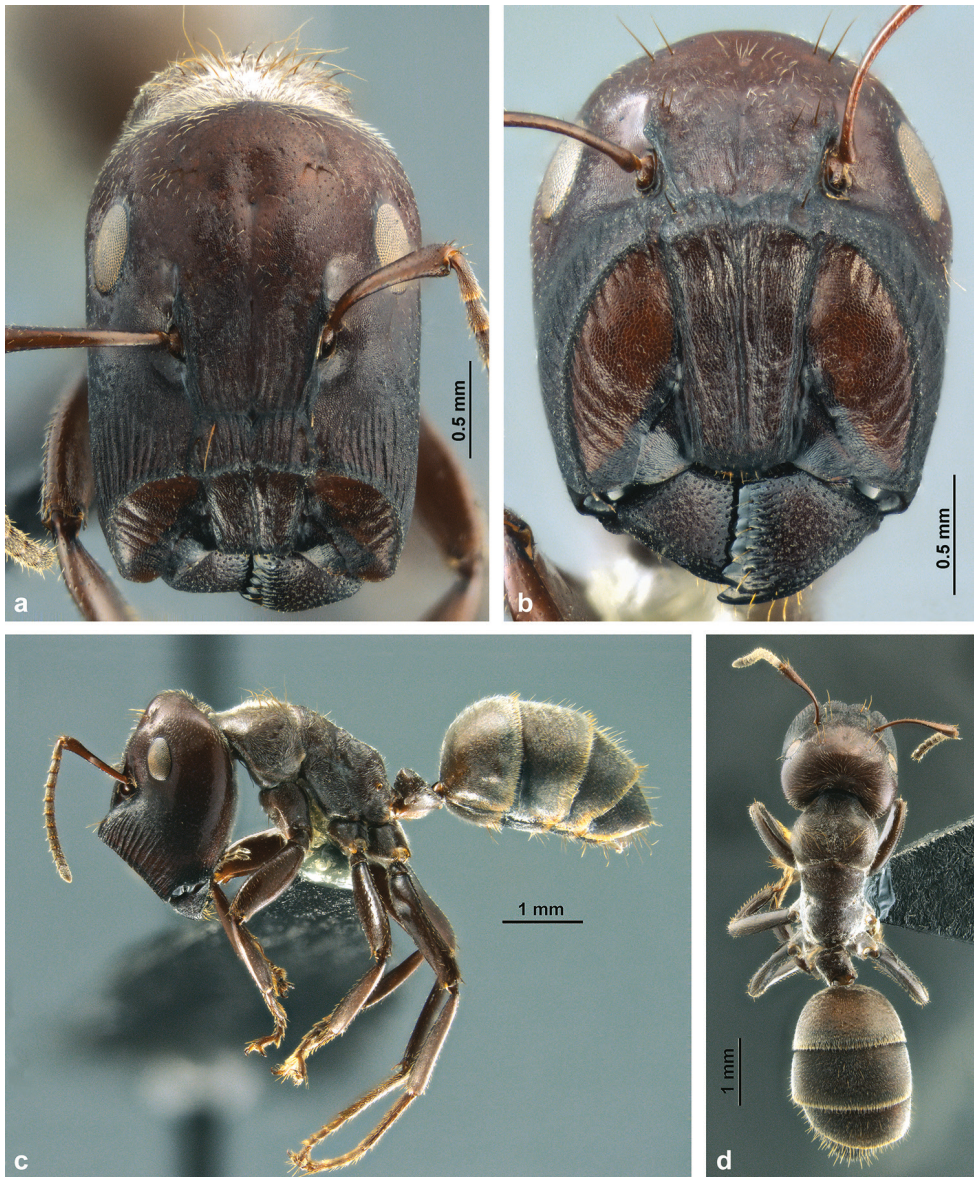
Measurements of paratype minor workers: (n = 59): TL 4.74–7.21; HW 1.22–1.57; HL 1.30–1.78; HS 1.27–1.67; PS5 0.21–0.25 (20); PS6 0.20–0.26 (21); EL 0.33–0.43; SL 1.21–1.39; SW 0.11–0.16; ML 1.50–2.22; HaL 0.08–0.19; PH 0.41–0.56 (44); PL 0.33–0.49 (47); NH 0.24–0.38 (52); FeL 1.73–2.10. Indices: CI 85–94; SI 87–104; SWI 9–12; EI 27–29; PI 112–133 (41); FeI 123–151; PSI 28–35 (20).

**Structures:** Head (Fig. 2a) subovate, longer than wide, narrower anteriorly; sides posteriorly convex, posterior cephalic margin roundly convex; microstructure consisting of



**Figure 2.** Habitus of *C. explodens* sp. n., holotype, minor worker; **a** full-face **b** lateral, and **c** dorsal view.

very fine, isodiametric or transverse mesh-like reticules; intermixed punctures very fine and inconspicuous on face, larger but shallow laterally and ventrally. Eyes small compared to other castes (EI 27–29, vs. 28–31 in major workers and 35–37 in gynes), flat, positioned dorsolaterally. Ocelli lacking, in some larger specimens position of median ocellus indicated by shallow impression. Frons with very fine impressed midline; frontal carinae slightly converging anteriorly, not elevated. Median carina of clypeus not reaching anterior clypeal margin, especially in small specimens. Mandibles mostly smooth, with rather dense punctures; masticatory margin with five teeth. Maxillary palpi long (PSI 28–35). Antennal scape long, its length roughly equal to head width (SI 87–104), moderately



**Figure 3.** Habitus of *C. explodens* sp. n., paratype, major worker; **a** full-face view **b** frontal shield **c** lateral, and **d** dorsal view.

flattened, slightly widened towards apex, integument punctate. Antenna 12-segmented; antennal segment III approx.  $1/5$  shorter than each IV and V, and approx.  $2/5$  shorter than II. Mesosoma slender, moderately low. Microreticulation isodiametric or slightly transverse, dorsally denser than laterally. Metanotal region delimited from mesonotum by a shallow groove; groove delimiting metanotum from propodeum indistinct or missing. Dorsal and posterior outline of propodeum rounded in lateral view, or meeting at

an obtuse angle, dorsal face slightly convex, posterior face flat to shallowly concave. Legs slender. Petiole with isodiametric reticulation; petiolar node moderately high, its short, slightly convex anterior and its rather straight posterior face forming a triangular shape in lateral view, its apex not truncated, rather rounded; node narrow in dorsal view, a crest indistinct; a medial depression indicated in most specimens. Gaster: dorsum of tergites I–III with extremely fine, dense, transverse microreticulation, slightly shiny (Fig. 4b); mesh-like reticulation wider on lateral areas of tergites I–III, tergite IV, and sternites, therefore meshes appearing not so strongly transverse, and the integument shinier (Fig. 4c). Exposed parts of tergite V and sternite V with dense, almost isodiametric reticulation, dull; base of tergite V (usually covered by tergite IV) sculptured as tergite IV.

**Colour:** Body mainly reddish brown. Vertex of head, margins of clypeus, masticatory and lateral margins of mandibles, dorsum and ventral margins of mesosoma, mid portion of gastral tergites I–III, and legs slightly darker brown in most specimens; some specimens with darker area extending medially from head vertex to frons. Gastral tergites and sternites with very narrow hyaline margins. All gastral sternites, lateral fourths and posterior margins of tergites I–III, as well as entire tergites IV and V black.

**Pilosity:** Dorsum of head with very short, inconspicuous, appressed and subdecumbent setae; a few very long, standing setae on frons near declivity to vertex, medial of frontal carinae, and on lateral portions of clypeus. Mesosoma and petiole with fine and short, whitish, velvety pilosity; long, standing, slightly undulated setae restricted to pronotum; declivity of propodeum and node of petiole with few very short standing setae. Gastral tergites with moderately dense, short whitish, decumbent setae and few slightly darker, longer standing setae, most of them in transverse rows near hind margins. Longest setae in transverse rows near hind margins of sternites and at base of gastral tergite V.

**Notes:** Minor workers of *Colobopsis explodens* sp. n. show a continuous size variation across a remarkably wide range, similar to that found in the undescribed *Colobopsis* sp. nrSA (Fig. 8; compare with Laciny et al. 2017).

**Phragmotic major worker** (Figs 3, 4a; Suppl. material 1: S1b).

Measurements of paratype major workers (n = 8): TL 7.30–8.71; HW 1.72–1.89; HL 2.25–2.58; HS 1.99–2.20; PS5 0.15–0.17 (6); PS6 0.15–0.17 (6); EL 0.50–0.56; SL 1.15–1.26; SW 0.17–0.20; ML 2.22–2.74; HaL 0.11–0.20; PH 0.59–0.69 (6); PL 0.45–0.51 (6); NH 0.40–0.45 (6); FeL 1.50–1.70. Indices: CI 71–77; SI 64–69; SWI 14–17; EI 28–31; PI 125–143 (6); FeI 87–95; PSI 14–17 (6).

**Structures:** Integument mostly dull, only head and legs shiny. Head (Fig. 3a) large, subcylindrical, anteriorly truncated. On posterior areas of face punctation slightly stronger than in minor worker. Eyes somewhat larger and more distant from vertex compared to minor worker. Ocelli lacking, their positions often indicated by shallow grooves (Fig. 4a). Anterior part of head forming a large shield (Fig. 3a, b) formed by clypeal and genal components, limited by a sharp and elevated crest so that the shield surface appears concave in lateral view. Shield with fine isodiametric reticulation and rather variable, mostly longitudinal rugae; most prominent are a pair of rugae along sides of clypeus and a single median carina that does not reach the anterior margin,



**Figure 4.** Cuticular microsculpture of *C. explodens* sp. n.; **a** vertex of major worker (paratype) **b** gastral tergite I, and **c** gastral tergite IV of minor worker (holotype) **d** labels of holotype, minor worker.

often reduced towards base. Genal part with curved rugae of variable number, length, and distinctiveness, but only exceptionally reaching onto the anteromedial triangle. Additional longitudinal rugae on clypeus often present, including a usually distinct pair of paramedian rugae running from base of clypeus over the crest anteriorly towards middle of shield; in specimens with short median carina, the area between these carinae more or less grooved. Longitudinal striation more regular and pronounced on frons and genae up to level of antennal insertions, laterally on genae similarly long and strong. Mandible with sharp and high ventrolateral ridge, coarsely punctate, its lateral face weakly rugose-striate; masticatory margin with acute apex and few (1–3) more or less distinct, very blunt teeth in distal half (Fig. 3b). Maxillary palpi very short (PSI 14–16). Antenna considerably shorter than head width (SI 64–69) and stouter than in all other morphs (Figs 3a, 8b); antennal scape distinctly widened towards apex. Mesosoma stouter and higher than in minors, especially promesonotum expanded; in lateral view dorsal and posterior face of propodeum forming an obtuse angle, somewhat less rounded than in minor workers, dorsally without concavity. Legs much shorter and stouter than in minors (Fig. 3c). Shape of petiole similar to minor workers, somewhat wider in dorsal view. Structures of gaster similar as in minor worker.

**Colour:** Overall slightly darker than minor worker; head, legs and mesosoma reddish brown; gaster slightly darker chocolate-brown, becoming darker towards caudal apex, hyaline margins yellowish; elevated crest of frontal shield, anterior clypeal margin, frontal carinae, and masticatory and lateral margin of mandibles blackish brown.

**Pilosity:** As in minor worker, except long setae on clypeus sides restricted to the area behind clypeal shield; mesonotum with standing setae which are approx. half the length of those on pronotum.

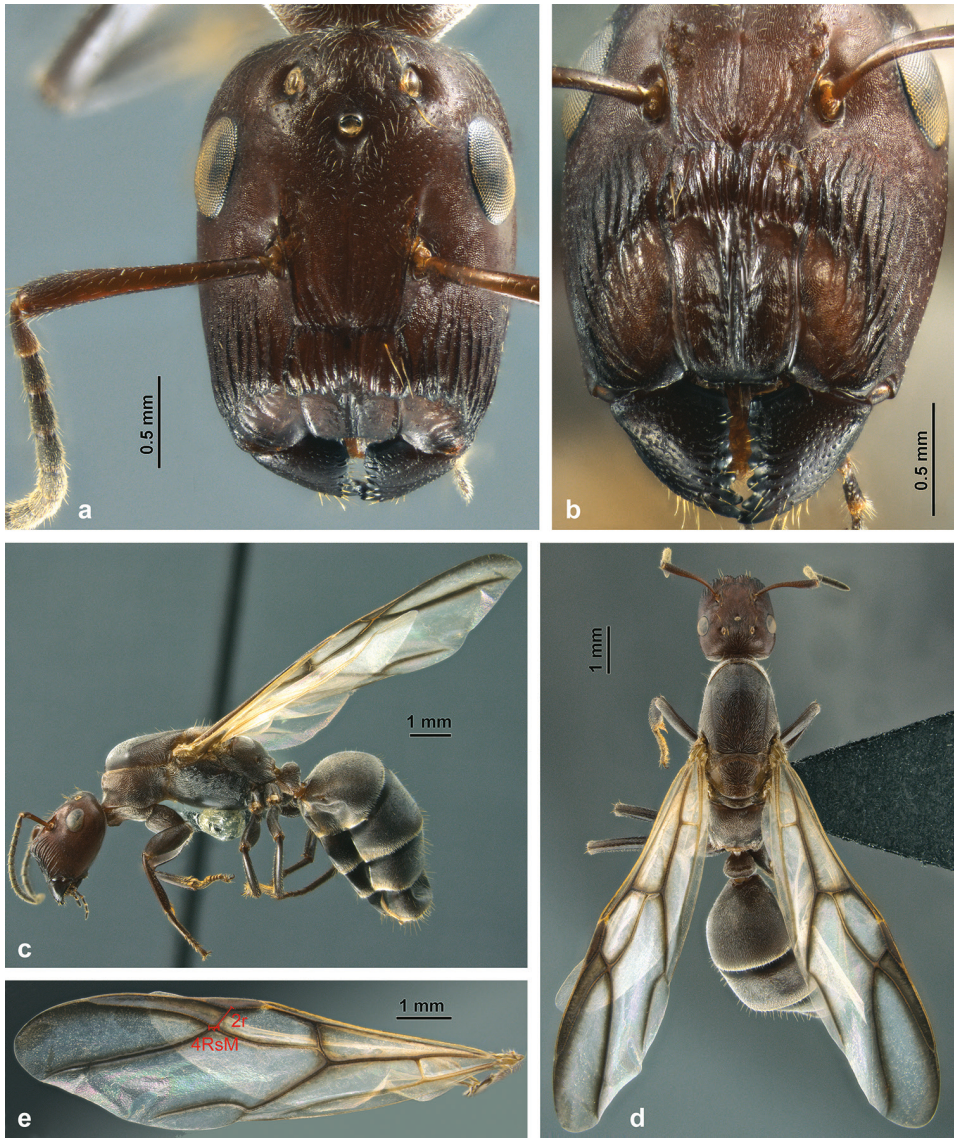
**Notes:** The head shield with a sharp, elevated crest is typical for majors of the *Colobopsis saundersi* complex (Fig. 3b).

**Gyne** (Fig. 5, Suppl. material 3: S3d).

Measurements of paratype gynes ( $n = 16$ ): TL 10.50–12.16; HW 1.74–1.83; HL 2.28–2.45; HS 2.02–2.14; PS5 0.19–0.21 (13); PS6 0.19–0.23 (13); EL 0.62–0.66; SL 1.33–1.45; SW 0.20–0.22; ML 4.11–4.63; HaL 0.14–0.29 (15); PH 0.77–0.92 (11); PL 0.54–0.67 (10); NH 0.40–0.54 (11); FeL 2.25–2.35; OcW 0.13–0.16; OED 0.34–0.38; OcD 0.54–0.64; FWL 9.72–10.50 (11); MSW 1.68–2.15; 2r 0.50–0.64 (12); 4Rs+M 0.14–0.32 (12). Indices: CI 73–77; SI 75–80; SWI 15–16; EI 35–37; PI 123–150 (8); FeI 125–132; PSI 18–20 (13); OI 54–69; WVI 26–58 (12).

**Structures:** Head (Fig. 5a) large, subcylindrical, anteriorly truncated, similar to that in major worker with the following exceptions: eyes larger than in workers (EI 35–37) and breaking outline of head in full-face view. Ocelli fully developed, their colour ranging from almost clear to reddish amber. Head shield sharply delimited, but slightly smaller than in major worker, distinctly narrower than head width. Striation of clypeus, frons, and genae similar as in major, though somewhat more strongly developed on lateral parts of shield. Mandible with sharp ventrolateral ridge; its lateral face weakly rugose-striate, narrower than in major; dorsal-anterior face punctured; masticatory margin with acute apex and 3–4 blunt teeth in distal half, mandible basally with blunt ridges (Fig. 5b). Maxillary palpi moderately long (PSI 18–20). Antennal scape moderately long, slightly shorter than head width (SI 75–80), somewhat widened towards apex (Figs 5a, 8b). Mesosoma large, structures as typical for caste; propodeum large and evenly convex in lateral view. Cuticular microstructures dorsally consisting of very fine punctation, with intermixed larger punctures, laterally finely reticulated. Legs stout, but not as short as in major (Fig. 5c). Forewing venation strongly reduced, as in most Camponotini; M-Cu absent; Mf2+ interstitial (Fig. 5e). Petiole distinctly wider than in workers; node more rounded in lateral view, in some specimens its apex shallowly impressed medially, in others with two shallow lateral impressions forming a trilobed outline. Gastral tergites I–IV and sternites I–IV with extremely fine and dense microstructures consisting of strongly transverse meshes; only sides of tergites with wide mesh-like reticulation and shiny; tergite V with dense isodiametric reticulation.

**Colour:** Chiefly as in major worker. Head and pronotum reddish brown; ventral and posterior mesosoma, petiole, legs and gaster somewhat darker chocolate-brown; mandibles and ridges of clypeal shield blackish brown. Pronotum and mesonotum with very narrow yellow margins. Gastral tergites medially with very narrow hyaline margins; sternites with relatively broad posterior margins. Wings hyaline, but forewing



**Figure 5.** Habitus of *C. explodens* sp. n., paratype, alate gyne; **a** full-face view **b** frontal shield **c** lateral **d** dorsal view **e** forewing with indicated measurements 2r and 4RsM.

cells along veins, as well as pterostigma darkened to brownish. On hind wing all veins pale yellow (Fig. 5c–e).

**Pilosity:** Short pilosity and distribution of long setae on head, petiole, and gaster similar as in major worker, but that of mesosoma different; pronotum with few long, undulated setae. Medial part of mesonotum (between parapsidal furrows) with numerous long erect setae, scutellum with few long erect setae; lateral part of mesonotum in front of tegulae without setae.

*Notes:* The head shield with a sharp, elevated crest is typical for gynes of the *Colobopsis saundersi* complex (Fig. 5b).

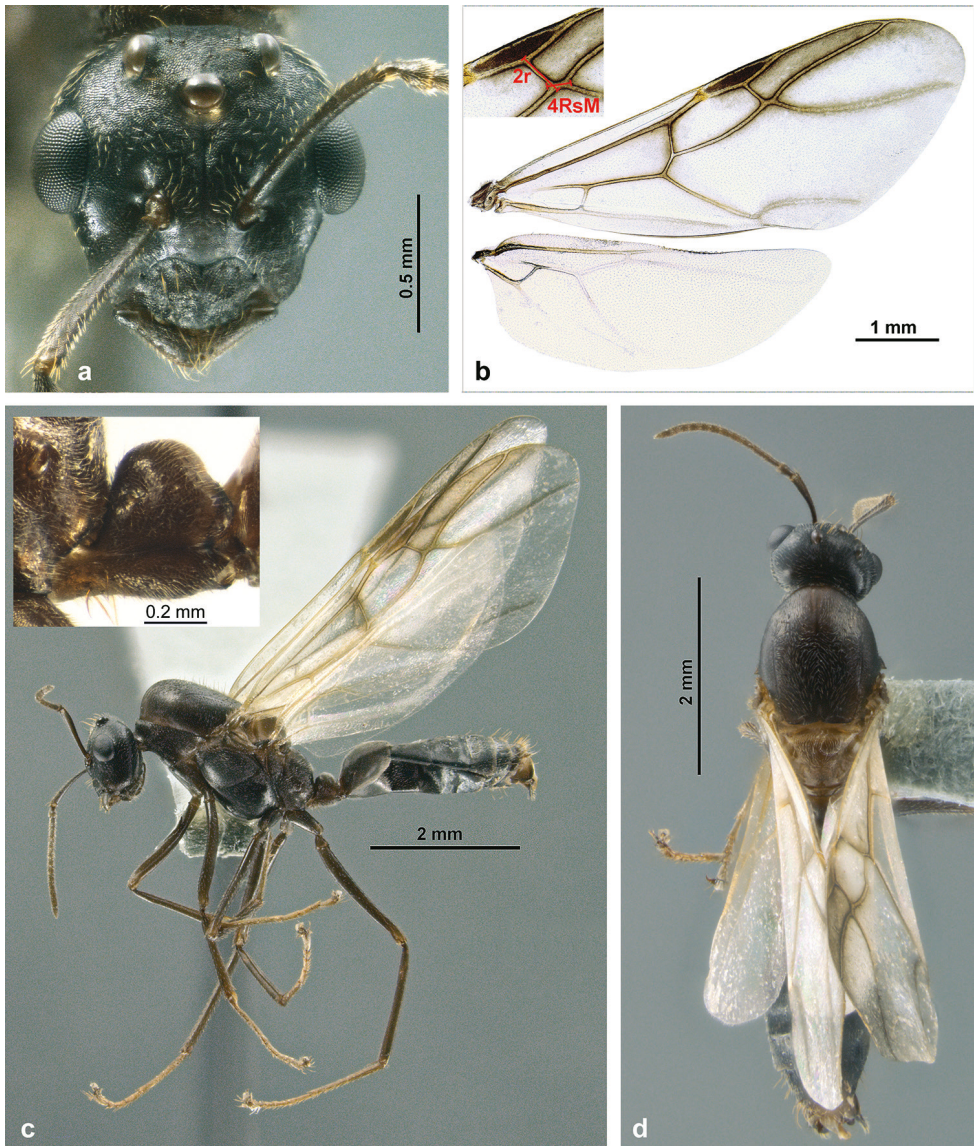
**Male** (Figs 6, 7). This is the first detailed description and illustration of males from the *C. cylindrica* group.

Measurements of allotype male: TL 7.11; HW 1.26; HL 1.20; HS 1.23; PS5 0.20; PS6 0.15; EL 0.44; SL 0.84; SW 0.11; ML 2.54; HaL n.a.; PH 0.46; PL 0.40; NH 0.29; FeL 1.83; OcW 0.18; OED 0.27; OcD 0.43; FWL 6.33; MSW 1.37; 2r 0.38; 4Rs+M 0.27. Indices: CI 105; SI 66; SWI 13; EI 35; PI 116; FeI 145; PSI 28; OI 62; WVI 70.

Measurements of paratype males (n = 5): TL 6.46–6.85; HW 1.24–1.29 (4); HL 1.14–1.24; HS 1.20–1.27; PS5 0.17–0.21 (4); PS6 0.13–0.17 (4); EL 0.43–0.46; SL 0.80–0.85; SW 0.10–0.12; ML 2.38–2.87; HaL n.a.; PH 0.45–0.49 (4); PL 0.38–0.40 (4); NH 0.26–0.33 (4); FeL 1.71–1.86; OcW 0.18–0.19; OED 0.25–0.27; OcD 0.43–0.46; FWL 5.87–6.33; MSW 1.17–1.50; 2r 0.38–0.47; 4Rs+M 0.14–0.22. Indices: CI 104–110 (4); SI 64–67 (4); SWI 12–15; EI 35–36 (4); PI 113–123 (4); FeI 136–151 (4); PSI 27–30 (4); OI 53–62; WVI 31–53.

*Structures:* Head (Fig. 6a) small, subtrapezoidal, eyes very large, round and protruding, EL more than one third of HL (EI 35–36). Ocelli very large, diameters larger than in gynes. Integument of head rather matt. Frons and genae finely reticulated, genae additionally finely punctured. Clypeus with some stronger punctures at margins (at base of setae), median carina weakly developed, present in proximal third of clypeus or entirely obsolete. Frons with impressed midline from median ocellus to level of antennal insertions. Frontal carinae weakly developed, converging more strongly than in minor worker. Mandible short with reduced dentition, masticatory margin with 2–3 blunt teeth; dorsal surface finely punctate. Maxillary palpi long (PSI 27–30). Antenna 13-segmented; scapes short (SI 64–67) and relatively slender (Fig. 8b). First funicular segment conspicuously enlarged distally, pear-shaped, 30–50% wider and ca. 20% longer than the following segment (Fig. 6a); all other funicular segments cylindrical, without modifications. Mesosoma large, structures as typical for alate ants. Mesoscutum anteriorly strongly convex with narrow impressed midline in posterior tenth. Scutellum moderately elevated; propodeum evenly convex. Cuticular microstructures of mesosoma consisting of a very fine reticulation with intermixed minute punctures at bases of short hairs, additionally with larger punctures dorsally at bases of erect setae. Legs very long and slender (FeI 136–151). Forewing venation strongly reduced, as in most Camponotini. M-Cu absent; 4Rs+M shortly developed or (more rarely) Mf2+ interstitial (Fig. 6b). Petiole small; in lateral view node more bluntly rounded than in female castes, anterior and posterior faces straight, not convex, apex not impressed medially in dorsal view. Gastral tergites I–IV and sternites I–IV with fine and dense microreticulation consisting of moderately transverse meshes; only sides of tergites with wide meshes and shiny; tergite V with almost isodiametric reticulation. Sternite VI posteriorly emarginated, sternite VII truncated.

*Genital structures* (Fig. 7): Genital capsule (Fig. 7a–c) approx. as long as wide in dorsal aspect (Fig. 7a), ventrally longer than dorsally, protruding from apex of gaster. Gonopod high, distally broadly rounded. Gonostylus (Fig. 7c) elongated and acuminate, with reticulated microstructure (only visible at very high magnification) and



**Figure 6.** Habitus of *C. explodens* sp. n. allotype, male; **a** full-face view **b** wings (see insert for illustration of measurements 2r and 4RsM) **c** lateral (see insert for detailed view of petiole), and **d** dorsal view.

some long setae. Basivolsella (Fig. 7e) dorsally with roundish structure, ventrally with evenly distributed, comparatively short setae. Digitus (Fig. 7e) large, evenly widened towards apex; apex rounded but with rectangular corner ventrally. Penis valvae (Fig. 7d) in dorsal aspect broad at base, but very narrow distally. Valviceps leaf-shaped in lateral view, apically rounded; surface smooth; ventral margin with very fine serration.

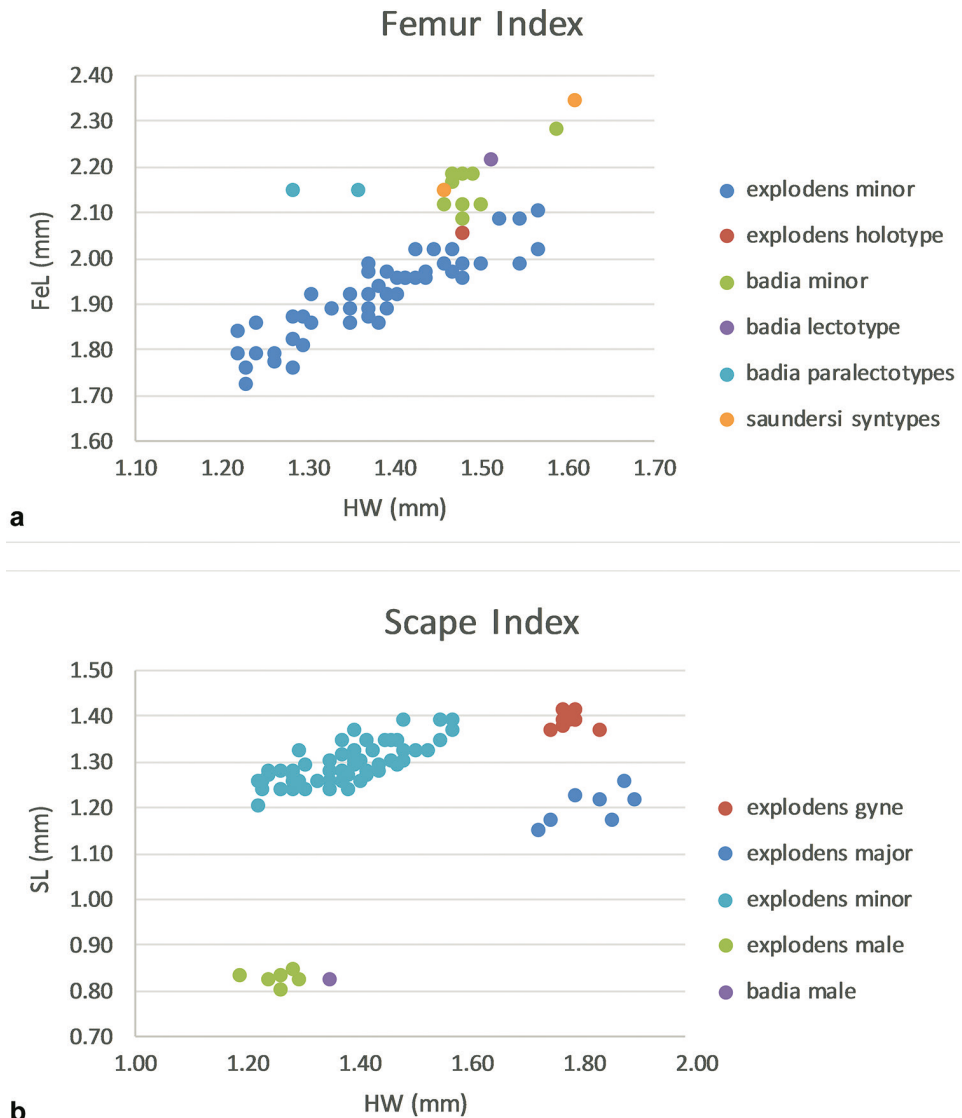
**Colour.** Mainly dark chocolate-brown. Head somewhat darker; eyes pale grey to blackish; ocelli translucent, ranging from almost clear to reddish amber. Antennae



**Figure 7.** Genital structures of *C. explodens* sp. n. paratype, male; genital capsule in **a** dorsal **b** ventral, and **c** lateral view **d** left penis valve **e** right volsella and gonostylus.

and legs lighter brown, fading into yellowish towards apices. Margins of mesoscutum, scutellum, and metanotum lighter yellowish brown. Gastral tergites medially with very narrow hyaline margins; sternites with relatively broad, indistinctly separated posterior margins. Wings almost hyaline, with a slight whitish tinge, but forewing cells along veins, as well as pterostigma darkened to brownish, all veins pale yellowish brown. On hind wing all veins pale yellow.

**Pilosity:** On head setae sparsely distributed, short, inconspicuous, appressed, sub-decumbent; a few very long standing setae on frons near vertex, and on anterior and posterior clypeal margins. Mandibles with dense short pilosity on lateral face, and few moderately long setae on anterolateral margin. Short pilosity and distribution of long



**Figure 8.** Variation of relevant morphometric measurements for the distinction of castes and species; **a** Metafemur length (FeL) in relation to head width (HW) for minor workers of *C. explodens* sp. n., as well as minor workers of *C. badia* and *C. saundersi* (type specimens plotted separately) **b** Scape length (SL) in relation to head width (HW) for minor workers, major workers, gyenes and males of *C. explodens* sp. n., as well as male of *C. badia*.

setae on mesosoma, petiole, and gaster similar as in gyne, but pronotum lacking long, undulated setae. Medial part of mesonotum (between parapsidal furrows) with numerous long erect setae, scutellum with few long erect setae; lateral part of mesonotum in front of tegulae without setae. Tegulae with dense brush of setae. Petiole with a few stout setae anteroventrally (see insert Fig. 6c). Petiolar node lacking any standing setae;

gastral tergite I without or with few subdecumbent, moderately long setae. Posterior gastral tergites and sternites (segments II and following) with sparse, relatively long, obliquely standing setae.

### Biological notes on *Colobopsis explodens* sp. n.

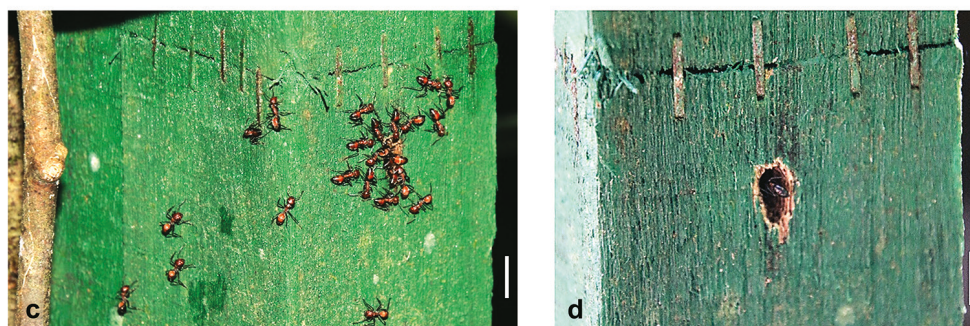
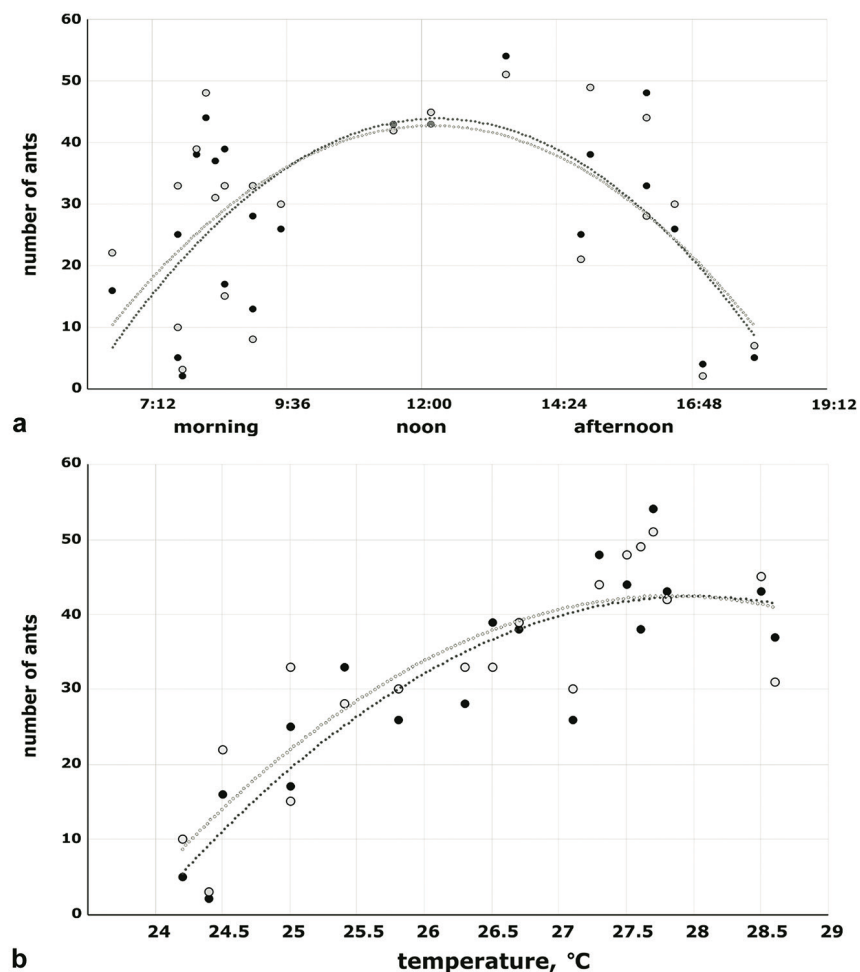
Colonies of *C. explodens* sp. n. observed in the Ulu Temburong National Park are commonly polydomous and polygynous. This species was selected as a model for the study of the “exploding ants” because among the species with advanced autothysis behaviour it was the most abundant COCY taxon in the vicinity of KBFSC.

*Colobopsis explodens* sp. n. frequently nests on dipterocarp trees and its colonies can contain thousands of individuals. The largest part of the studied colony lived on a 60 m tall *Shorea johorensis* Sym. (Dipterocarpaceae) tree identified morphologically and by DNA barcoding (matK, identical to GenBank accession number KY973022, E-value is zero; Heckenhauer et al. 2017). The colony’s foraging ground included the canopy of the main tree, its direct vicinity, and also covered canopies of a 25 m tall *Horsfieldia wallichii* (Hook.f. & Th.) Warb. (Myristicaceae) tree and a smaller tree of *Shorea maxwelliana* King (9 m). Colony fragments on all trees were connected by ant trails either through the canopy or on the forest floor in the litter layer. The total area occupied by the colony was estimated to be at least 2500 m<sup>2</sup>.

The colonies are distributed three-dimensionally, occupying any suitable nesting ground within the colony boundaries. On the main tree, we found four nesting sites of the examined colony in dead branches at heights ranging between 35 and 55 m above ground and two nesting sites in the living stem 50–60 m above ground. No nests in living branches were observed. At least five nest entrances were also seen in the stem of *S. maxwelliana*. No signs of necrosis of the plant tissue were observed around stem entrances.

The translocation of a nest fragment in a fallen branch to the laboratory’s terrace, 30 m away from its original location, resulted in the expansion of the colony’s foraging ground to a neighbouring *Shorea* sp. tree where these ants were not previously present, while the connection to the colony on the original host tree was maintained.

If provided with an appropriate artificial nest (Fig. 9c–d), *C. explodens* sp. n. ants will inhabit it within several weeks up to several months and even use it to rear brood. One artificial nest, mounted on the main host tree, was colonized one week after it was installed. For the activity assessment, the easily accessible artificial nest #38 was observed. During behavioural monitoring, *C. explodens* sp. n. was observed to be mainly diurnal, foraging between 6:00 and 18:00 hrs, with peak activity around 9:00 and 16:00 hrs (Fig. 9a). The activity correlated positively with the temperature with lowest values at 24.2 °C and highest at 28.6 °C (Fig. 9b). The atmospheric pressure and clouds did not influence the activity of *C. explodens* sp. n. (Suppl. material 6 “activity”); humidity was constant over the period of observations ranging from 86 to 88%. A slight rain on a warm day did not reduce the activity of ants near the nest but no activity was observed during heavy rains. However, if a shelter was provided, *C. explodens* sp. n. remained active



**Figure 9.** Activity of *C. explodens* sp. n. at the entrance to the artificial nest #38. **a** Scatter plot of the number of minor workers entering (black dots) and leaving (open dots) the nest depending on the time of day **b** Scatter plot of the number of minor worker ants entering (black dots) and leaving (open dots) the nest depending on the air temperature. Polynomial trend lines on a and b are shown for the numbers of entering (black) and exiting (grey) minor workers **c** and **d** show high and low worker activity near the entrance of nest #38, respectively; white bars denote 1 cm.

also during the rain and even after sunset. A drastic reduction in the number of minor workers at the nest entrance was observed on the days of nuptial flight, when several alate gynes and males left the nest in the early evening (Suppl. material 6 “activity”). Between one and six minor workers (“guards”) were frequently positioned at the nest entrance, touching all incoming and outgoing workers with their antennae and seemingly monitoring the activity of foragers. In the early afternoon of the day with the highest activity, larvae were carried out of the nest. No carrying of larvae into the nest was ever observed.

Remarkably, during all observations the numbers of the minor workers leaving and entering the nest were almost equal. The fact that this proportion did not change over the day (Fig. 9a) suggests a tendency to maintain a constant number of individuals present inside the nest.

After dusk, other species of Camponotini such as *Polyrhachis* spp., *Camponotus* spp., and *Dinomyrmex gigas* (Latreille, 1802) were observed on the trees in the vicinity of the artificial nest.

Within the colonies, minor workers were by far the most abundant caste of *C. explodens* sp. n., whereas major workers (soldiers) were rare and almost never seen outside the nest. Alate gynes and males were observed leaving the nest during nuptial flight after dusk on two occasions during our field observations (Suppl. material 6 “activity”). Several more alate sexuals were found inside a detached nest fragment (Suppl. material 3). The same nest fragment also contained symbiotic ant crickets of the genus *Camponophilus* Ingrisch, 1995. DNA barcoding of the cricket based on COI sequence resulted in the highest value of 80 % similarity to COI sequences of insects from several groups including Mann’s ant cricket *Myrmecophilus manni* (Schimmer, 1911) (EU938370, Fenn et al. 2008). Thus, the molecular identification of these crickets is currently not possible due to lack of reference sequences.

Observations have shown that minor workers of *C. explodens* sp. n. display a characteristic, possibly defensive pose with raised gaster (Suppl. material 1: S1a) (compare with Davidson et al. 2007) and are extremely prone to self-sacrifice when threatened. The mandibular gland content is released during autothysis by contracting the gaster until the integument ruptures, leading to the death of the ant (Suppl. material 4). The secretion is slightly viscous, sticky, and has a species-specific bright yellow colour and a distinctive spice-like odour (Hoenigsberger et al., in prep.).

Minor workers of *C. explodens* sp. n. spend significant time on leaves, which has previously been hypothesized to contribute to their nutrition (compare with Davidson et al. 2007, 2016). However, the exact purpose of their activity on leaves is yet to be understood. Observations suggest a patrolling or monitoring behaviour aiming at the removal of debris from the phyllosphere (mainly adaxial leaf surface but also abaxial leaf and petiole surfaces) and possible deterrence of intruding arthropods. Similar “cleaning” behaviour was observed *in vitro*, as well.

Another very specific behaviour was exhibited on the tree bark: Minor workers “graze” on the layers of epiphytes (mosses, lichens, algae, filamentous fungi, and yeasts) with their mandibles, often for up to 60 minutes. This behaviour differs from the cleaning behaviour on leaves and presumably contributes to the ants’ nutrition.

Preliminary feeding experiments using cultures of filamentous fungi isolated from the phyllosphere of the host trees remained unsuccessful, no fungal feeding was observed. Only a suspension of yeast in water was accepted *in vitro* (M. Rahimi, pers. obs.). However, minor workers of *C. explodens* sp. n. have been observed to feed on small dead insects, fruit, and fish when offered on the foraging grounds (A. Kopchinskiy, A. Laciny & M. Hoenigsberger pers. obs.).

Commonly observed modes of behaviour of *C. explodens* sp. n. *in situ* and *in vitro* as well as a variety of nesting sites are documented in the Suppl. material 7 (Video S7).

The molecular analysis of the mandibular gland (MG) content of *C. explodens* sp. n. resulted in PCR amplification and sequencing of the 16S rRNA fragment of the bacteria *Blochmannia* sp. (Gammaproteobacteria), a genus of obligate symbiotic bacteria found in carpenter ants (Williams and Wernegreen 2015). We revealed four identical mOTUs originating from two different DNA extracts from samples composed of five pooled MG reservoirs of the minor workers each. The sequences of 728 nt were 99 % similar (11 SNP sites) to the “uncultured bacterium clone 193-11” KC136854 from *Camponotus* sp. voucher KC-A017-01 defined as *Blochmannia* sp. in Russell et al. (2012).

More detailed data on autothysis, composition of mandibular gland secretion, biodiversity of the COCY-associated microorganisms, and experimental assessment of nutrition will be presented in future publications.

### ***Colobopsis badia* (Smith, 1857)**

Figs 8, 10

*Formica badia*: Smith 1857: 54.

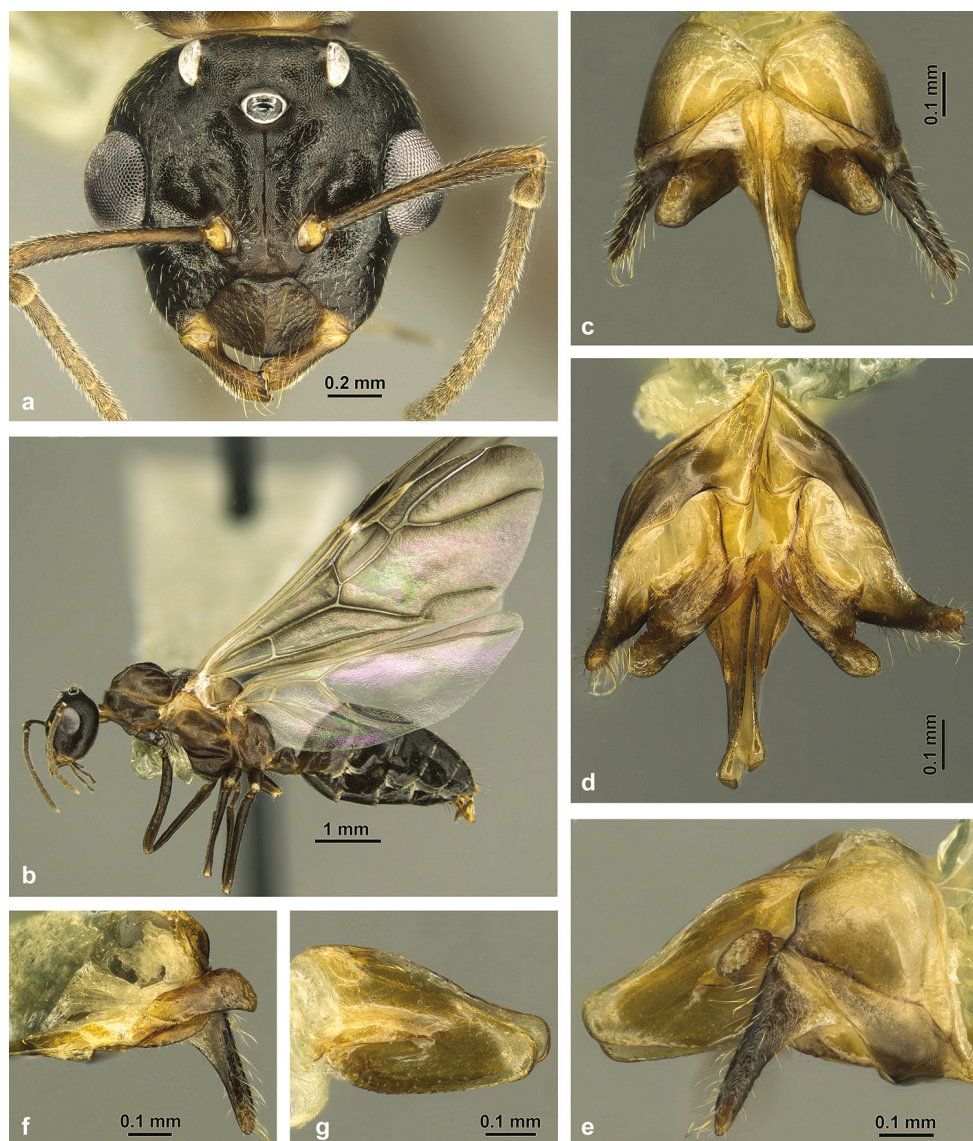
*Camponotus badius*: Roger 1863: 3.

*Colobopsis badia*: Ward et al. 2016: 350. Bolton 2017.

**Type material** examined. 1 lectotype minor worker (Oxford University Museum of Natural History, present designation), Singapore, “*Formica badia*”, “Syntype”, CASENT 0901897, “Lectotypus *Formica badia* Smith, 1857 des. Laciny & Zettel, 2017”, 2 paralectotype minor workers (Oxford University Museum of Natural History) mounted on the same card, Sarawak (“Sar 32”), “*Formica badia*”, “Syntype”, “Paralectotypes *Formica badia* Smith, 1857”.

**Additional material examined.** 1 male (Natural History Museum Vienna), Thailand, Trang Province, Nayong District, Khao Chong Botanical Garden, at light of head quarter, 7°33'N, 99°46'E, 60 m a.s.l., 1–7.VI.2016, leg. H. Zettel (68); 10 minor workers (Natural History Museum Vienna), Thailand, Trang Province, Nayong District, Khao Chong Botanical Garden, trail to Ton Pliw Waterfall, N07°32'34", E99°47'33", 150 m a.s.l., 1–7.VI.2016, leg. H. Zettel (66–4).

**Description notes on the type specimens.** *Lectotype*: Minor worker glued to a square cardboard, in relatively good condition; right hind leg missing; tarsi of middle



**Figure 10.** Habitus of *C. badia*, male; **a** frontal **b** lateral; genital capsule in **c** dorsal **d** ventral, and **e** lateral view **f** right volsella and gonostylus **g** left penis valve.

legs and left hind leg broken; erect setae on dorsum probably lost. Structures agree well with other species of the *C. saundersi* complex, a few characteristic features are given: Setae on scape more decumbent than in *C. explodens* sp. n. Dorsal outline of mesosoma almost straight, only with weak indentation at meso-metanotal suture. Propodeum forming a distinct obtuse angle in lateral view. Petiolar node relatively short, apex acute in lateral view, its crest slightly indented in middle. Tergites I–III with very fine, strongly transverse microsculpture (lateral parts not visible). Colour relative-

ly dark brown; appendages strongly infusate; antennal segments III–XII, meso- and metafemora almost black.

**Paralectotypes:** Two minor workers glued to the same square cardboard, in relatively poor condition. Left specimen with damaged head and gaster, lacking right middle leg; major parts of body covered by dirt or glue; most erect setae probably lost. Right specimen with slightly damaged head, lacking gaster and right hind leg; some parts of body covered by dirt or glue; most erect setae probably lost. The two specimens are probably conspecific, but conspecificity with the lectotype is uncertain. The combination of morphological features is intermediate between *C. badia* and *C. explodens* sp. n.: setae on scape similar to *C. explodens* sp. n., more erect than in the lectotype; dorsal outline of mesosoma intermediate, more structured than in the lectotype, but propodeum with angle; shape of node intermediate, apex more acute than in *C. explodens* sp. n. Colour almost as dark as in the lectotype.

Measurements of lectotype minor worker: TL 6.13; HW 1.51; HL 1.63; HS 1.57; PS5 n.a.; PS6 n.a.; EL 0.40; SL 1.43; SW 0.15; ML 1.96; HaL 0.17; PH 0.54; PL 0.36; NH 0.32; FeL 2.22. Indices: CI 93; SI 95; SWI 10; EI 26; PI 150; FeI 147; PSI n.a.

Measurements of paralectotype minor workers\* (n = 2): TL 6.13, n.a.; HW n.a., 1.36; HL n.a., 1.52; HS n.a., 1.44; PS5 n.a., 0.21; PS6 n.a., 0.25; EL 0.36, 0.37; SL 1.39, 1.40; SW 0.12, 0.13; ML 1.89; HaL 0.13, n.a.; PH n.a., 0.51; PL 0.37, 0.42; NH 0.27; FeL 2.15. Indices: CI n.a., 89; SI n.a., 103; SWI 8, 9; EI n.a., 27; PI n.a., 121; FeI n.a., 158; PSI n.a., 32. \*One specimen with strongly damaged head, one with missing gaster.

Measurements of non-type minor workers (n = 10): TL 5.64–6.23; HW 1.46–1.59; HL 1.63–1.72; HS 1.54–1.65; PS5 0.24–0.25 (3); PS6 0.24 (3); EL 0.38–0.40; SL 1.37–1.43; SW 0.13–0.14; ML 1.96–2.22; HaL 0.13–0.19; PH 0.51–0.56 (7); PL 0.41–0.45; NH 0.31–0.37 (9); FeL 2.09–2.28. Indices: CI 88–92; SI 90–96; SWI 9–10; EI 25–27; PI 118–130 (7); FeI 141–148; PSI 30–31 (3).

**Male.** *Notes on collecting and identification:* A single male collected at light was identified as a specimen of the *C. cylindrica* group. DNA barcoding revealed specific identity with a nest series of *C. badia* from the same botanical garden. The morphological identification of this nest series (Col. 66-4) was carried out by direct comparison to the lectotype of *C. badia*.

*Description* (Fig. 10): Overall very similar to *C. explodens* sp. n. and differing by the following characters:

Measurements of male (n = 1): TL 8.28; HW 1.35; HL 1.26; HS 1.30; PS5 0.20; PS6 0.15; EL 0.48; SL 0.83; SW 0.10; ML 3.07; HaL n.a.; PH 0.47; PL 0.41; NH 0.31; FeL 1.96; OcW 0.19; OED 0.29; OcD 0.46; FWL 7.43; MSW 1.37; 2r 0.41; 4Rs+M 0.37. Indices: CI 107; SI 61; SWI 13; EI 36; PI 116; FeI 145; PSI 26; OI 61; WVI 91.

*Structures:* Size larger (TL ca. 8.3 mm). Integument rather shiny (Fig. 10a, b), especially on mesosoma. Clypeus with distinctly developed median carina, almost reaching anterior margin. Maxillary palpi (PSI 26) and antennal scapes (SI 61) relatively short. First funicular segment slightly more enlarged (30% wider than the following segment, Fig. 10a). Vein 4Rs+M of forewing long. Petiolar node slightly more widely rounded in lateral aspect.

*Genital structures* (Fig. 10c–g) very similar to *C. explodens* sp. n., with the following exceptions: Gonostylus very narrow, with weaker reticulation of lateral surface (Fig. 10e). Basivolsella with extremely short ventral setae (Fig. 10f). Digitus with rounded apex, without ventroapical corner (Fig. 10f). Valviceps with slightly coarser ventral serration (Fig. 10g).

*Colour*: Head chiefly dark brown, with lighter area comprising frons between antennal insertions and clypeus. Eyes grey. Ocelli clear, almost colourless. Posterior and anterior clypeal margins, as well as proximal fourth of clypeal carina black. Gaster dark brown. Mesosoma, petiole, mandibles, antennae, and legs lighter brown, appendages becoming yellowish towards apices. Antennal insertions, mandibular bases, margins of thoracic sclerites (especially below tegulae) creamy yellow. Gastral tergites medially with very narrow hyaline margins; sternites with relatively broad posterior margins. Wings hyaline, forewing with a slight brownish tinge and cells along veins, as well as pterostigma darker brownish, all veins pale brown. On hind wing all veins pale yellow.

*Pilosity*: Appressed and subdecumbent setae comparatively shorter and sparser, but difference less obvious on gaster. Standing setae on mesonotum and gaster shorter, on mesonotum less numerous.

*Comparative notes*: The male of *C. badia* can be distinguished from males of *C. explodens* sp. n. by larger body size, differing colour pattern, more shiny integument, well-developed clypeal carina, differing proportions of wing venation, and relatively shorter scapes (Fig. 8b). In the genitalia, the most striking differences are in the narrower gonostylus and the more rounded digitus apex (compare Figs 7e, 10f).

## Discussion

### Molecular results

In this study, three mitochondrial DNA loci and one nuclear DNA fragment were applied for the construction of a molecular phylogenetic tree (Fig. 1). The evolutionary analysis based on four loci showed that *C. explodens* sp. n. is clearly genetically distinct from morphologically similar species. A minor level of infraspecific polymorphism within the specimens from Brunei was observed for COI, COII, and in particular for cytB marker. While tree topologies based on single mitochondrial loci were concordant, the cad tree (nuclear locus) was not resolved for the entire COCY group. The comparison of COI sequences with several hundred COCY sequences available in our local database and 13 sequences deposited in NCBI (Nov. 2017) suggests that this marker can be used for the reliable molecular identification (DNA barcoding) of *C. explodens* sp. n., as COI sequences of the nearest COCY taxa share only 91% similarity (*C. badia*), and the similarity to the selected non-COCY species *C. aruensis* is 83%.

The DNA extraction from the gastral parts of the mandibular gland reservoirs of *C. explodens* sp. n. minor workers resulted in drastically low yields indicating no abundant microbial symbionts present there. The successful 16S rRNA PCR amplification

gave a sharp band that was sequenced with high reproducibility. The 16S rRNA fragment corresponding to the whole genome sequenced *Blochmannia* endosymbiont of North American *Colobopsis obliquus* strain 757 (NCBI GenBank accession number CP010049, Williams and Wernegreen 2015) was 92 % similar to mOTU revealed in this study resulting in 56 polymorphic sites. This confirms that *C. explodens* sp. n. also harbours these bacteria that usually colonize the midgut of Camponotini workers (Sauer et al. 2002) and are considered to be beneficial for N-nutrition of these ants; they may also contribute to the general health of the workers and gynes. Thus, the detection of cf. *Blochmannia* bacteria rather indicated the contamination of the MG sample by fragments of the digestive system. In this respect, it is interesting to note that no *Wolbachia* (Alphaproteobacteria) mOTUs were recovered, but neither digestive tract nor ovaries were specifically investigated.

### Taxonomy

The treatment of *Colobopsis* as a genus separate from *Camponotus* is supported by molecular, morphological, and biological data (Blaimer et al. 2015, Ward et al. 2016). Naked pupae (Wheeler 1904; see Suppl. material 5) and presence of phragmotic soldiers and gynes are important features of *Colobopsis*, although unknown in many of the 94 valid species assigned to this genus by Ward et al. (2016). The morphological separation of minor workers of *Colobopsis* and *Camponotus* is chiefly based on head morphology, but complicated by extensive evolutionary changes within each group (Ward et al. 2016); however, the phylogeny of *Colobopsis* species has not been studied to date. The molecular data published by Blaimer et al. (2015), obtained from only four species, do not allow an interpretation of the relationships of intrageneric clades. Attempts to classify the species by morphological characters (Emery 1925, McArthur 2012), although useful for a rough sorting of species, probably hardly reflect their evolutionary relationships.

A first attempt of a comprehensive classification of the species of *Colobopsis* (as a subgenus of *Camponotus*) was done by Carlo Emery. In his outstanding treatment of Formicinae (Emery 1925) he treated 58 species and established six groups to hold 49 of them (nine remained unclassified). He defined the [*Camponotus* (*Colobopsis*)] *cylindricus* group by a gradual variation between worker and soldier, interspecific variation of head in soldiers and females (from concave and marginate to oblique and obtuse), and generally large size. Emery included eight species presently classified as *Colobopsis* (Ward et al. 2016), of which *Colobopsis calva* Emery, 1920, *C. quadriceps* (Smith, 1859), and *C. smithiana* (Wheeler, 1919) are not presently assigned to this group (see below), whereas *C. badia* and *C. corallina* were not included (listed under incertae sedis). Although Emery (1925) correctly recognized the size variation of workers, he failed to recognize the unique characteristics of the soldier caste (see Laciny et al. 2017).

More species of the COCY group were subsequently described by Stitz (1925), Menozzi (1926) and Karawajew (1929, 1935). A second attempt at classification was

made by McArthur (2012): His *Camponotus* (*Colobopsis*) *cylindricus* group consists of species with “neck attached to head well below vertex” and is broader than Emery’s (1925) *cylindricus* group. It includes the following species (according to current classification) that do not fit the characteristics of the COCY group in the present sense: *Colobopsis anderseni* (McArthur & Shattuck, 2001), *C. brachycephala* Santschi, 1920, *C. cotesii* (Forel, 1893), *C. desecta* (Smith, 1860), *C. excavata* (Donisthorpe, 1948), *C. hosei* (Forel, 1911a), *C. mutilata* (Smith, 1859), and *C. quadriceps*, as well as *Camponotus dedalus* Forel, 1911b, and *Camponotus kutteri* Forel, 1915.

According to our morphological studies the COCY group can be defined as such: polymorphic *Colobopsis* with at least three distinct female castes: (i) winged, phragmotic gynes, (ii) phragmotic soldiers (doorkeepers), and (iii) minor workers with a considerable size variation; intermorphic workers may occur in addition (Laciny et al. 2017). Minor workers: Vertex highly raised above foramen. Eyes of minor worker small and flat, not breaking head sides in full-face view. Entire trunk with dense, reticulated microstructures; punctures of integument often reduced. Head with moderate, mesosoma with dense pubescence. Mesosoma (at least the pronotum, except in *C. clerodendri* Emery, 1887) dorsally with long undulated setae, never arranged in distinct rows. Gaster with appressed pubescence and two or three types of setae of different lengths (not arranged in rows, except at hind margin). Soldiers (not known of all taxa): differing from minor workers by enlarged heads and short appendages (antennae, palpi, legs); in most species with a clearly circumscribed head shield for phragmosis. Microsculpture and pilosity similar to minor worker.

Following this definition, the COCY group presently comprises 17 names in the rank of species, subspecies or variations, which are partly in synonymy to each other. The strong intraspecific variation of minor workers, the frequently lacking knowledge on soldiers (or gynes), and the historical descriptions of taxa from different morphs (either workers or gynes) make the species taxonomy a true challenge. A preliminary analysis of morphological and molecular data (unpublished) supports the division of the group into four species complexes (molecular data of one complex not available). We restrict the following analysis to the species of the *C. saundersi* complex, which includes *C. explodens* sp. n. and can be defined by the following combination of characters observable in minor workers and soldiers: head always red or brown (not black); mesosoma moderately elongated and dorsally with some long undulated setae, at least on pronotum; node of petiole without long setae; gastral tergites with dense (in most species strongly transverse) micro-reticulation and with small hair pits (without large grooves). Soldiers and gynes (not known of all species) have a strongly truncated head, in most species with a well-defined (crested) head shield. The following names are available in this group (listed chronologically): *Colobopsis badia* (Smith, 1857), *C. corallina* Roger, 1863, *C. saundersi* Emery, 1889, *C. badia* var. *krama* Forel, 1912, *C. badia saginata* Stitz, 1925, *C. solenobia* (Menozzi, 1926), and *Colobopsis trieterica* (Menozzi, 1926), comb. n.

*Colobopsis corallina* (= *C. solenobia* syn. n., = *C. trieterica* syn. n.) is an endemic species from the Philippines. Soldiers and gynes differ strongly from *C. explodens* sp. n.

and other taxa of the complex (as far as such morphs are known) by a very obtusely margined, not crested head shield. Minors have a bright orange colour on head, mesosoma, and petiole, often extending to gastral tergite I. Morphometrically, the examined minor workers of *C. corallina* (n = 31) mainly differ from those of *C. explodens* sp. n. by a greater average length of appendages (SI 92–109 vs. 87–104; FeI 136–159 vs. 123–151; PSI 30–39 vs. 28–35).

The greatest similarity is observed between *C. explodens* sp. n. and *C. saginata* (stat. n.), a taxon only known from a single alate gyne from Northern Borneo. The important structures of the head shield are almost identical. Although strongly different from *C. explodens* sp. n. by pale orange brown colour, this gyne differs only by subtle morphometric characters of which the long and distally wide scape seems to be the most reliable (SI 83 vs. 78–80). The length of vein 4Rs+M is considerably longer in *C. saginata* than in *C. explodens* sp. n. (WVI 65 vs. 26–58).

*Colobopsis badia* var. *krama*, described from a soldier from Java (Forel 1912) is a very poorly known taxon. We have not been able to study any further material from Java yet. The type (illustrated by AntWeb.org under CASENT0910610) differs from *C. explodens* sp. n. by a pale red head that strongly contrasts with the dark brown mesosoma, by a well-developed median carina of the head shield that reaches the foremargin of the clypeus, and by a stronger punctuation of the preocellar area.

*Colobopsis badia* was described by Smith (1857) from Singapore and Sarawak (Borneo). However, the original description is too brief to draw any meaningful taxonomic conclusions. Viehmeyer (1916) describes workers of this species in more detail, also noting the secretion of a sticky liquid upon capture. He mentions a strong variability in colouration (from red to almost black with reddish head) and propodeal shape. This raises the question whether all examined specimens were truly members of the same species or perhaps belonged to one of the other, similar species of the *C. saundersi* complex. We examined the three syntype minor workers of *C. badia* in the Oxford University Museum of Natural History. To fix the identity of this taxon, we have chosen the syntype from Singapore (imaged by AntWeb.org under CASENT0901897) as the lectotype of *Formica badia*. The two syntypes from Sarawak are in a relatively poor condition, which does not allow a complete morphometric analysis, and therefore the conspecificity with the *C. badia* type remains doubtful. We were not successful in obtaining fresh material of *C. badia* from Singapore, but a nest sample (minors only) from southern Thailand (Trang Province) which agrees well with the lectotype in morphology, especially morphometry, was available for a molecular analysis. It shows that *C. badia* and *C. explodens* sp. n. are closely related, but distinct (Fig. 1). Although very similar to *C. explodens* sp. n. in overall habitus and colouration, the examined *C. badia* minor workers are on average somewhat larger with less size variation (HW 1.22–1.57 vs. 1.46–1.59) and possess longer appendages (e.g., FeI 123–151 vs. 141–168; see Fig. 8a).

We examined two syntype minor workers of *Colobopsis saundersi* from Myanmar (“Tenasserim, Thagata”, one illustrated by AntWeb.org under CASENT0905463). Morphometric analysis revealed no differences between the types of *C. saundersi* and *C. badia*, suggesting that the two species should be synonymized. *Colobopsis saundersi*

was considered a junior synonym of *C. badia* by Carlo Emery himself (Emery 1896) but revived from synonymy by Bingham (1903) without providing a reason. The large geographic distance of the type locality of *C. saundersi* and some minor differences in morphology led us to the decision to refrain from a formal synonymization at this time. A comparative molecular analysis of specimens from the type localities (Myanmar, Singapore) would most likely be necessary to corroborate this synonymy.

## Morphology of males

The morphology of males of the tribe Camponotini is insufficiently studied, so that a complete comparison at generic level is not possible. The modified (enlarged) first funicular segment is presumably characteristic for males of *Colobopsis*. This characteristic has been described in the type species, *Colobopsis truncata* (Spinola, 1808), by Kutter (1977) and has been equally observed in several species of the *C. cylindrica* group.

Males of the COCY group have previously been described for three species (see below). However, these descriptions largely lack the necessary details to meaningfully compare taxa. No previous accounts of genital morphology or illustrations of male specimens have been found in the literature.

*Colobopsis badia*: Viehmeyer (1916) gives a brief description of a male from Singapore. Colouration, size, proportions of head and ocelli, as well as the enlarged first funicular segment correspond well to the examined male from Thailand.

*Colobopsis severini* (Forel, 1909): The extremely brief description of a male from the island Labuan (near Borneo) is not sufficient to draw any meaningful taxonomic conclusions.

*Colobopsis leonardi* (Emery, 1889): Karawajew (1929) gives a rather detailed description of males collected within a nest-series on Sumatra. The correct species identification by Karawajew is uncertain; the series may belong to another species of the *C. leonardi* complex as well. The pattern of pilosity on the gaster, with standing setae only present on the posterior half, also corresponds to our observations in males of the *C. saundersi* complex.

According to our knowledge, males of the *C. cylindrica* group can be distinguished from other Southeast Asian *Colobopsis* species by the relatively rich subdecumbent pilosity and the dense microreticulation of gastral tergites.

## Biology

The behavioural observations on *C. explodens* sp. n. conducted at KBFSC revealed multiple modes of behaviour that are either poorly studied or new to science.

The diurnal activity pattern, as well as the positive influence of high temperatures correspond to the results of previous studies in related taxa (see Hamdan et al. 2013). Similarly large colonies containing several thousand individuals and extend-

ing to multiple trees and / or artificial nesting structures have also been described for other members of the genus (Federle et al. 1998, Laciny et al. 2017). However, it is still unclear whether individual workers are linked to certain parts of the colony or whether all foragers can move freely through the entire territory of the colony. An interesting and hitherto undescribed phenomenon in this regard is the presence of one or multiple “guards” at the artificial nest’s entrance: These minor workers were frequently observed to touch any incoming or leaving workers with their antennae. In some instances, returning foragers were delayed or altogether denied entrance by the guarding ants. One reason for this may be that some workers are linked to different parts of the colony. Alternatively, the observed guarding behaviour may be related to the limited capacity of the artificial nest, which is also suggested by the conspicuously balanced numbers of workers entering and leaving the nest during times of foraging activity. These behavioural patterns are hitherto undescribed and must be investigated in future studies.

A further noteworthy activity observed in foraging workers was so-called “grazing” behaviour, in which minor workers were frequently seen using their mandibles to pluck and chew at various mosses, lichens, and other epiphytes on the bark of trees or other surfaces. While this activity can last for up to one hour at a time, its exact purpose remains unclear. It is possible that minor workers cut and consume parts of the plants and microorganisms or merely ingest fluids. As previous analyses of nitrogen isotopes (Davidson et al. 2016) suggest a largely plant-based diet for COCY ants, it seems likely that “grazing” contributes to their nutrition. However, other previously hypothesized modes of nutrition, such as tending of scale insects (Davidson et al. 2016) were not observed, and recent investigations on *Colobopsis leonardi* (Emery, 1889) (Zettel et al., ms submitted to Asian Myrmecology) even suggest a higher prevalence of carnivory in COCY ants than previously suspected.

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## Supplementary material I

### Figure S1. Living workers of *C. explodens* sp. n. on a detached branch containing a nest fragment

Authors: Laciny A, Zettel H, Kopchinskiy A, Pretzer C, Pal A, Salim KA, Rahimi MJ, Hoenigsberger M, Lim L, Jaitrong W, Druzhinina IS

Data type: multimedia

Explanation note: **a** Minor worker with characteristically raised gaster **b** Major worker with phragmotic head to close nest entrances. Photo: H. Wiesbauer

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Link: <https://doi.org/10.3897/zookeys.751.22661.suppl1>

## Supplementary material 2

### Figure S2. Natural nest of *C. explodens* sp. n. in a dead branch in the high canopy of *S. johorensis* (the main host tree for the model colony)

Authors: Laciny A, Zettel H, Kopchinskiy A, Pretzer C, Pal A, Salim KA, Rahimi MJ, Hoenigsberger M, Lim L, Jaitrong W, Druzhinina IS

Data type: multimedia

Explanation note: **a** Camera setup in the canopy, white arrow marks the nest entrance  
**b, c** Entrance and minor workers.

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Link: <https://doi.org/10.3897/zookeys.751.22661.suppl2>

## Supplementary material 3

### Figure S3. Interior of the natural nest of *C. explodens* sp. n. found in a dead tree branch of *S. johorensis* on the forest floor

Authors: Laciny A, Zettel H, Kopchinskiy A, Pretzer C, Pal A, Salim KA, Rahimi MJ, Hoenigsberger M, Lim L, Jaitrong W, Druzhinina IS

Data type: multimedia

Explanation note: **a** Longitudinal section of the nest **b** Nest cavity. Sawdust is an artefact due to cutting. **c** Enlarged view of the nest cavity with a chamber made of dark carton **d** Dealate gyne and eggs found inside the chamber shown on **c**. The nest contained at least three such chambers.

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Link: <https://doi.org/10.3897/zookeys.751.22661.suppl3>

## Supplementary material 4

### Figure S4. Autothysis as defensive behaviour in an experimental setting

Authors: Laciny A, Zettel H, Kopchinskiy A, Pretzer C, Pal A, Salim KA, Rahimi MJ, Hoenigsberger M, Lim L, Jaitrong W, Druzhinina IS

Data type: multimedia

Explanation note: A worker of the predatory species *Oecophylla smaragdina* is attacked by three minor workers of *C. explodens* sp. n.; all four animals died after the encounter. Black arrows indicate the yellow secretion expelled by rupture of the mandibular gland reservoirs.

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Link: <https://doi.org/10.3897/zookeys.751.22661.suppl4>

## Supplementary material 5

### Figure S5. Pupa of *C. explodens* sp. n. found inside the opened natural nest

Authors: Laciny A, Zettel H, Kopchinskiy A, Pretzer C, Pal A, Salim KA, Rahimi MJ, Hoenigsberger M, Lim L, Jaitrong W, Druzhinina IS

Data type: multimedia

Explanation note: Note the absence of a pupal cocoon which is diagnostic for *Colobopsis*.

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Link: <https://doi.org/10.3897/zookeys.751.22661.suppl5>

## Supplementary material 6

### Table S6.

Authors: Laciny A, Zettel H, Kopchinskiy A, Pretzer C, Pal A, Salim KA, Rahimi MJ, Hoenigsberger M, Lim L, Jaitrong W, Druzhinina IS

Data type: specimens data

Explanation note: “measurements”: Complete dataset of measurements, indices, locality data and type status of all measured specimens of *C. explodens* sp. n.

“activity”: Activity of minor workers of *C. explodens* sp. n. at the entrance of the artificial nest #38.

“accession numbers”: Complete list of accession numbers at NCBI GenBank for all organisms treated in this study.

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Link: <https://doi.org/10.3897/zookeys.751.22661.suppl6>

## Supplementary material 7

### Video S7. Video depicting habitat, nesting sites and behaviour of *C. explodens* sp. n.

Authors: Laciny A, Zettel H, Kopchinskiy A, Pretzer C, Pal A, Salim KA, Rahimi MJ, Hoenigsberger M, Lim L, Jaitrong W, Druzhinina IS

Data type: multimedia

Explanation note: Video depicting habitat, nesting sites and behaviour of *C. explodens* sp. n. accessible under <http://explodingants.com/index.php/publications/colobopsis-explodens>

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Link: <https://doi.org/10.3897/zookeys.751.22661.suppl7>

#### Supplementary material Study 4

Supplementary Table S6 and Supplementary Video S7 are provided in the digital version of this dissertation.



**Figure S1. Living workers of *C. explodens* sp. n. on a detached branch containing a nest fragment. a** Minor worker with characteristically raised gaster **b** Major worker with phragmotic head to close nest entrances. Photo: H. Wiesbauer



**Figure S2. Natural nest of *C. explodens* sp. n. in a dead branch in the high canopy of *S. johorensis* (the main host tree for the model colony). a Camera setup in the canopy, white arrow marks the nest entrance b, c Entrance and minor workers. Photo: A. Kopchinskiy**



**Figure S3. Interior of the natural nest of *C. explodens* sp. n. found in a dead tree branch of *S. johorensis* on the forest floor. a** Longitudinal section of the nest **b** Nest cavity. Sawdust is an artefact due to cutting. **c** Enlarged view of the nest cavity with a chamber made of dark carton **d** Dealate gyne and eggs found inside the chamber shown on **c**. The nest contained at least three such chambers. Photo: A. Kopchinskiy



**Figure S4. Autothysis as defensive behaviour in an experimental setting.** A worker of the predatory species *Oecophylla smaragdina* is attacked by three minor workers of *C. explodens* sp. n.; all four animals died after the encounter. Black arrows indicate the yellow secretion expelled by rupture of the mandibular gland reservoirs. Photo: A. Kopchinskiy



**Figure S5. Pupa of *C. explodens* sp. n. found inside the opened natural nest.** Note the absence of a pupal cocoon which is diagnostic for *Colobopsis*. Photo: NHM Vienna

## **Caste-specific morphological modularity in the ant tribe Camponotini (Hymenoptera, Formicidae)**

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### **Abstract**

**Background:** The morphological structures of organisms form tightly integrated but mutually independent character complexes (modules) linked through common development and function. Even though their abundance, diversity, and complex caste systems make camponotine ants ideal subjects to research developmental modularity and phenotypic integration, no studies investigating these phenomena have been conducted in this taxonomic group. This study attempts to identify and visualize integrated character complexes in female castes of the genera *Camponotus* and *Colobopsis* using statistical analyses of morphometry.

**Results:** The identified modules differ between castes: Minor workers have small heads and long extremities, while major workers have enlarged heads modified for defence, and short, thick appendages; extremities (legs and antennae) are strongly correlated in both worker castes. Gynes show weaker integration of extremities, but a strong correlation of mesosoma and eyes, and highly variable median ocellus size. Gynes infested by mermithid nematodes exhibit aberrant proportions due to reduction of gyne-specific characters.

**Conclusion:** The integrated character complexes described herein can largely be interpreted as functional, caste-specific modules related to behavioural ecology and task allocation within ant colonies. This modular nature of the body plan is hypothesized to facilitate the evolution of novel phenotypes and thus contribute to the tremendous evolutionary success of ants. The study of these modules can help to further elucidate the evolution and ontogeny of castes in camponotine ants, as well as the effects of parasite infestation on the phenotype.

**Keywords:** cluster analysis, division of labour, modules, morphometry, PCA, social insects, phenotypic integration

## Background

### Phenotypic Integration and Modularity

The concept of phenotypic integration was first formulated by Olson & Miller [1]. It states that, when viewing an organism as a whole, certain morphological structures covary more strongly than others, forming spatially and temporally interdependent modules due to commonalities in development and function. It thereby becomes evident that the concept of phenotypic integration is closely linked to the topic of modularity: In most known organisms, certain groups of elements - morphological, genetic, or otherwise - exhibit strong interconnection and correlation, but are relatively independent of other such groups, thereby dividing organisms into distinct, heterogeneous parts or “modules”. The concept of modularity has gained great interest in the fields of evolutionary, developmental, and systems biology within the last decade [2, 3, 4]; for reviews see [5, 6, 7, 8].

While most research on the phenomenon has been performed on vertebrates [1, 3, 9, 10, 11] and flowering plants [12, 13], insects are rather poorly studied in this regard [14]. Depending on the investigated traits and applied methodology, previous authors have reported either intermediate [15, 16] or very high [14] levels of phenotypic integration in holometabolous insects.

To date, there have been very few studies directly investigating how patterns of integration and modularity differ between castes and species of the family Formicidae and none in the diverse tribe Camponotini. Those few studies investigating integration and modularity in ants are either conceptual in nature [17, 18] or focus on single phenotypes or species [19, 20, 21]. This is surprising, as researching these phenomena across ant castes and taxa is especially intriguing: Firstly, the workers of a colony are closely related and the diversity of castes has been attributed to high degrees of phenotypic plasticity and modularity [17, 18, 22, 23]. Secondly, phenotypically integrated modules frequently correspond to structures associated with particular functions and behaviours, e.g. locomotion or foraging [10, 11]. This is especially noteworthy in terms of the functional specialization and division of labour commonly observed in ants exhibiting morphologically distinct castes. Comparing integration and modularity within and between ant species can therefore not only elucidate patterns of

caste- and species specific morphology, but also help to understand the role of morphological structures and modules in functionality, ecology and behaviour.

### Caste-specific morphological specializations in Camponotini

The ant tribe Camponotini currently includes 1875 species in eight genera [24, 25, 26]. Females of the camponotine species are particularly known for their diversity of morphologies within the worker caste [27, 28, 29, 30]. The most prominent cases of worker caste dimorphism are found in the genera *Camponotus*, *Colobopsis* and *Dinomyrmex*: most commonly, the smaller minor workers (Fig. 1) engage in foraging, brood care and nest-maintenance, while the major workers (often termed “soldiers”) have large heads and mandibles specialized for defence [26, 28, 31] (Fig. 2a, b, d, f).

Alate sexuals (gynes and males) in the Camponotini are typically characterized by large eyes and ocelli, and an enlarged mesosoma to accommodate the flight muscles [32, 33]. In contrast to the larger gynes (Fig. 2c), males usually have a small body and head, as well as long and fragile extremities [31]. Their short lifespans [34], and mating flights make them much harder to sample than their conspecific females, leading to their scarcity in museum collections and low number of publications describing male phenotypes. As males of the Camponotini are still insufficiently studied and taxonomic relationships in older descriptions are partially doubtful, this study uses the recently described males of *Colobopsis explodens* Laciny & Zettel, 2018 (see fig. 6 in [30] as an example).

In his seminal work, Wilson [35] used morphometric data to characterize different forms of allometry and polymorphism in various species of ants. For the genus *Camponotus* in its current state, his results indicated a gradual transition from monophasic allometry to complete dimorphism of the worker caste, which has arisen independently multiple times. He especially mentioned the pronounced dimorphism within the current genus *Colobopsis*, where the specialized major workers bear plug-shaped heads used to close the small entrance-holes in wooden nests. This phenomenon of animals using parts of their bodies to obstruct their nests is termed “phragmosis”. Several cases of independent evolution of diverse phragmotic structures have also been described in at least five other genera and subgenera of ants [31].

Most traditional theories assume the origin of the major worker or soldier caste from minor workers [35, 36]. Other authors, however, have strongly disputed this, regarding majors as a caste derived from queens. This assumption is based on many morphological similarities and observations of larval development [37, 38]. More recent publications, however, propose the

evolution of specialized castes as mosaics of preexisting or ancestral developmental patterns [17, 22, 39]. Others consider the influences of genetic and epigenetic mechanisms [40] and disruptive selection [41] on caste evolution, leaving the issue open to further investigation. In addition to the genus-specific phragmotic major workers, minor workers of the Southeast Asian *Colobopsis cylindrica* (COCY) group (Figs. 1a, b, d; 2a, c, e) have evolved a peculiar specialization as well: in territorial combat with other arthropods, they can rupture their enlarged mandibular gland reservoirs and their gastral integument, thereby covering their enemy in a sticky, toxic defensive secretion and sacrificing their own lives in the process [29, 30, 42, 43, 44, 45, 46]. The roughly 15 species included in the group have been preliminarily divided into several subgroups (complexes) based on molecular and morphological data (H. Zettel, A. Laciny, I. Druzhinina; unpublished). Members of the *Colobopsis saundersi* (Figs. 1a; 2a, c, e), *C. leonardi* (Fig. 1b) and *C. cylindrica* (s. str.) (Fig. 1d) complexes are treated in the present study.

One especially intriguing aspect of the hitherto investigated COCY colonies is the frequent presence of so-called “interlopers” [29, 45] – presumably inquiline or parabioc ants belonging to *Camponotus* (*Myrmamblys*) (Zettel et al, in prep.; Figs. 1e, 2f), whose minor workers are often found inside COCY nests. Their morphology is characterized by a domed head, long appendages, and very long setae on the posterior mesosoma (Fig. 1e). The minor workers appear to be mimetic, as their colour pattern (reddish-yellow, black, or red head with black body) always matches that of their host species. Their biology is still poorly understood and their taxonomic placement is the subject of an ongoing study (Zettel et al., in prep.). The inclusion of one example species (*Camponotus* “*inquilinus*”, manuscript name sensu Zettel et al. in prep.) in this study aims to further assess their morphology and compare it to that of their putative hosts.



Fig. 1: Example minor worker specimens of investigated taxonomic groups, lateral view; a) *Colobopsis explodens*, minor worker (from [30]); b) *Colobopsis* sp. “BBQ”, minor worker (© A. Laciny); c) *Colobopsis truncata*, minor worker (from [58], CASENT 0179881); d) *Colobopsis cylindrica*, minor worker (© A. Laciny); e) *Camponotus* “inquilinus”, minor worker (© P. Balàka); f) *Camponotus singularis*, minor worker (from [28]).

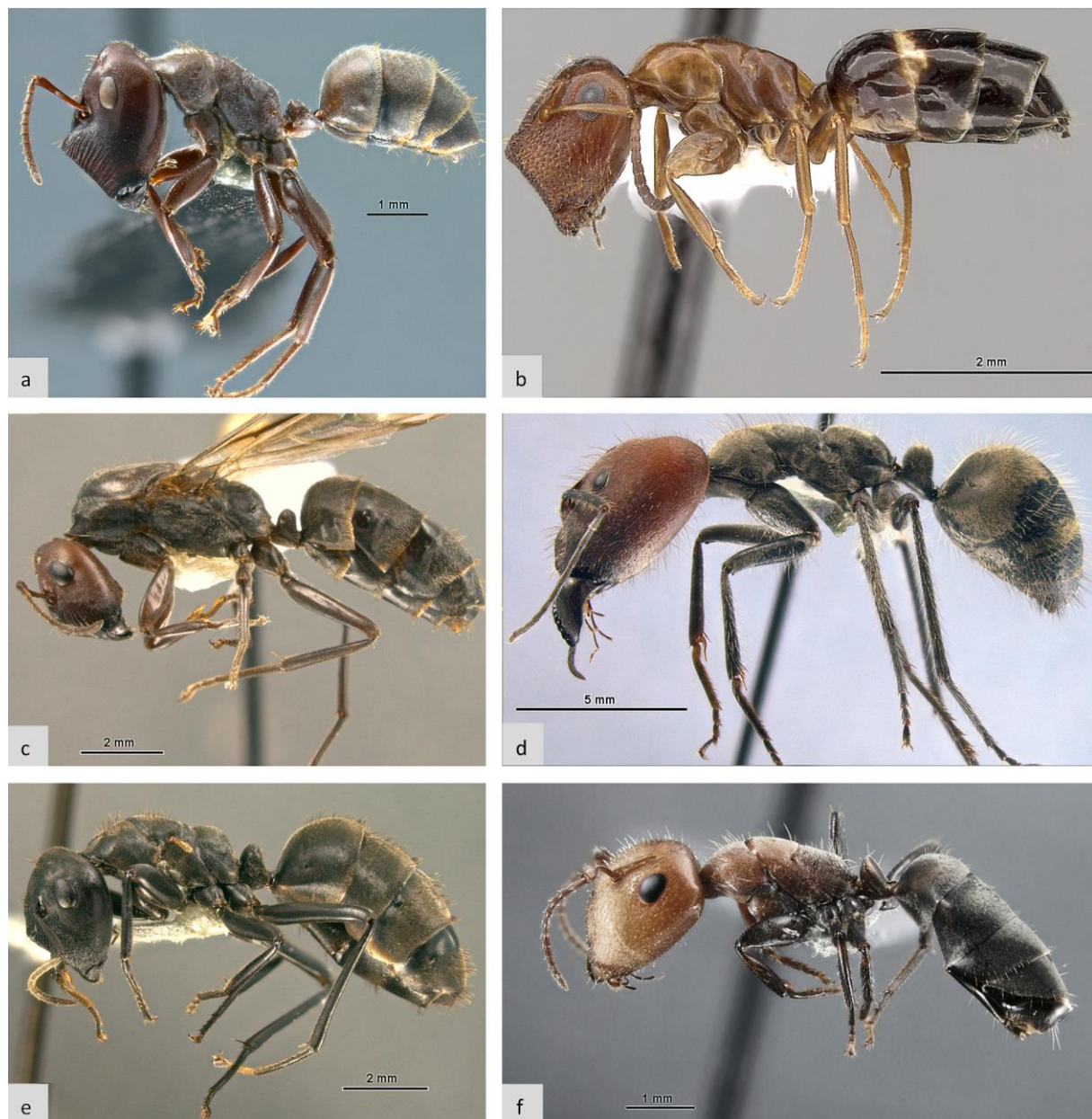


Fig. 2: Example major worker, gyne and MIC specimens of investigated taxonomic groups, lateral view; a) *Colobopsis explodens*, major worker (from [30]); b) *Colobopsis truncata*, major worker (from [58], CASENT 0249998); c) *Colobopsis* sp. “nrSA” alate gyne (from [29]); d) *Camponotus singularis*, major worker (from [28]); e) *Colobopsis* sp. “nrSA” mermithogyne (MIC) (from [29]); f) *Camponotus “inquilinus”*, major worker (© Pia Balàka, from Zettel et al., in prep.).

### Mermithogenic phenotypes

The influences of parasitic nematodes of the family Mermithidae on ant-hosts have long been a subject of scientific study [47], especially as the infestation is often accompanied by aberrant morphologies in the hosts. Such parasitized individuals are known from several

subfamilies of ants [47, 48] and have been observed to infest specimens of the genus *Colobopsis* [29, 49]. Studies on *Lasius* [50, 51, 52] and *Myrmica* [53] revealed that all known mermithogenic phenotypes develop from larvae destined to be sexuals, i.e., gynes or males, and found no infestation of workers. Parasitized individuals are often morphologically intermediate between castes, resulting in so-called intercaste phenotypes [47]. Characteristic changes include reduced body size, elongated extremities, enlarged gaster (due to distension by the nematode), reduced size of head, deviations in pilosity and sculpture, as well as reduction of all sexual characters (wings, thoracic sclerites, ovaries, and ocelli, Fig. 2e) [29, 47, 50, 51, 54, 55]. The study of morphological changes brought about by this form of parasitism can provide insight into phenotypic plasticity and developmental modularity in the studied species [17, 18, 22, 29]. Following previous studies in *Colobopsis* and *Lasius* [29, 52], this study hopes to further clarify how mermithid parasites influence host-morphology and developmental patterns in gynes of the studied species.

## Methods

### Material examined

In the course of this study, 332 specimens from 14 taxa were measured and examined. The sample was composed of 231 minor workers, six males, 36 major workers, 54 gynes, two intercaste specimens (IC) and three mermithogenic intercaste phenotypes (MIC). To simplify the analyses, specimens of the COCY group were sorted into species complexes as accurately as current taxonomic knowledge allows; example images for each species or complex are shown in Figs. 1 and 2. The complete list of specimens and depositories is provided in Additional file S1.

Notes on caste definition: While the distinction between minor workers, major workers, gynes and males is based on clear morphological characters (i.e., wings, ocelli, genitals, phragmotic or enlarged heads) and therefore self-evident in most of the examined camponotine species, some classifications had to be performed according to the individual perception of the researchers. Workers of the *leonardi* and *cylindrica* COCY complexes exhibit a continuous transition from minor to major worker; major workers were therefore only classified as such, when a clear phragmotic modification of the head (clypeal shield, cuticular ridges) was evident. Intercaste (IC) specimens were defined as those individuals whose morphology presented as a combination of two or more of the known castes. In three specimens, intercaste

morphology was known to be caused by infestation with mermithid nematodes; they were thus denoted as mermithogenic intercaste (MIC) phenotypes.

## Measurements

Seven morphological measurements (modified from [29, 30]) were taken from all specimens. In addition, median ocellar width (OcW) was measured in all gynes and MIC specimens possessing ocelli. All examined specimens were card-mounted or pinned, individually numbered, and measured at magnifications from  $25.6\times$  up to  $256\times$  with a Nikon SMZ1500 binocular microscope. All measurements were taken in millimetres and logarithmically transformed for statistical analyses. For a visual depiction of measurements, see also Figs. 15, 17, and 19. The complete dataset of measurements is provided as a supplementary table (Additional file S2).

HW Head width. Maximum width of head in full-face view, including eyes if laterally protruding.

HL Head length. Maximum length of head in full-face view, excluding mandibles, measured parallel to midline from anterior-most point of clypeus to midpoint of occipital margin or to midpoint of an imaginary line connecting the apices of posterior projections (major workers and some gynes of *C. singularis*).

EL Eye length. Maximum diameter of compound eye.

SL Scape length. Maximum length of antennal scape in dorsal view excluding basal neck and condyle.

SW Scape width. Maximum width of antennal scape in dorsal view, usually in distal third.

WL Weber's length. Maximum diagonal length of mesosoma. Measured laterally from anterior surface of pronotum proper (excluding collar) to posterior extension of propodeal lobes.

FeL Femur length. Maximum length of hind femur, measured from base to apex.

OcW Ocellar width. Maximum width of median ocellus (if present).

## Imaging of specimens

### *MicroCT imaging*

A selected sample of specimens (*Colobopsis explodens* minor worker, *Colobopsis saundersi* major worker, *Colobopsis* sp. “BBQ” gyne) was used for x-ray microtomographic imaging to visualize caste-specific morphology [56, 57] (Figs. 15, 17, 19). All specimens were stored in 70% ethanol prior to imaging and prepared according to the following protocol:

Specimens stored in 70% EtOH, transferred to 1:1 Bouins solution (96-100% EtOH) 3h – overnight; 70% EtOH (> 30 min.); 96% EtOH (> 30 min.); 100% EtOH (> 30 min.); I2E stain (1% iodine, 96-100% EtOH) overnight; stored in 100% EtOH until scan.

Fixed and stained specimens were then scanned in 100% EtOH or embedded in agarose. To mount specimens for scanning, they were inserted into pipette tips that were glued onto plastic blocks and sealed with Parafilm and / or wax. X-ray microtomographic images were made with a high-resolution microCT system (Xradia, MicroXCT-200, Zeiss X-Ray Microscopy, Pleasanton, CA) with a tungsten microfocus X-ray source and variable secondary optical magnification. Scans were made with an anode voltage setting of 60 – 80 kV and 50 – 130  $\mu$ A with an exposure time of 5 – 25 seconds for projection images every 0.25 or 0.20 °. Tomographic reconstructions were exported as image stacks with isotropic voxel sizes of 2.2 – 15.0  $\mu$ m. Using 4X optical magnification, the minor worker specimen was scanned as a whole, major worker and gyne specimens were scanned in two parts (anterior and posterior half of body) and stitched after reconstruction. Additional scans using 1X magnification were performed for whole body scans of large specimens in some cases. Images were reconstructed with Xradia software, and processed with Amira 6.3.0 and Adobe Photoshop 7.0.

### *Stacking photography*

To visualize species- and caste-specific morphology, including colouration, pubescence and cuticular microsculpture, selected specimens of all investigated species-groups (Figs. 1, 2) were imaged photographically. Images of *C. truncata* minor and major workers were obtained from AntWeb.org [58].

Digital stacked images (Figs. 1a, b, d-f; 2a, c-f) were taken with a Leica DFC camera attached to a Leica MZ16 binocular microscope with Leica Application Suite v3 and stacked with Zerene-Stacker 64-bit. All images were processed with Adobe Photoshop 7.0.

### *Statistical analysis*

All statistical analyses were conducted in Past3 [59] using log-transformed morphometric data.

A linear discriminant analysis of the entire sample based on a variance-covariance matrix, using the hypothetical caste-assignments (minor, major, male, gyne, intercaste) as groups was conducted. The resulting confusion matrix based on Jackknife resampling was used to assess the validity of the chosen measurements for caste classification.

To assess the expression of phenotypic integration over the complete character set, and identify the characters and character-combinations most relevant to total variance, a principal component analysis (PCA) based on a correlation matrix with 1000 bootstrap resamplings was conducted on the entire sample. To enable visual distinction in the resulting scatterplots, the respective castes were assigned a colour, and species (species groups) were allocated a shape, each consistent throughout all featured graphs.

To assess the interdependences of characters and allow an alternative, hierarchical visualization, a cluster analysis of the overall sample was conducted. To this end, the R-mode clustering function in Past3 was used on the transposed character matrix, applying the UPGMA algorithm and correlation as similarity index, with 1000 bootstrap resamplings.

Identical analyses (PCA and cluster analysis) were subsequently conducted using only the sample of minor workers and gynes including MIC to identify caste-specific morphological patterns. The sample of major workers proved to be too small for PCA and was only analysed via cluster analysis. A separate analysis of the remaining castes was omitted due to insufficient sample size. Graphical representation of results were created in Past3 [59] and processed with Adobe Photoshop 7.0.

## Results

### Linear discriminant analysis (LDA)

The linear discriminant analysis (LDA) of all specimens using seven characters and five caste-assignments as groups, yielded a correct classification of 93.37% after Jackknifing (Fig. 3, Tab. 1). Axes 1 and 2 shown on Fig. 3 explained 64.59% and 30.91% of total variance, respectively (Tab. 2). Based on the given morphometric data, all males were correctly classified, as well as all IC / MIC phenotypes except one (classified as a minor worker) and all gynes except two of *C. singularis* (classified as major workers). Most interestingly, three major workers and 14 minor workers of the *C. leonardi* complex, as well as two minor workers of *C. singularis* were classified as intercastes.

Tab. 1: Confusion matrix after linear discriminant analysis (LDA).

Caste	Hypothesis	LDA assignment	classification by LDA				
			Minor	male	major	gyne	IC/MIC
minor	231	216	215	0	0	0	16
male	6	6	0	6	0	0	0
major	36	35	0	0	33	0	3
gyne	54	52	0	0	2	52	0
IC/MIC	5	23	1	0	0	0	4

Caste-assignments of 332 specimens before and after LDA with Jackknifing; confusion matrix showing subsequent classifications resulting in 93.37% congruence with the hypothetical assignments.

Tab. 2: Axes 1 to 4 of linear discriminant analysis (LDA).

Axis	Eigenvalue	% of total variance
1	10.641	64.59
2	5.0914	30.91
3	0.639	3.88
4	0.1023	0.62

LDA based on 332 specimens, presented with eigenvalues and the percentage of total variance explained by each axis.

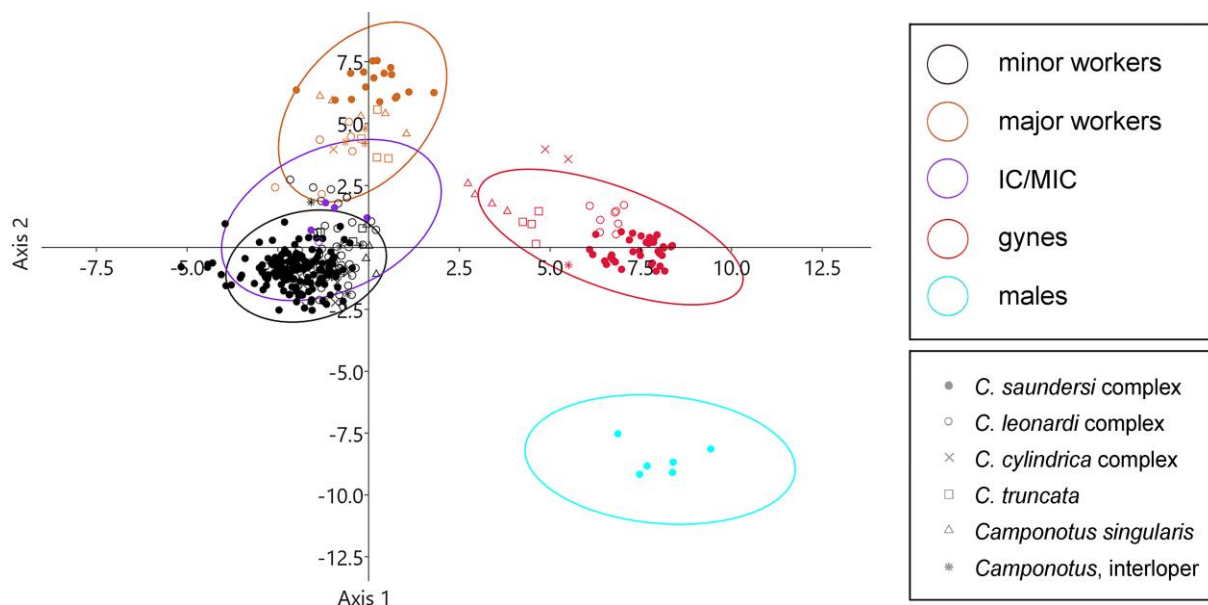


Fig. 3: LDA (axis 1 and 2) based on the complete sample (332 specimens, 7 characters) assigned to five groups, showing 95% ellipses.

## Principal component analysis (PCA)

Subsequently, a principal component analysis was conducted on the entire sample as well as for the subsamples of gynes and minor workers, to identify which characters or character combinations contributed most meaningfully to the principal component axes and their respective variances.

### *Complete sample*

PC1 contributed 84.53% of the variance and exhibited overall similarly high, positive loadings of all characters. This pattern is often considered a “size axis” [21, 60]. PC2 accounted for 10.16% of the variance (Tab. 3) and exhibited the highest positive loadings for SL and FeL, and high negative loadings for EL and WL (Fig. 6a, Additional Tab. S3). Thus, all specimens included in the sample can be roughly classified in a space ranging from “small body vs. large body” on PC1 – the most extreme cases being *C. truncata* minor workers (Fig. 1c) as the smallest and *C. singularis* gynes and major workers (Fig. 2d) as the largest – and “small eyes and mesosoma with long appendages vs. large eyes and mesosoma with short appendages” on PC2 – the extremes exemplified by *C. singularis* minor workers and *C. truncata* gynes and majors, respectively (Fig. 4). A further noteworthy result of this analysis was the placement of *C. leonardi*-complex and *C. cylindrica* major workers: While in all other taxa the major workers were clearly set apart from their conspecific minor workers by a lower placement on PC2 (e.g., due to shortened appendages), this was not the case for these major workers, which mainly differed from their conspecific minors in average body size (higher loadings on PC1). A very similar pattern was also observed for all the IC / MIC specimens. Males were clearly set apart from female castes due to their small size, and enlarged mesosoma and eyes, resulting in low placement on PC1 and PC2 (Fig. 4).

Tab. 3: First three principal components (PC) for the complete dataset.

PC	Eigenvalue	% of total variance	Lower 2.5% conf. Limit	Upper 2.5% conf. limit
1	5.916900	84.5270	81.7020	86.9210
2	0.711179	10.1600	8.5819	12.1580
3	0.193333	2.7619	2.1806	3.4525

PC 1-3 are presented with their eigenvalues, percentages of total variance, and 95% confidence intervals following 1000 bootstrap resamplings for the complete dataset (332 specimens, 7 characters).

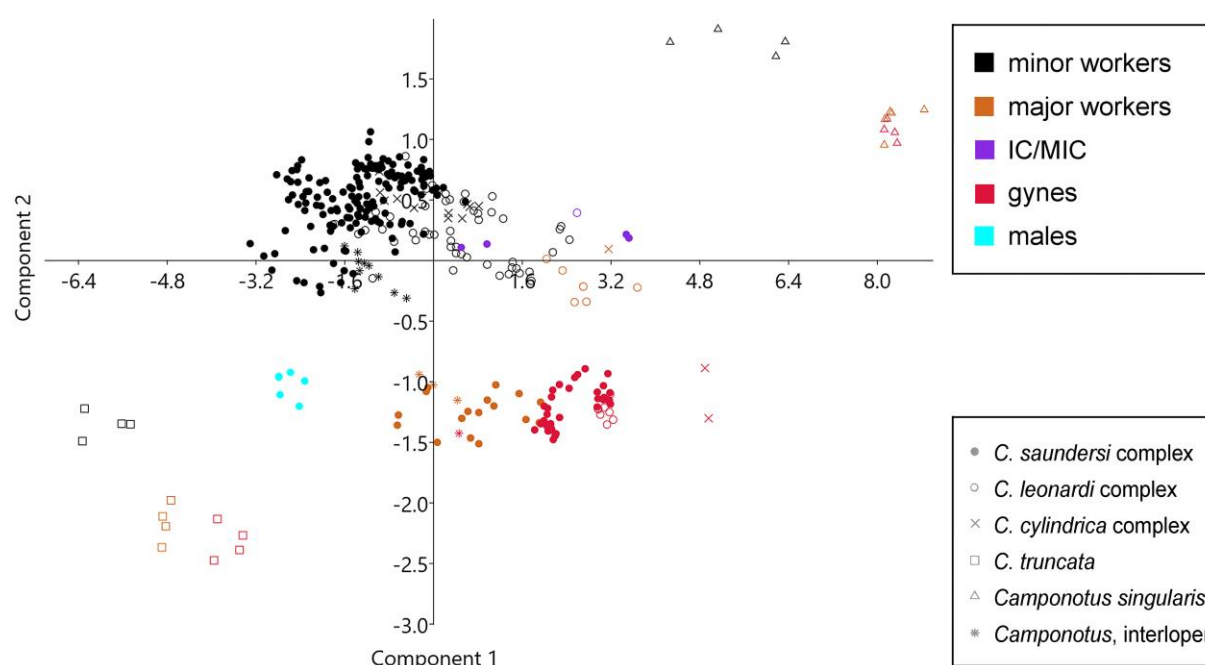


Fig. 4: PCA of the complete dataset (332 specimens, 7 characters), showing PC1 and PC2.

PC3 accounted for 2.76% of the total variance (Tab. 3) and was characterized by strong negative loadings for HW, HL and SW, as well as strong positive loadings for EL, FeL and WL (Fig. 6b, Additional Tab. S3). PC3 is especially suitable to better distinguish between conspecific gynes and major workers of the investigated species: Major workers are consistently placed lower on PC3, due to the combination of large heads, thick scapes, short femora, and a comparatively short mesosoma, while gynes exhibit the contrasting traits (Fig. 5). Male specimens were again set apart from their conspecific females by very high placement on PC3, as a result of their small head, enlarged mesosoma and elongated legs (Figs. 5, 6b).

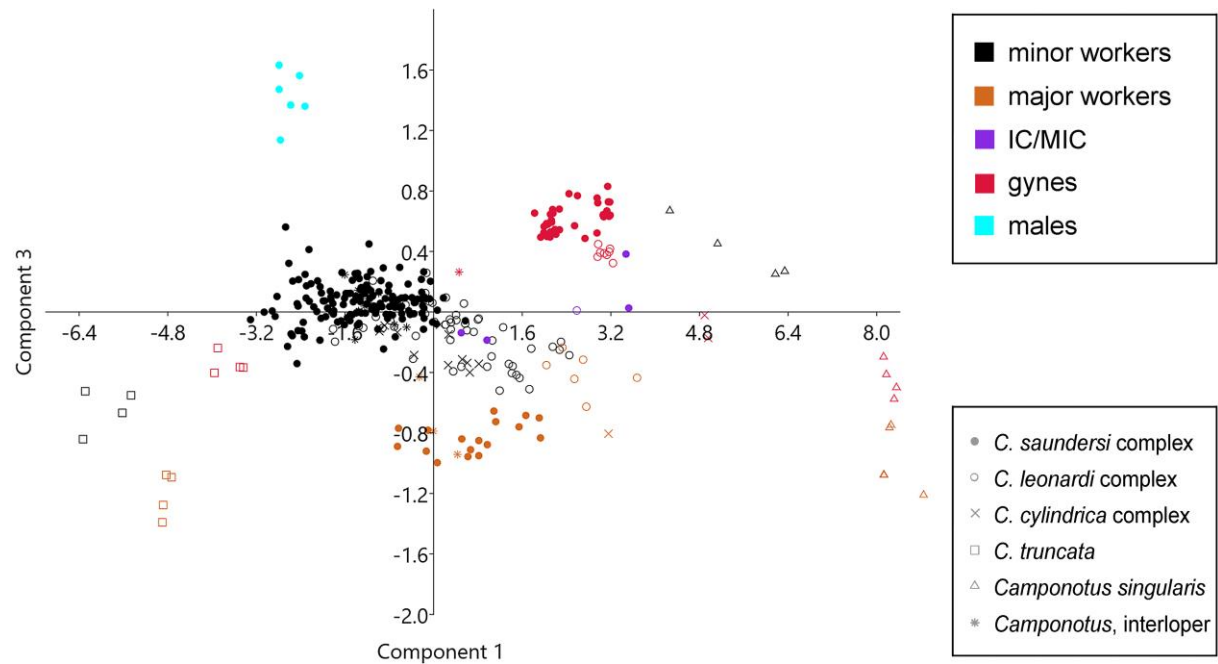


Fig. 5: PCA of the complete dataset (332 specimens, 7 characters), showing PC1 and PC3.

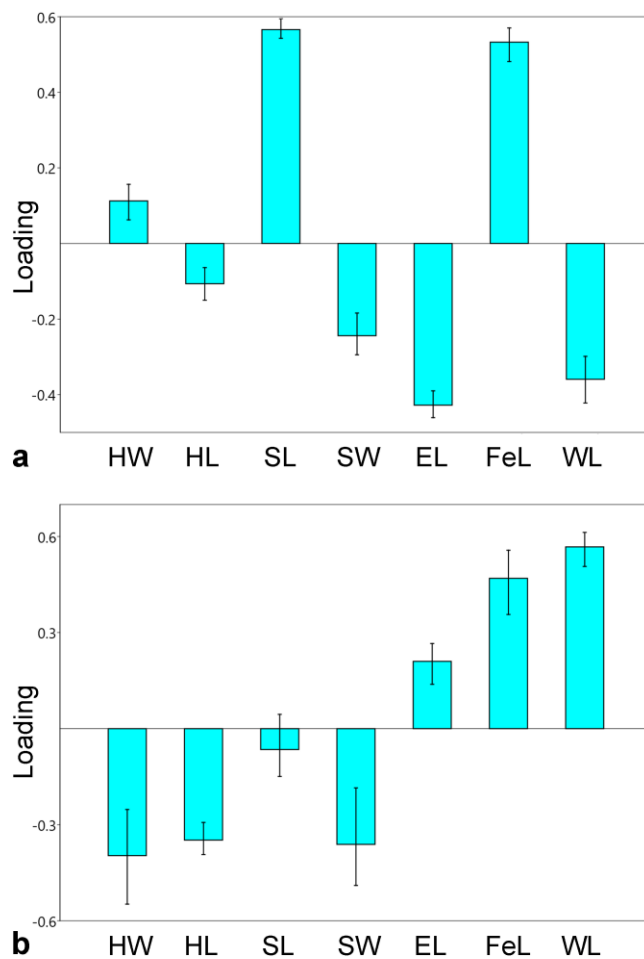


Fig. 6: Character loadings on PC2 (a) and PC3 (b) of the complete dataset (332 specimens, 7 characters)

*Minor workers*

Considering only the 231 specimens of the minor worker caste, the PCA yielded 88.9% variance for PC1, 5.64% for PC2, and 2.29% for PC3 (Tab. 4). PC1 was again considered mainly an indicator of overall size. PC2 exhibited very strong positive loadings for appendage lengths (SL and FeL) and strong negative loadings for SW and EL (Fig. 9a, Additional Tab. S3). A two-dimensional space created by PC1 and PC2 (Fig. 7) for characterization of the minor workers of different camponotine taxa could thus be approximated as “small body, short and thick appendages, large eyes” (e.g., *C. truncata*) vs. “large body, long and thin appendages, small eyes” (e.g., *C. singularis*), with the majority of COCY ants occupying an intermediate space. An interesting outlier from this trend are the minor workers of the *C. leonardi* and *C. cylindrica* complexes: Especially the larger individuals exhibit short and thick appendages, more often seen in major workers of other taxa. A second noteworthy observation is the position of so-called “interlopers” (Fig. 1e): Their very large eyes and comparatively wide scapes place them very low on PC2, but they are not entirely distinguishable from their known hosts (COCY species of the *saundersi* clade, Fig. 1a) within the conducted PCA (Fig. 7). However, they are clearly distinct from the sample of all COCY minor workers when PC3 is also taken into consideration, due to their higher loadings on this axis. PC3 differed from PC2 mainly by the strong positive loading of SW and the weakly negative loading of FeL (Fig. 9b, Additional Tab. S3). Minor workers of the *C. cylindrica* clade show a similar pattern, due to their conspicuously wide scapes (Fig. 8).

Tab. 4: First three principal components (PC) for the minor worker sample.

PC	Eigenvalue	% of total variance	Lower 2.5% conf. Limit	Upper 2.5% conf. limit
1	6.223370	88.9050	85.7630	91.2110
2	0.394777	5.6397	4.3348	7.3820
3	0.160217	2.2888	1.6316	2.8592

PC 1-3 presented with their eigenvalues, percentages of total variance, and 95% confidence intervals following 1000 bootstrap resamplings for the minor worker sample (231 specimens, 7 characters).

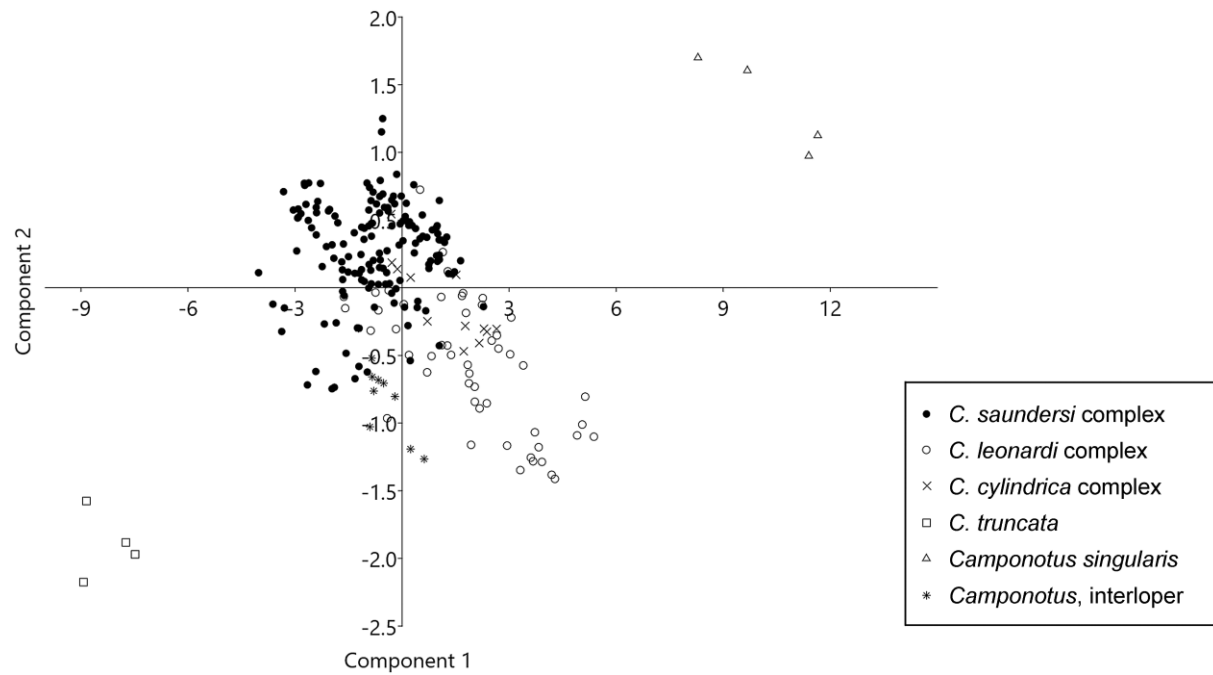


Fig. 7: PCA of minor worker subsample (231 specimens, 7 characters), showing PC1 and PC2.

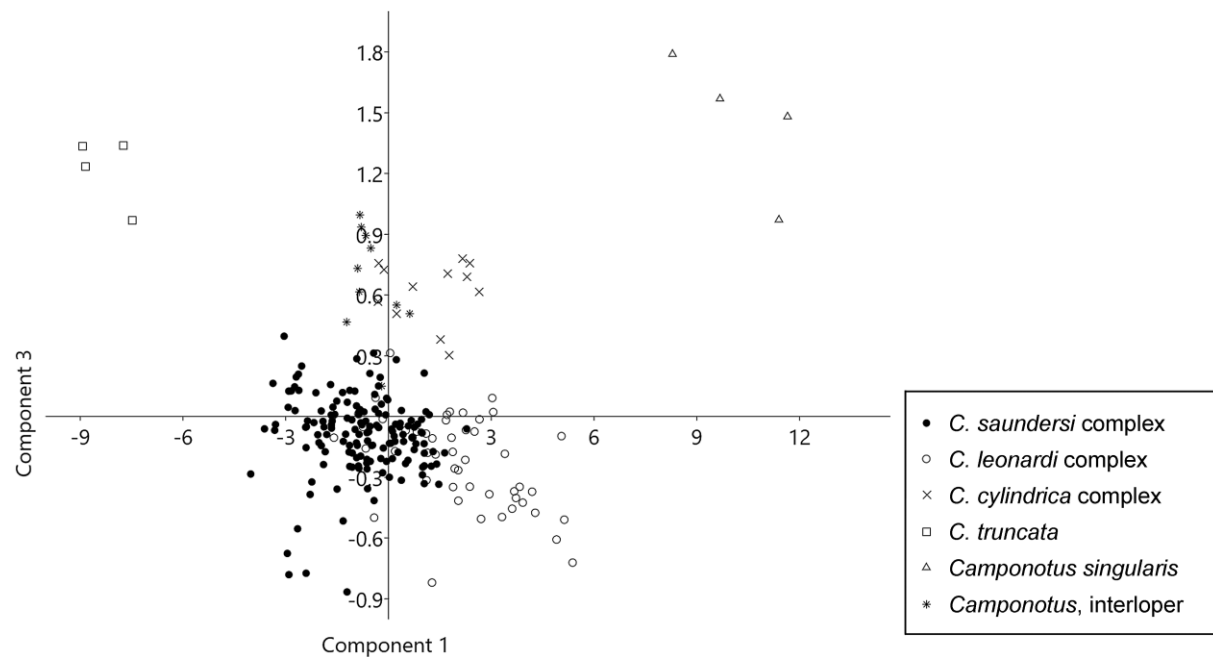


Fig. 8: PCA of minor worker subsample (231 specimens, 7 characters), showing PC1 and PC3.

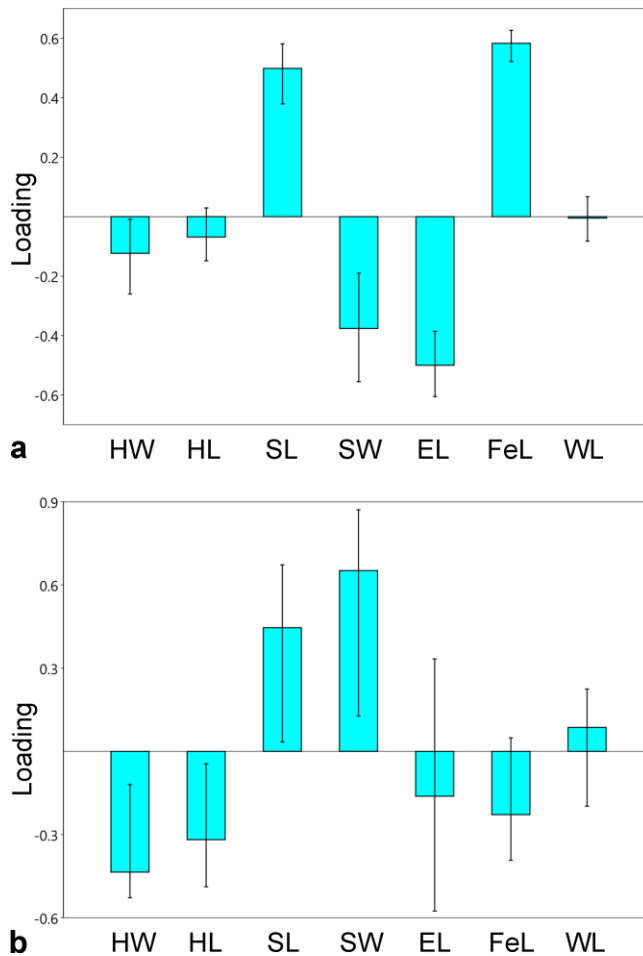


Fig. 9: Character loadings on PC2 (a) and PC3 (b) of the minor worker subsample (231 specimens, 7 characters).

### Gynes

For the PCA of all gynes (54 specimens) and two *C. sp.* “nrSA” MIC specimens with visible ocelli, the set of characters was expanded to include the width of the median ocellus (OcW). PC1 held 84.16% of the total variance (Tab. 5) and was again considered a size-axis, albeit with a slightly weaker loading for OcW (Additional Tab. S3). PC2 accounted for 10.91% (Tab. 5) and had the strongest positive loadings for OcW and WL, and strong negative loadings for SL and HW (Fig. 12a, Additional Tab. S3). PC3 accounted for 2.28% of total variance (Tab. 5) and exhibited strong positive loadings for SW and strong negative loadings for EL (Fig. 12b, Additional Tab. S3). Ocellar width is known to be a highly variable character in all examined taxa [28, 29, 30]. Eye length and Weber’s length (a measure of mesosoma size) are typically increased and correlated in gynes to accommodate flight. The extraordinarily long and thick scapes of *C. cylindrica* gynes are indicated by their position on PC2, which contrasts with the morphology of all other examined COCY gynes (Fig. 10).

Although similar in overall body size, the MIC specimens were clearly set apart from morphologically typical gyne by their placement on both PC2 and PC3 (Figs. 10, 11), due to reduction of their gyne-specific characters (ocelli, mesosoma).

Tab. 5: First three principal components (PC) for the gyne and MIC sample.

PC	Eigenvalue	% of total variance	Lower 2.5% conf. limit	Upper 2.5% conf. limit
1	6.732670	84.1580	77.1360	91.507
2	0.873139	10.9140	5.8896	15.845
3	0.182741	2.2843	0.5510	4.117

PC 1-3 presented with their eigenvalues, percentages of total variance, and 95% confidence intervals following 1000 bootstrap resamplings for the gyne and MIC sample (56 specimens, 8 characters).

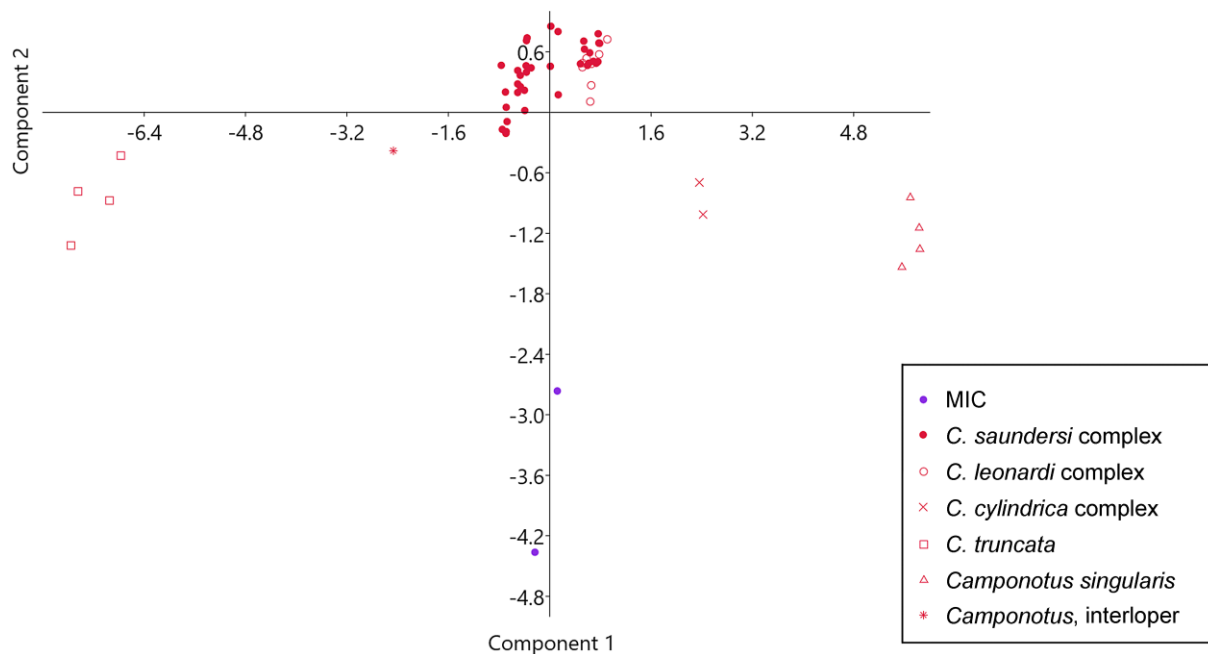


Fig. 10: PCA of gyne and MIC subsample (56 specimens, 8 characters), showing PC1 and PC2.

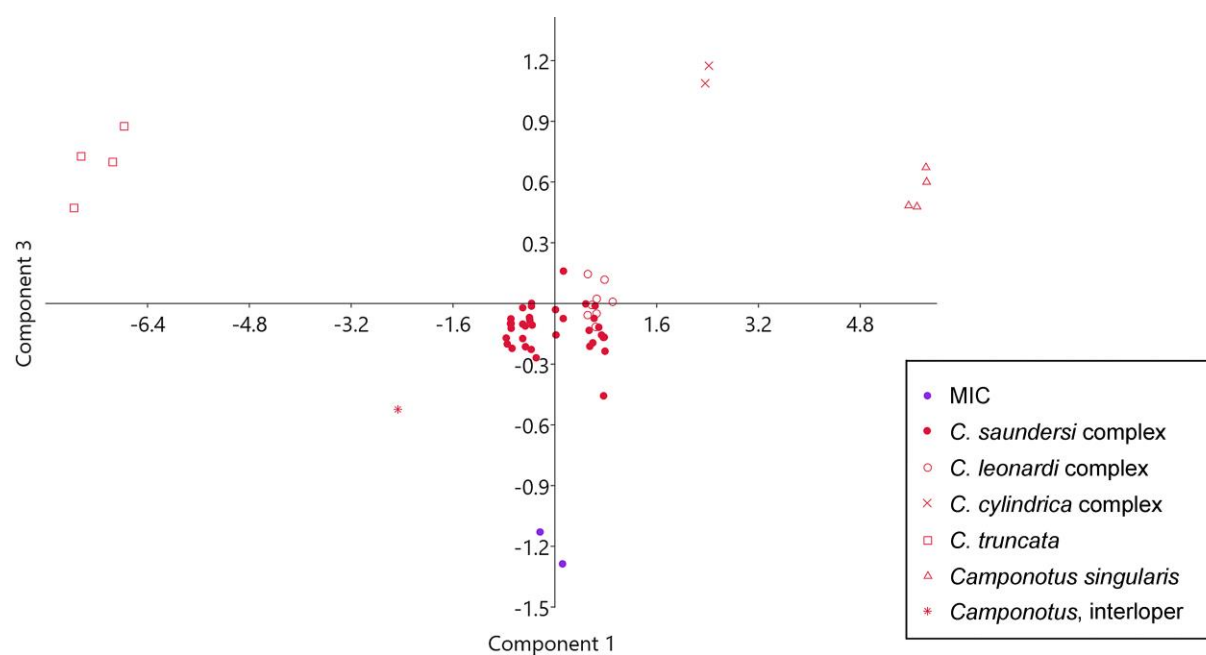


Fig. 11: PCA of gyne and MIC subsample (56 specimens, 8 characters), showing PC1 and PC3.

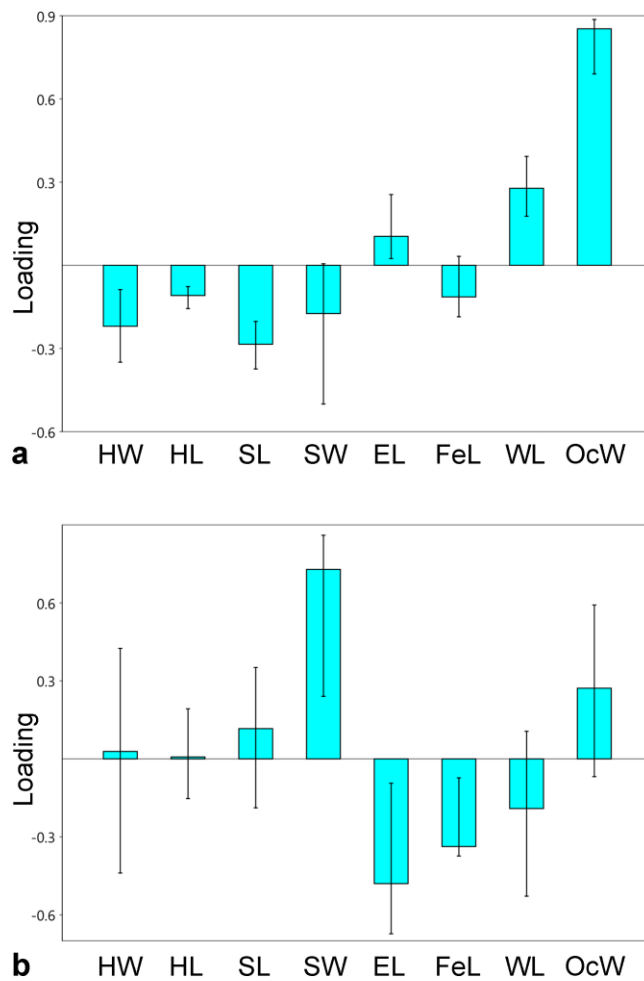


Fig. 12: Character loadings on PC2 (a) and PC3 (b) for gynes and MIC (56 specimens, 8 characters).

### Cluster analysis

To further assess which of the examined morphological characters could be considered as parts of phenotypically integrated modules in development and evolution, R-mode cluster analyses based on a UPGMA algorithm and using correlation as a similarity index were conducted. Bootstrap values resulting from 1000 resamplings are shown in character tree diagrams (Figs. 13, 14, 16, 18, 20). In addition, morphological measurements are shown on microCT images for a representative of each female caste (Figs. 15, 17, 19); colours of the measurements correspond to colours of integrated modules in the character trees.

### Complete dataset

The cluster analysis of the complete sample corroborated the results of the corresponding PCA and visualized which of the investigated traits may form integrated modules: HL and HW, the two size-indicators of the head, were strongly correlated, as were the indicators of

appendage length, SL and FeL. Scape width (SW) was included in the same cluster as the head characters, thus contrasting with the remaining cluster constituted by EL and WL – the two characters most closely associated with gyne-specific morphology (Fig 13).

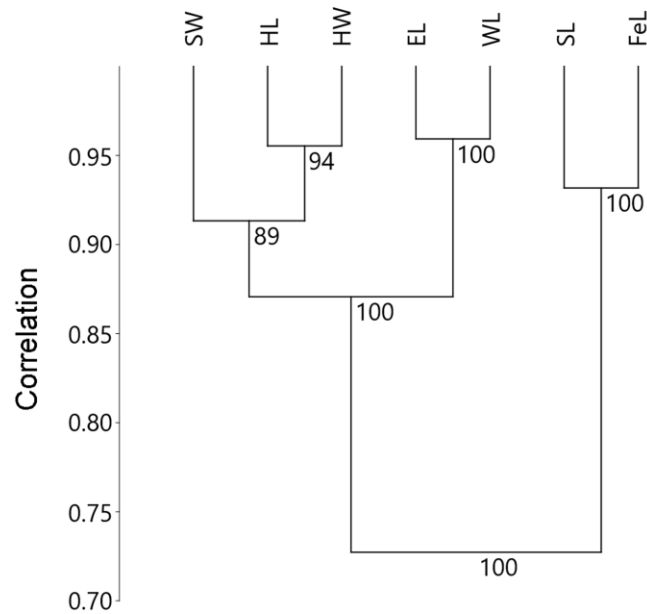


Fig. 13: Results of the cluster analysis of the entire dataset (332 specimens, 7 characters), showing the correlation of main integrated modules supported by bootstrap values.

#### *Minor workers*

Although differing from the clusters found in the complete dataset by structural details, the cluster analysis conducted on the minor workers yielded overall similar results to that of the complete sample: Again, the appendages (SL, FeL) formed one strongly correlated module, distinct from all remaining characters. The characters of head-size (HW, HL) were again strongly linked but now clustered with mesosoma length (WL), which can all be considered as aspects of “body size”. SW and EL were highly variable and set apart as distinct modules (Figs. 14, 15)

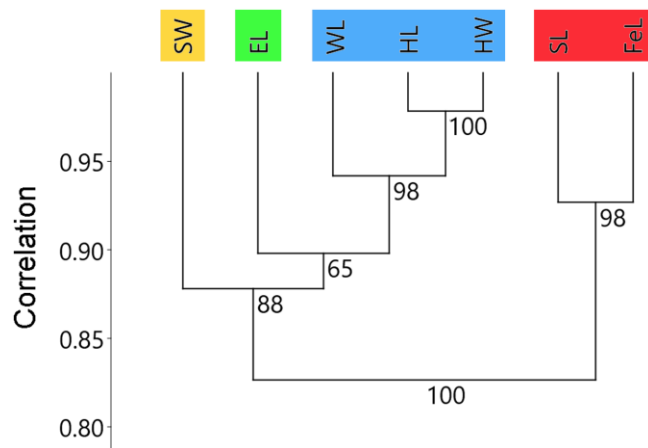


Fig. 14: Results of the cluster analysis of the minor worker subsample (231 specimens, 7 characters), showing the correlation of main integrated modules supported by bootstrap values; colours correspond to Fig. 15.

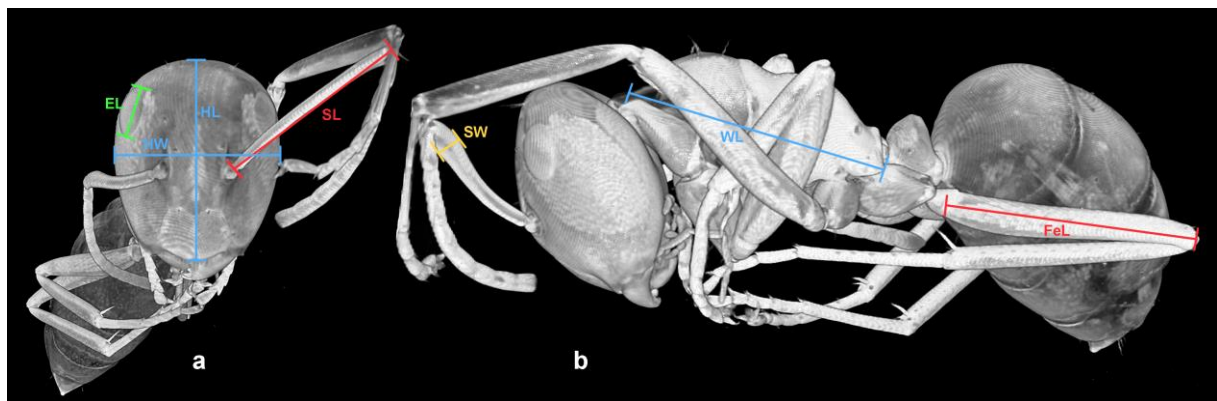


Fig. 15: Integrated character modules colour coded on an example minor worker specimen of *C. explodens*; a) frontal, b) lateral view.

### Major workers

The cluster analysis of the major worker subsample (36 specimens, 7 characters) yielded a character tree differing from the topology of the minor workers (Figs. 16, 17). The characters SW and EL were strongly variable and rather independent from other characters. The characters defining extremity length, FeL and SL, were here clustered with HW, while the second character of head size (HL) correlated strongly with mesosoma length (WL).

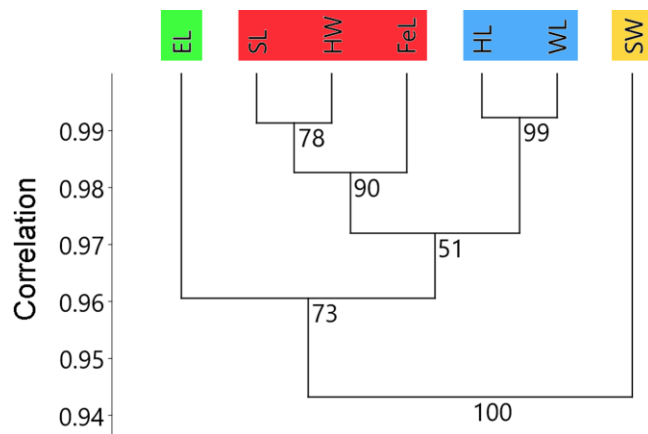


Fig. 16: Results of the cluster analysis of the major worker subsample (36 specimens, 7 characters), showing the correlations of main integrated modules supported by bootstrap values; colours correspond to Fig. 17.

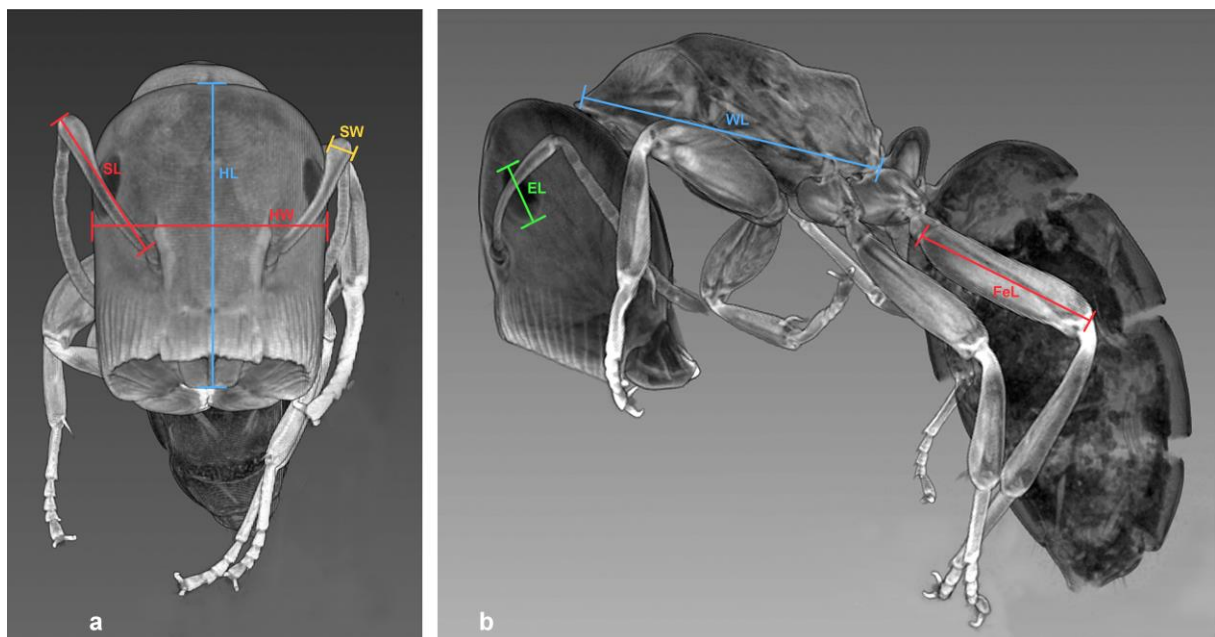


Fig. 17: Integrated character modules colour coded on an example major worker specimen of *C. saundersi*; a) frontal, b) lateral view.

### Gynes

For the subsample of gynes, two different cluster analyses were conducted: 1) One analysis used eight characters (including OcW), based on a sample of all gynes including the two MIC specimens with ocelli (56 specimens in total). 2) For better comparison with the overall and worker samples, only the original character set (seven characters, without OcW) was used and MIC specimens were omitted.

The first analysis (56 specimens including MIC, 8 characters, Figs. 18, 19) yielded results differing from those of the complete and minor worker samples: The highly variable OcW was set apart from all other characters. In contrast to all other analyses, the gyenes did not present such strong correlation of appendages. Instead, the appendage characters were each strongly correlated to a measurement of head-size – SL was linked to HW, FeL to HL; SW was again set apart from these modules. The influence of the characteristic gyne-specific morphology with both eyes and mesosoma enlarged for flight was also apparent, thus linking EL, WL.

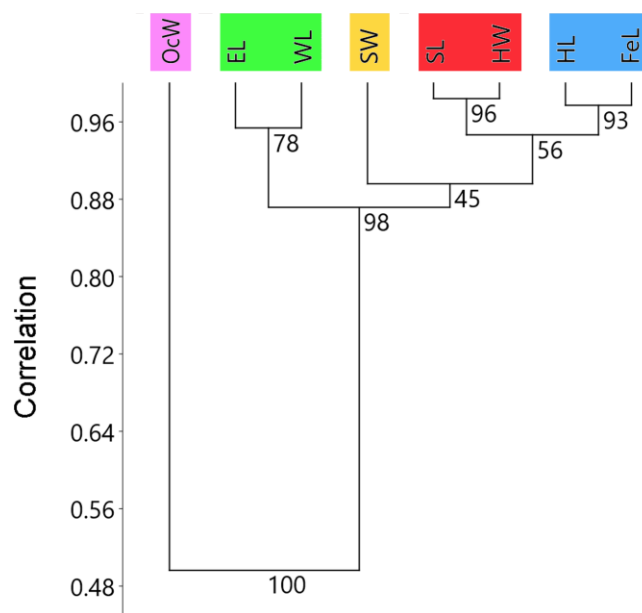


Fig. 18: Results of the cluster analysis of the gyne subsample including two MIC specimens and OcW (56 specimens, 8 characters), showing the correlations of main integrated modules supported by bootstrap values; colours correspond to those in Fig. 19.

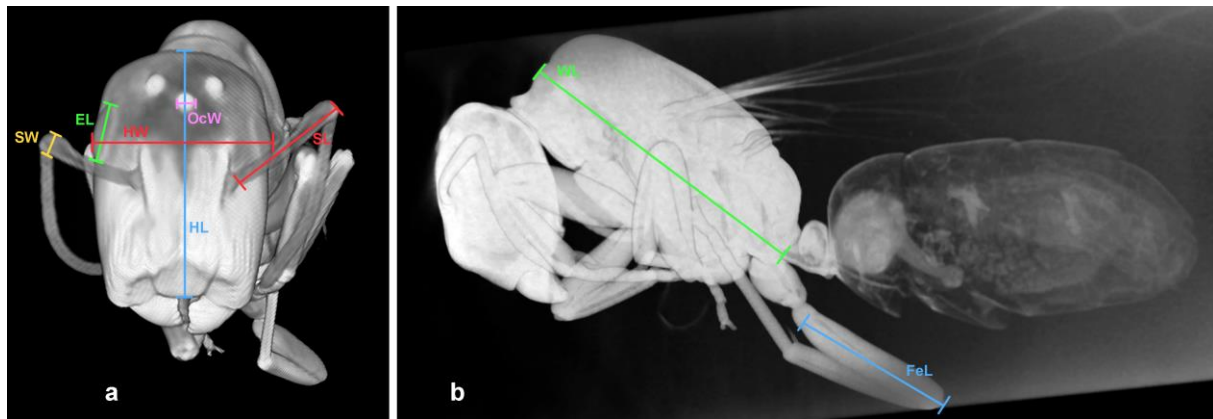


Fig. 19: Integrated character modules colour coded on an example gyne specimen of *C. sp.* “BBQ”; a) frontal, b) lateral view.

When removing the two mermithogenic specimens and the character of median ocellar width from the sample, the resulting character tree (54 specimens, 7 characters, Fig. 20) was only marginally different from the previously examined sample: The strongly correlated HL and FeL were now more closely associated to the cluster of EL and WL than to the one composed of SL and HW; SW was again set apart as a distinct character.

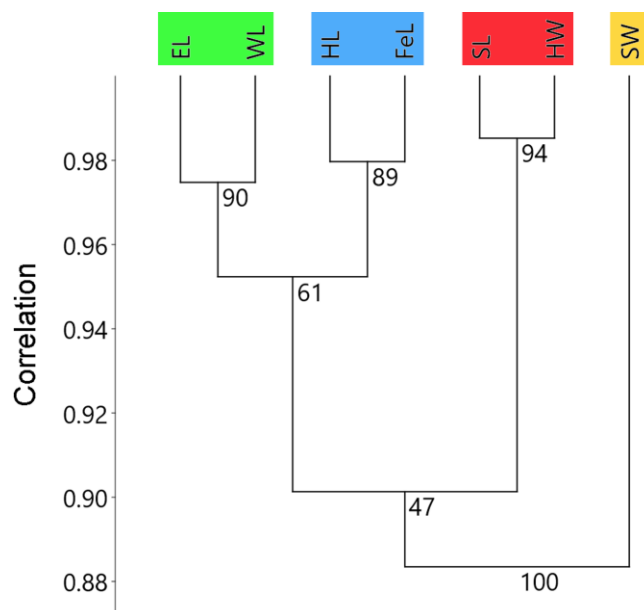


Fig. 20: Results of the cluster analysis of the gyne subsample specimens, excluding OcW and MIC specimens (54 specimens, 7 characters), showing the correlations of main integrated modules supported by bootstrap values.

## Discussion

The merits of studying phenotypic integration

First proposed in 1958 by Olson & Miller [1], the concept of studying integrated groups of characters in organisms is not a scientific novelty. However, modern methods and insights from such diverse fields as molecular genetics, developmental biology, evolutionary ecology, palaeontology, and philosophy of science [5] now make interdisciplinary studies on the origin and evolution of complex patterns of covarying traits more interesting and promising than ever before.

An important question in terms of evolutionary biology is whether phenotypic integration acts as a facilitator for natural selection and adaptation or whether it should rather be regarded as an evolutionary constraint [5, 8]. While strong interdependence among traits certainly channels and limits the evolutionary trajectory of the species in question [5, 61], authors of previous studies have alternatively considered the possibility that functional integration is in itself an adaptation. Since functionally related parts show coordinated variability [10], integration may serve to increase evolvability by making the population more capable of evolving in likely directions of selection [8, 62].

It thereby becomes evident that a better understanding of how a complex phenotype is composed out of tightly integrated, but mutually relatively independent modules can be an important factor in shedding light on the ontogenetic development and evolution of all organisms, as well as the adaptive value and functionality of specific morphological structures.

With the exception of honeybees [15, 16] and a select few species of myrmicine ants [19, 20, 21], social insects have been largely overlooked in previous publications. A noteworthy result of a recent comparative study [14] is the high degree of integration in adult holometabolous insects in comparison to plants, vertebrates and hemimetabolous insects. The authors attribute this to the relative lack of spatial and temporal variation during the pupal phase. In vertebrates, this same study also reported the strongest mean correlations within the group of limb characters. In addition, investigations on birds (Carduelidae, Columbiformes) [3, 10] showed strongly integrated modules corresponding to structures necessary for optimal flight and foraging. Interestingly, this corresponds to the results of the study at hand: Particularly the limb characters (scape length and femur length) tend to be closely integrated in workers, whereas gynes show higher levels of correlation between eyes and mesosoma. This can be

interpreted regarding functional aspects of structures necessary for foraging and flight, as well as the common genetic underpinnings by homologous developmental control genes [63, 64]. In light of these findings, it seems worthwhile to further investigate patterns of phenotypic integration in the family Formicidae: Not only are their caste systems excellent subjects for the study of phenotypic plasticity and modularity [17, 18, 23], they also exhibit patterns of phenotypic integration similar to those found in previously studied vertebrates. These functionally and developmentally integrated modules may have been shaped by functional demands common to many different organisms in the course of natural selection.

#### Linking specialized caste morphology and division of labour

Ants and other eusocial insects are commonly compared to superorganisms, in which each animal is considered less as an individual and more like a cell within a larger body [31]. In this context, the evolution of functionally and morphologically specialized female castes is the foundation for division of labour within the colony and thus the enormous evolutionary success of ants and other eusocial Hymenoptera [65]. In the context of phenotypic integration and modularity, members of distinct castes themselves have been compared to other “functionally specialized modules” such as different cells within one organism or the different limbs of arthropods [66].

The number of different tasks that need to be performed in order to maintain a functioning colony ranges from 20 to 40, yet only 20% of ant genera possess more than one worker caste and no known ant genus contains more than three distinct physical worker castes [67]. Therefore, the behavioural repertoire resulting from functional specialization must be sufficient to perform the necessary tasks efficiently, but may not be too narrow to allow for multiple different tasks to be executed by each colony member [66].

#### *Minor workers*

The subsample of minor workers was by far the largest at 231 specimens. Across all examined taxa, minor workers were the most abundant and most readily available caste. This is also corroborated by previously published data, which place optimal caste ratios for camponotine species at 80% minor workers vs. 20% major workers [68, 69]. These high numbers, together with the apparent intraspecific variability in size [28, 29, 30], suggest a possible division of labour not only between minor and major workers, but even within the minor worker caste itself. Although behavioural data on the examined taxa are still sparse,

similar results have been described for *Camponotus japonicus* var. *aterrimus* [70] and *Camponotus sericeiventris* [71].

Considering the results of the PCAs and cluster analyses, the most strongly integrated modules for the minor worker caste are the complexes of head size (HL, HW) and the extremities exemplified by SL and FeL (Figs. 9, 14, 15, Additional Tab. S3). As shown in previous studies on three of the individual taxa [28, 29, 30], long legs and scapes tend to be correlated with well-developed, elongated mouthparts (maxillary and labial palpi) as well. This module thus contains structures for locomotion (legs) as well as sensory function (palpi, antennae). This is likely linked to minor workers' tasks within the colony (e.g., foraging, brood care), which rely on mobility as well as well-developed sensory abilities. As shown for two species of *Camponotus* [72], gynes and major workers possess shortened antennae and maxillary palpi, as well as smaller corresponding brain structures than foraging minor workers of the same species [73]. All appendages are further linked in evolution and development through a common genetic underpinning by developmental control genes, such as homothorax (*hth*) and extradenticle (*exd*) [64], which may explain the modular patterns observable in many of the examined taxa.

The minor worker caste is also the protagonist in two fascinating phenomena observed in connection with the group of COCY ants – suicidal explosion in the COCY group itself [30, 42, 45] and inquiline behaviour of *Camponotus* (*Myrmamblys*) “*inquilinus*” minor workers (“interlopers” sensu Zettel et al., in prep) in nests occupied by COCY species [29, 45]. Interestingly, this study has found no specialized adaptations of outer morphology or proportions in either group. Considering only the “interlopers” and their hosts of the *C. saundersi* complex, both occupy intermediate positions within the scatterplots and are only distinguishable by the proportions of SW and EL. When examined under a microscope, they also differ in structural details of the integument, hair length and head shape (domed in *C. “inquilinus”*), but are strikingly similar in size and colouration (Zettel et al., in prep.). Although the biology of the *C. “inquilinus”* group is largely unstudied, their known association and even mimetic resemblance to ants of the COCY group may well enable them to benefit from the ecological dominance and suicidal defence of the “exploding ants” [29, 30, 43, 45]. How they avoid detection by their host colony via putative alterations to their cuticular hydrocarbon profile will be the subject of future studies (M. Hoenigsberger et al., in prep.).

### *Major workers*

Although the limited number of major workers available for this study limited their suitability for statistical analysis, their placement in the PCA based on the entire sample of specimens permits a number of meaningful conclusions. Major workers of all examined species were consistently placed lower on PC2 and PC3 than their conspecific minor workers and gynes (Figs. 4, 5). Taking into account the character loadings on the respective axes, it becomes clear that this is the result of a caste-specific set of traits: A large head (HW, HL) and very thick scapes (SW) result in a low placement on PC3, short extremities (SL, FeL) contribute to strong negative loads on PC2. Exceptions to this pattern are only found in the basal COCY groups (H. Zettel, I. Druzhinina et al., unpublished) represented by the *C. leonardi* and *C. cylindrica* complexes: Here, major and minor workers are not as clearly set apart from each other, as these groups exhibit intermediate morphologies not clearly definable as major or minor workers. This is also reflected in the classification yielded by the LDA (Fig. 3, Tab. 1), which labelled three major workers and 14 minor workers of the *C. leonardi* complex as intercastes. As disruptive selection is thought to be at least partially responsible for the evolution of distinct castes [41], it is possible the distinct minor and major worker phenotypes observable in e.g. the *C. saundersi* complex arose later in the evolution of COCY ants, thus leading to the varying degrees of caste dimorphism observable today. As phragmosis in the major worker caste is also known from the *C. truncata* group, this trait may have evolved multiple times within the genus *Colobopsis* (H. Zettel, unpublished).

Overall, the major worker caste in Camponotini is morphologically adapted to its two main functions within the colony: nest defence and storage of nutritious liquids, leading to enlargement of the head and mandibles, as well as the gaster [28, 74, 75]. Within the genus *Colobopsis*, the defensive function is shifted from active, aggressive “soldiers” with enlarged mandibles towards passive phragmotic plugging of nest entrances with specialized shield-shaped heads [29, 30, 35, 76]. Both, active combat as well as phragmotic behaviour can possibly benefit from a heavy-set body with short, thick appendages to reduce the probability of injury [28].

### *Gynes and MIC*

Considering the morphology of the gynes examined within this study, several noteworthy parallels can be drawn to the results of previously conducted investigations: Both, the high loadings on PC2 and the modules apparent in the cluster analysis point to a high level of

integration between EL and WL in gynes (Figs. 10, 11, 12, 18, 20, Additional Tab. S3). This is most probably linked to aspects of life history and behavioural ecology relevant for alate camponotine gynes: Known flight-specific morphological adaptations in sexual castes of the Formicidae include the presence of ocelli, enlarged eyes, and a large mesosoma to accommodate the well-developed flight musculature [32, 33]. Thus, the characters of EL and WL may form a functional module in gynes, explained by their crucial role in complex behaviours, such as mating flights, dispersal, and mate-choice. In contrast, gynes showed lower variability of femur length and less pronounced integration of the extremities (SL, FeL) than minor workers (compare Figs. 9a, 12a). This previously unreported finding may also be connected to the life history of alate gynes, which lacks the selective pressures for ambulatory locomotion and sensing of chemical cues relevant for minor workers. In the case of the antennae, this is known to be reflected in the corresponding neurological structures of the brain in two investigated species of *Camponotus* [72]. The results of the PCA and cluster analysis point to an extremely high variability of median ocellar width (OcW) (Figs. 12, 18, Additional Tab. S3). This phenomenon was also observed in studies focusing on three of the individual camponotine species examined in the present study: *C. singularis* [28], *Colobopsis* sp. nrSA [29], and *C. explodens* [30]. Current literature yields no evolutionary or developmental explanation for this fact. The authors presume a high level of plasticity for this trait, possibly linked to size-dependent developmental thresholds. This is also suggested by the fact that median ocelli or vestiges thereof have been observed in particularly large major workers and intercaste specimens [28, 29, 38].

In the case of MIC specimens (Fig. 2e), vestiges of ocelli, wings and gyne-like mesosomal architecture are due to their origin from queen-presumptive larvae [53]. The infective larval stages of parasitic nematodes of the family Mermithidae infect ant larvae via oral uptake of a paratenic host (e.g. oligochaetes) and develop in synchronicity with them [50]. Whether mermithogynes of the studied species have specific tasks within their native colonies is hitherto unknown. As they are usually flightless and have reduced ovaries [47, 51, 77], mermithogynes cannot take part in reproduction. However, there are no reports of antagonistic behaviour by nestmates against infested individuals. On the contrary, they have been observed to stay with their native colony, aid in brood care and beg workers for food [77]. More recent theories suggest that the survival of intercaste phenotypes may be facilitated by mechanisms of colonial buffering [17]. Eventually, the mature parasite will compel its host to seek a moist habitat for its release and engage in suicidal behaviour (drowning) [49, 50, 77].

Phenotypes produced by mermithid infestation can lie anywhere on a range from almost normal worker morphology (“mermithergates”) to gyne-like specimens with only slightly reduced features (“mermithogynes”) [53]. This is also apparent in the studied sample: Although their reduced ocelli and mesosoma placed them consistently lower on PC2 and PC3 compared to normally developed gynes, the two conspecific mermithogynes differed considerably from each other (Figs. 10, 11). Within the LDA and PCA of the complete sample (Figs. 3, 4), IC and MIC specimens were consistently plotted as intermediate between minor and major workers, further corroborating their status as “intercaste” specimens. How exactly mermithid nematodes influence the morphology of their hosts – whether by hormonal disruption [17, 53] or through a different mechanism – and why they can present as such a wide range of phenotypes, must be investigated in future studies. Uncovering the developmental mechanisms behind mermithogenic phenotypes may even lead to a better understanding of the evolution of specialized castes: Previous studies have proposed a link between the developmental programs facilitating this plastic response to environmental perturbations and the evolution of novel phenotypes [17, 18].

## Conclusions

While the investigation of phenotypic integration and modularity of the body plan has a long tradition in the fields of vertebrate biology and botany [1, 12], studies on insects are rare [14] and social insects have been largely overlooked. In this study, we present the first detailed investigation of phenotypic integration in the ant tribe Camponotini, based on morphometric data of two camponotine genera – *Camponotus* and *Colobopsis*. As these ants possess complex caste systems with distinct phenotypes (minor worker, major worker, gyne, and male), special focus was placed on caste-specific morphology and character complexes (modules) presumably related to behaviour and division of labour within the colony. The selected characters were shown to be suitable for caste characterization, resulting in over 93% correct classification by LDA. Using multivariate statistical methods (PCA, cluster analysis), we were able to detect species- and caste-specific proportions and visualize multiple tightly integrated modules composed of covarying characters specific to each female caste: For minor workers, strong integration of extremities (legs, antennae) was a distinctive feature. In major workers, extremities also clustered with head width. Gynes differed from workers by a strong correlation of eye length with mesosomal length. In mermithogynes (gynes infested with parasitic nematodes), gyne-specific characters were reduced and overall proportions consequently altered. The caste-specific character modules are interpreted in light of their

putative functional relevance to task-allocation for each caste: Locomotion and sensory perception are crucial for foraging activity in minor workers [72], while major workers are morphologically equipped for phragmosis (*Colobopsis*) or active defence (*Camponotus*) [35], and gynes rely on well-developed visual abilities and flight musculature for dispersal and mating [32, 33]. Similar character modules linked through common development and function have previously been described in vertebrates [10, 14]. Furthermore, modularity of the body plan and plastic responses to environmental stress (e.g., parasites) are thought to have contributed to the diversity and ecological dominance of the family Formicidae [17, 18]. Further studies on the subject could therefore lead to valuable insights into the factors facilitating the evolution of novel phenotypes in ants and even uncover ontogenetic and evolutionary principles common to a wider range of organisms.

### **Abbreviations**

**COCY:** *Colobopsis cylindrica* group

**EL:** eye length

**EtOH:** ethanol

**FeL:** hind femur length

**HL:** head length

**HW:** head width

**IC:** intercaste

**LDA:** linear discriminant analysis

**MIC:** mermithogenic intercaste

**microCT:** x-ray microtomography

**OcW:** median ocellus width

**PCA:** principal component analysis

**PC:** principal component

**SL:** scape length

**SW:** scape width

**WL:** Weber's length (diagonal length of mesosoma)

### **Declarations**

#### **Ethics approval and consent to participate**

Not applicable.

#### **Consent for publication**

Not applicable.

### **Availability of data and material**

The dataset supporting the conclusions of this article is included within the article and its additional files. The datasets of measurements and specimens are available as Supplementary Files S1 and S2. The investigated specimens are deposited and available for study or loan at the respective institutions as described in S1.

### **Competing interests**

The authors declare that they have no competing interests.

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### **Authors' contributions**

AL and HLN conceptualized the study and performed statistical analysis. AL compiled the set of specimens and conducted morphometric measurements. BDM and AL conducted microCT scans. HZ and AL constructed figures and conducted stacking photography. ISD organized funding, sampling expeditions and availability of materials. AL, HLN and HZ wrote the manuscript text, to which BDM and ISD contributed.

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### **Additional files**

*Additional file 1:* Additional PDF file S1: “S1 Depositories and Material.pdf”. Complete list of depositories, taxonomic placement, caste assignment, and collection data for the specimens analysed in this study.

*Additional file 2:* Additional MS Excel table S2: “S2 Measurements.xls”. Complete list of measurements (in mm) for all specimens examined in this study.

*Additional file 3:* Additional MS Excel table S3: “S3 Character Loadings.xls”. Loadings on principal components (PC) 1-3, for the complete dataset (332 specimens, 7 characters), the minor worker sample (231 specimens, 7 characters), and the sample of gynes and MIC (56 specimens, 8 characters).

## Supplementary material Study 5

### Supplement S1 Depositories and Material

#### Depositories

BRM	Brunei Museum, Kota Batu, Brunei Darussalam
CLA	Coll. A. Laciny, Vienna, Austria
CZW	Coll. H. Zettel, Vienna, Austria
MCSN	Museo Civico di Storia Naturale Giacomo Doria, Genova, Italy
NHMW	Natural History Museum Vienna, Austria

#### Material examined

In the course of this study, 332 specimens from 14 taxa were measured and examined. The sample was composed of 231 minor workers, six males, 36 major workers (soldiers), 54 gynes, two intercaste specimens (IC) and three mermithogenic intercaste phenotypes (MIC) from the following taxonomic groups:

#### *Colobopsis*

##### *Colobopsis saundersi* complex:

*Colobopsis* sp. “nrSA” (NHMW, BRM): 10 alate gynes, 6 major workers, 42 minor workers, 2 mermithogynes, 1 intercaste; Brunei Darussalam, Ulu Temburong National Park, Kuala Belalong Field Studies Center (KBFSC) 4°33' N, 115°09' E, 60 m a.s.l., artificial nest #27, 16.IV.2015, leg. A. Kopchinskiy; 4 alate gynes, same locality except top of Engkiang trail, “nrSA colony #1”, I.2011, leg. D. W. Davidson.

*Colobopsis* sp. “RHYG” (NHMW, BRM): 10 minor workers, Brunei, KBFSC, 4°33' N, 115°09' E, 60 m a.s.l., “KB02-118”, 2002, leg. D. W. Davidson; 4 minor workers, same data except “RHYG A790”, 16.VI.2004; 3 minor workers, 2 major workers, same data except “KB02-72”, 2002; 6 minor workers, same locality, vial “E 26”, V.2017, leg. A. Kopchinskiy.

*Colobopsis* sp. “AR” (NHMW, BRM): 17 minor workers, 1 major worker, 1 alate gyne, Brunei, KBFSC, 4°33' N, 115°09' E, 60 m a.s.l., V.2017, leg. A. Kopchinskiy.

*Colobopsis* sp. “CASA” (NHMW, BRM): 6 minor workers, Brunei, KBFSC, 4°33' N, 115°09' E, 60 m a.s.l., vial “G 29” V.2017, leg. A. Kopchinskiy.

*Colobopsis* sp. “RHOG” (NHMW, BRM): 6 minor workers, Brunei, KBFSC, 4°33' N, 115°09' E, 60 m a.s.l., vial “B 27”, V.2017, leg. A. Kopchinskiy.

*Colobopsis* sp. “cf. YG” (NHMW, BRM): 6 minor workers, 1 intercaste, Brunei, KBFSC, 4°33' N, 115°09' E, 60m a.s.l., V.2017, leg. A. Kopchinskiy.

*Colobopsis* sp. “LCY” (NHMW, BRM): 4 alate gynes, Brunei, KBFSC, 4°33' N, 115°09' E, 60 m a.s.l., artificial nest “LCY 23 (367)”, IV.2015, leg. A. Kopchinskiy.

*Colobopsis explodens* Laciny & Zettel, 2018 (NHMW, BRM): 16 alate gynes, 3 major workers, 20 minor workers, 6 males; Brunei, KBFSC, 4°33' N, 115°09' E, 60 m a.s.l., “Vienna Colony”, 10.XI.-05.XII.2015, leg. A. Laciny & A. Kopchinskiy; 1 major worker, “YG 373 main natural nest”, same data except 17.IV.2015, leg. A. Kopchinskiy; 2 major workers, “cf. YG 39 (351) artificial nest”, same data except 20.IV.2015; 8 minor workers, 2 major workers, same locality, 370 m along Ashton trail, KB02-108, 2002, leg. D.W. Davidson; 4 minor workers, same locality and collector as previous, no collection date, “YG 2025”; 5 minor workers, same locality and collector as previous, “YG T-trail (202)”, I.2012; 5 minor workers, same locality and collector as previous, “YG-2 (73)”, 15.V.2014; 7 minor workers, same data as previous, “YG-2 (49)”.

*Colobopsis leonardi* complex:

*Colobopsis* sp. “BBQ” (NHMW, BRM): 4 minor workers, 3 major workers, 7 alate gynes, 1 mermithogyne; Brunei, KBFSC, 4°33' N, 115°09' E, 60 m a.s.l., cut nest fragment “Vienna Colony”, V.2017, leg. A. Kopchinskiy; 6 minor workers, same data except colony BBQ-2017-4, vial #25; 5 minor workers, same data except vial “A”, 6 minor workers, same data except vial “C”; 8 minor workers, same data except “400 m Ashton Trail”, 10.XI.-5.XII.2015, leg. A. Laciny & A. Kopchinskiy; 3 major workers, 1 dealate gyne, same data as previous except “BBQ Mata Ikan Tree 1”; 2 major workers, same data except “LE Visitor Canopy Walkway”.

*Colobopsis* sp. “AB” (NHMW): 15 minor workers, 1 major worker, Brunei, KBFSC, 4°33' N, 115°09' E, 60 m a.s.l., “AB KB02-100”, 2002, leg. D.W. Davidson; 2 minor workers, same data except “AB 1200 (203)”, I.2012; 5 minor workers, same data except “AB-1 (69)”, 14.V.2014.

*Colobopsis cylindrica* complex (currently under revision, all specimens tentatively identified as *C. cylindrica* (Fabricius, 1798) s.str.): 4 minor workers, 1 major worker (NHMW), Brunei,

KBFSC, 4°33' N, 115°09' E, 60 m a.s.l., “CH-1 (61)”, 13.-22.V.2014, leg. D.W. Davidson; 8 minor workers, same data except “CH-1 (79)”; 1 alate gyne (MCSN), Sumatra, Siboga, “*Camponotus doriae*”, X.1890-III.1891, leg. E. Modigliani; 1 alate gyne (MCSN), same data except Sumatra, Pangherang Pisang.

*Colobopsis truncata* (Spinola, 1808): 1 dealate gyne (CZW), Austria, Vienna, 14<sup>th</sup> district, Steinhofgründe, 08.IV.2009, leg. H. Zettel; 1 dealate gyne (CZW), Austria, Vienna, Lainzer Tiergarten 18/7, 1992, leg. H. Zettel; 2 dealate gynes (NHMW), Austria, Lower Austria, Laxenburg, 03.III.1972, leg. Faber; 1 major worker (NHMW), Austria, Lower Austria, Donauauen, IV.1884, leg. Loew (?); 2 major workers (CLA), Austria, Vienna, Prater, Schwarzenstockallee near Lusthaus, 16.X.2015, leg. A. Laciny & H. Zettel; 1 major worker, 1 minor worker (CZW), Austria, Lower Austria, Theresienfeld, Wienerstr. 63, III.2014, leg. W. Hansely; 1 minor worker (CZW), Austria, Lower Austria, Perchtoldsdorfer Heide, G-Mitte, PH 19, 03.VI.2012, leg. H. Zettel; 1 minor worker (NHMW), Austria, Vienna, Lainzer Tiergarten, 06.VII.1946, leg. Scheerpeltz; 1 minor worker (NHMW) Austria, Lower Austria, Weinviertel, Mistelbach, Zayawiesen (#189), 11.VI.1997, leg. Schödl.

### *Camponotus*

*Camponotus singularis* (Smith, 1858): 5 alate gynes (NHMW), Indonesia, Java, 1894, leg. Adensamer; 1 major worker (NHMW), Indonesia, Java, leg. Vollenhov; 1 major worker (NHMW), Luang Nam Tha Province, ca. 20 km SE of Muang Sing, 950 m a.s.l., 12–13.VI.1996, leg. H. Schillhammer (#25); 1 major worker (NHMW), Luang Nam Tha Province, ca. 10 km E of Muang Sing, 600 m a.s.l., 19.VI.1996, leg. H. Schillhammer (#32); 1 major worker (MCSN), Kayin State [Carin Cheba], 900–1100 m a.s.l., V.1888, leg. L. Fea; 1 major worker, 4 minor workers (CZW), Hua Phan Province, Ban Saleui, Phou Pan (Mt.), 1300–1900 m a.s.l., 20°12'N 104°01'E, 7–29.IV.2014, leg. C. Holzschuh.

*Camponotus (Myrmamblys) “inquilinus”* (“interlopers”, sensu Zettel et al. in prep.) (NHMW, BRM): 9 minor workers (including holotype) Brunei, KBFSC, artificial nest #29, 5.IV.2015, leg. A. Kopchinskiy, #346/347; 3 major workers, 1 dealate gyne, same nest series; 1 minor worker, same locality, artificial nest #27 (occupied by *Colobopsis* sp. “nrSA”), 16.IV.2015, leg. A. Kopchinskiy.

## Supplement S2 Measurements

Table S2 is provided in the digital version of this dissertation.

## Supplement S3 Character loadings

	Complete Dataset (n = 332)			Minor Workers (n = 231)			Gynes (n= 56)		
	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
<b>HW</b>	0.39517	0.11264	-0.39648	0.38981	-0.12336	-0.43528	0.36452	-0.21985	0.02852
<b>HL</b>	0.40001	-0.10654	-0.34796	0.39189	-0.06832	-0.3182	0.38098	-0.10899	0.00722
<b>SL</b>	0.35379	0.56601	-0.06513	0.3681	0.49876	0.44596	0.36574	-0.28462	0.11619
<b>SW</b>	0.38726	-0.24405	-0.36146	0.36729	-0.37614	0.65179	0.35465	-0.17405	0.72875
<b>EL</b>	0.37665	-0.42788	0.21019	0.36652	-0.49972	-0.16157	0.37045	0.10445	-0.48001
<b>FeL</b>	0.35415	0.53272	0.46957	0.36785	0.5832	-0.22807	0.3772	-0.11409	-0.3373
<b>WL</b>	0.37602	-0.35939	0.56758	0.39296	-0.00496	0.08636	0.36477	0.2779	-0.19117
<b>OcW</b>	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.22332	0.85283	0.27174

### 3. Discussion

#### 3.1. Novel insights and consequences

The following chapter will once more address the four initially established research questions and assess, which insights may be gained from the cumulative results of the publications included herein.

##### **3.1.1. Which measurements and proportions of external characters are suitable to characterize castes of the camponotine genera *Camponotus* and *Colobopsis*?**

Within the present dissertation, satisfying results have been produced using a set of relatively simple, traditional morphometric measurements, ranging from seven (study 5) to 23 (study 4) characters per specimen. The number of measured characters and calculated indices varied depending on caste of the specimen (e.g., wing- and ocelli-characters in gynes and males) and focus of the respective study. Even a reduced character-set of seven measurements (head width, head length, scape width, scape length, eye length, femur length, Weber's length) proved sufficient to assign over 90% of specimens to their correct caste (study 5). Overall, this method proved effective, while keeping the necessary time and expenses at a minimum and facilitating comparisons with other, often more traditional, taxonomic and morphometric studies.

While the conspicuous differences between female castes in overall body size and head shape have long been known as important markers of caste-specific morphology (e.g., Wilson 1953), the studies included herein also describe several other, novel characters and proportions useful for caste-characterization. This is especially noteworthy for the extremities: Across all studies, the scapes, legs-segments and maxillary palps of minor workers were consistently longer and more slender than those of conspecific majors, with gynes usually occupying intermediate proportions (studies 1, 2, 4). This was not only true relative to head-size (leading to potentially skewed proportions due to enlarged heads in gynes and majors), but also relative to body- or mesosoma-length and even considering only the absolute values (studies 1, 5). In certain cases, even taxonomic distinctions were based on extremity-length as an important character, e.g. *Camponotus singularis* vs. *rufomaculatus* (study 1) and *Colobopsis explodens* vs. *badia* (study 4). Conspicuously elongated extremities also played a role in the detection of mermithogenic phenotypes (studies 2, 3; see 3.1.3.).

In all taxa where individuals of multiple female castes were available, major workers possessed very short maxillary and labial palpi, short, thick and flattened scapes, as well as short, stout legs in comparison to conspecific minors. This character set (demonstrated by the low values of scape-, femur-, and palp segment-indices, and a high scape width index) was usually combined with an overall large body, a stocky, worker-like mesosoma and a very large, modified head. The head shape was always similar in majors and gynes – either heart-shaped with large mandibles (in *Camponotus singularis*, study 1) or truncated for phragmosis (in *Colobopsis* and *Camponotus inquilinus*, studies 2, 4, 5).

While these apparent caste-specific differences in proportions of all extremities have never been directly addressed in previous publications focusing on morphometry, some useful comparisons may be drawn to other taxa: Mysore et al. (2009) found shorter antennae with reduced sensilla in major workers of *Camponotus compressus* and *Camponotus sericeus*. These findings also corresponded to reductions in the corresponding neural structures in comparison to minors. The authors hypothesize that differentiation of chemical cues is essential for foraging minor workers, but less so in defensive majors. Leafcutter ants (*Atta colombica*) and army ants (*Eciton hamatum*) were also shown to possess shorter legs with increasing body mass, which was attributed to the mechanics of foraging (Feener et al. 1988). Selective pressure towards increased sensitivity to chemical signals, as well as highly developed locomotion abilities also seems plausible in the species treated within the present dissertation. In turn, short, thick appendages that can be flattened against the body may reduce the risk of injury in phragmotic or aggressive major workers. However, these hypotheses have yet to be supported by behavioural data from the species in question (see also 3.2.1, 3.2.2., 3.2.5.).

While gynes and males of the Camponotini are easily characterized by the presence of wings (or wing-scars) and ocelli, several new, potentially useful characters have come to light in the course of the studies at hand.

Especially regarding male morphology in the tribe Camponotini, current scientific literature is severely lacking. Many historical publications lack sufficient detail and illustrations to allow any meaningful comparisons across taxa (e.g., Viehmeyer 1916). While other authors do provide more information on male morphology (e.g., Karawaiew 1929), association of males with their native colonies and thus species assignment remains extremely difficult due to their dispersal during mating flights. The characters, illustrations, molecular and ecological data provided within the present dissertation for males of the species *Colobopsis explodens* and *C. badia* (study 4) may serve as a baseline for future studies on camponotine males.

For gynes, the combined results of all studies showed several characteristic traits, useful for delimiting castes and species: The enlarged mesosoma shows caste-specific adaptations to flight, with a hypertrophied mesothorax and a comparatively reduced prothorax. Previous authors (Keller et al. 2014) have ascribed this phenomenon to a morphological trade-off between the enlarged prothorax of foraging workers, which provides the necessary space to attach powerful neck muscles, and the mesothorax of gynes optimized for flight. In addition, gynes of all examined taxa had larger compound eyes relative to their head width (higher eye index) than their conspecific major and minor workers. Together with the presence of ocelli, this is interpreted as a further adaptation towards flight and dispersal, presumably linked to a necessity for increased visual abilities (compare Kalmus 1945). Median and lateral ocelli were present in all examined gynes, but showed astonishing intraspecific variability in size. Especially the variability of median ocellar width and the presence of ocelli or vestiges thereof in especially large majors and intercaste specimens (studies 1, 2, 4, 5) warrant further investigations of the underlying developmental mechanisms.

### **3.1.2. Which character-sets can be interpreted as phenotypically integrated modules?**

One main focus of the present dissertation – especially of study 5 – was to detect patterns within the selected set of measurements, which may be understood as “modules” in the sense of phenotypic integration (e.g., Olson & Miller 1958, Wagner et al. 2007). While holometabolous insects are known to exhibit overall tightly integrated phenotypes (Conner et al. 2014), no comparative analysis of ant castes has ever been performed in this regard. This is surprising, as their complex caste-systems are doubtlessly influenced and shaped by mechanisms of phenotypic plasticity and modularity (West-Eberhard 2003, Molet et al. 2014, Londe et al. 2015). Following a multivariate statistical approach (PCA, R-cluster analysis), those sets of characters were interpreted as modules, which showed the strongest correlations to each other, while remaining relatively independent of other examined characters. While body-size and details of proportions certainly differed between the examined species of *Camponotus* and *Colobopsis*, trends of caste-specific allometry showed striking similarities within each species.

#### **a) How do such modules differ between castes?**

While overall correlation of all examined morphological structures was high in all morphs (compare Conner et al. 2014), several modules may be highlighted as characteristic for each female caste, using the results of the R-cluster analysis (study 5): Minor workers are set apart

by tightly correlated extremities (scape length, femur length), a module of head and thorax size (head width and length, Weber's length; all linked to overall body size), as well as rather independent scape width and eye size. Though overall quite similar, major workers differed by an integration of head width with extremity length, and head length with thorax length. In contrast, gynes (including mermithogynes) were characterized by extremely variable median ocellus width, and a strong correlation of mesosoma and eye size. They also differed from workers by a conspicuous decoupling of the "extremities-module", whereby the scapes and femora were respectively more closely connected to characters of head size.

**b) Can the observed patterns be interpreted in relation to functional ecology and evolution of castes or division of labour within the colonies of the investigated species?**

Though little is known about the biology of many of the included taxa (see 3.2.5.), several of the detected modules appear functionally adaptive when extrapolating from the known life history and biology of related ants: In minor workers, characters involved in foraging and sensory perception (legs, antennae, mouthparts), tend to be strongly correlated (studies 1, 2, 4, 5). Thus, the uncovered patterns of modularity corroborate the results of other studies linking minor worker morphology to adaptations towards optimal locomotion and chemosensory abilities (e.g., Mysore et al. 2009). Interestingly, minor workers known to engage in highly specialized behaviours (suicidal "exploding" in the COCY group, presumed xenobiosis in *Camponotus inquilinus*) showed no apparent alterations of outer morphology that may be interpreted as adaptive to these particular phenomena (Zettel et al. 2018b, studies 4, 5).

The link between shortened extremities and increased head width in major workers or soldiers of the Camponotini is a novel finding of the present dissertation and may be interpreted as a set of adaptive traits common to these phenotypes: an enlarged head (for phragmosis or active combat) combined with thick, short appendages to increase stability and lower risk of damage.

The correlation of mesosomal size (Weber's length) and eye length apparent in the cluster analysis of gynes is in accordance with the results of previous studies: The muscles of the flight-apparatus necessitate enlargement of the mesosoma, particularly the mesothorax; well-developed eyes are thought to improve visual abilities crucial for flight, dispersal and mating (see 3.1.1., compare Kalmus 1945, Keller et al. 2014).

Perhaps most surprisingly, gynes were the only caste not exhibiting strong correlations between extremities (study 5). The proportions of their antennae, legs and mouthparts tend to be

intermediate between minor and major workers (studies 1, 2, 4) and lack obvious specializations – with the exception of especially broad scapes in gynes of the *Colobopsis cylindrica* s.str. clade (study 5, Zettel et al., in prep). This phenomenon is hitherto unknown and may be caused by relaxed selective pressures on ambulatory and sensory abilities in camponotine gynes, which remain relatively sedentary after colony foundation.

### **3.1.3. How does the influence of mermithid parasitism during ontogenetic development affect morphological proportions and patterns of phenotypic integration in the host ant, and are these changes comparable across host taxa?**

Within this dissertation, four mermithogynes were examined – three from species the COCY group (two of *Colobopsis* sp. nrSA, one of *Colobopsis* sp. BBQ; see studies 2, 5), and one from *Lasius niger* (study 3). Infestation by mermithid nematodes was confirmed either via microCT imaging (study 2) or by direct observation of the emerging parasite (study 3). As morphological species determination often relies on morphometric characters, as well as cuticular sculpture, pubescence and colouration (e.g., Seifert 2007) – all of which may be altered under parasitic influence – species identities were confirmed by DNA barcoding (studies 2, 3). The importance of correct species determination becomes all the more apparent, when considering that mermithogenic phenotypes have led to erroneous species descriptions and taxonomic confusion in the past (Czechowski et al. 2007, Csősz 2012).

Several general trends in the morphology of parasitogenic phenotypes are evident in all examined specimens, as well as in the results of previous publications: Reduced sexual characters (wings, thoracic sclerites, ocelli, gonads), a smaller head, a distended gaster and elongated appendages were similar across all taxa (Wheeler 1928, Kaiser 1986, Czechowski et al. 2007, Csősz & Majoros 2009, O'Grady & Breen 2011, Poinar 2012, studies 2, 3). Mermithogynes of the COCY group also exhibited reduced size of compound eyes and aberrations in colouration of the cuticle (study 2). Especially the increased length of all extremities proved to be a consistent result across all examined taxa: Mermithogynes were characterized by elongated metafemora (study 2), profemora (O'Grady & Breen 2011, study 3), metatibiae (Czechowski et al. 2007), maxillary palps (studies 2, 3), and antennal scapes (Czechowski et al. 2007, Csősz & Majoros 2009, studies 2, 3). Overall, the mermithogynes of *Lasius* (O'Grady & Breen 2011, study 3) were less drastically altered by parasitic influence than those of *Colobopsis* spp. (see 3.2.3.). In the COCY group, mermithogynes exhibited proportions rather similar to those of conspecific minor workers, despite resembling healthy

conspecific gynes in overall body size (studies 2, 5). Taking into account the caste-specific morphological modules proposed in study 5, a reduction of the gyne-like character complex consisting of mesosoma and eyes, as well as a shift towards a more worker-like pattern of strongly correlated and elongated extremities seems plausible. The parasite presumably causes a developmental switch in queen-presumptive larvae towards more worker-like growth programs, thereby creating the described intercaste- or mosaic-phenotypes (sensu Molet et al. 2012). The exact mechanisms of parasitic influence during development of the ant-host are hitherto unstudied for the ant-mermithid system, as discussed in 3.2.3, and will be the subject of further planned studies.

### **3.2. Open questions and avenues for further research**

Naturally, the contents of the present dissertation do not represent comprehensive answers to all the initial research questions, and – as the scientific process often goes – novel insights into one aspect may entail new unexplained phenomena and opportunities for future studies in another. The following chapters highlight some of the most apparent unresolved issues and areas for further investigations.

#### **3.2.1. Minor workers: Size-variation as an indication of intra-caste polyethism?**

One relatively consistent result across all studies (studies 1, 2, 4, 5) was the remarkable size-range of the minor worker caste. We used multiple different markers (head width, head size, Weber's length, and total body length) and methods of analysis (raw data, coefficients of variation, PCA) for the often imprecise character of "body size". In all these measurements, the variability of the minor worker caste always exceeded that of all other castes by far. Even in samples from one single colony – thus excluding different habitats as the source of variation – this held true for the examined species of the COCY group and *Camponotus singularis*.

The adaptive value of this noteworthy finding is still unclear. Given the vast number of tasks minor workers are known to perform in colonies (e.g., Hölldobler & Wilson 1990, Hasegawa 1997), it may be a valid assumption that the observed size polymorphism is correlated to polyethism and differential task allocation within the colony. While studies on other camponotine taxa have found such connections (e.g., Lee 1938, Busher et al. 1985, Espadaler et al. 1990), behavioural data on the taxa treated within this dissertation are either scarce or non-existent. It would therefore be desirable to see future research focussing on fieldwork and experimental setups to explore whether minor workers of different sizes really do perform different tasks within the colonies of the treated species.

### 3.2.2. Evolutionary origin of the major worker caste

The evolutionary origin of specialized major worker or “soldier” castes has been the subject of an ongoing scientific dispute for several decades: Traditional theories assume different developmental pathways for gynes and workers, followed by a second developmental switch to distinguish major and minor workers (Wilson 1953, Ward 1997). Other authors view majors as a caste derived from queens, based on similarities in morphology and larval development (Baroni Urbani 1998, Baroni Urbani & Passera 1996, Hasegawa 1997). Several recent publications propose the evolution of specialized castes as mosaics of pre-existing or ancestral developmental patterns (Molet et al. 2012, 2014, Rajakumar et al. 2012) or consider the influences of genetic and epigenetic mechanisms (see Favreau et al. 2018 for a review), disruptive selection (Planqué et al. 2016), or size-dependent developmental switches (Trible & Kronauer 2017).

The results of the studies included in the present dissertation are of course not sufficient to settle this complex issue. Overall, morphometric analyses of the examined taxa have shown apparent similarities between major workers and gynes – especially in overall size and head shape – but otherwise distinct allometric patterns for each female caste (studies 1, 2, 4, 5). Thus, contrary to the purely size-dependent model recently proposed by Tribble & Kronauer (2017), major worker morphology is much more complex than simply being “scaled down” gynes – in fact, in many of the examined taxa, major workers may even exceed conspecific queens in all measurements of total body size! The idea of mosaic-like caste evolution (sensu Molet et al. 2012, Londe et al. 2015), wherein a mix of developmental programs from gynes and minor workers is present in the major worker caste, therefore seems more plausible for the examined taxa.

However, a definitive answer to this question may not be possible for the entire tribe Camponotini, let alone for the entire family of ants. Taking into account the immense diversity of caste-systems and colony structures (e.g., Heinze 2008), the question of how such specialized castes arose in the course of evolution must surely be answered for each genus – possibly even each species-group – individually. Examples of this complexity are even found within the genus *Colobopsis*: Phragmosis in major workers may have arisen multiple times within the genus and is even differentially expressed within the different clades of the COCY group (Zettel et al., in prep; study 5).

Which developmental, biotic or abiotic factors underlie the different types of major worker morphology observed in the studied taxa may best be examined in experimental settings designed to induce caste-specific phenotypes under controlled conditions. As this was not possible within the framework of the present dissertation, many questions remain unanswered in this regard and should be addressed in future, more empirical studies.

### **3.2.3. Origin and plasticity of mermithogenic phenotypes**

While morphological aberrations caused by mermithid nematodes have been known for centuries (Gould 1747), it is still not clear which exact mechanisms are involved in the development of aberrant parasitogenic phenotypes and the behavioural manipulation ultimately leading to host suicide.

In all previously studied species, host infection takes place when ant larvae are fed a paratenic host (oligochaetes or aquatic insects) containing the infective mermithid larvae (Poinar 1981, Kaiser 1986, Poinar 2012, studies 2, 3). As larval feeding was not directly observed in the cases treated herein, the relevant intermediate hosts are unknown. However, the presence of mermithid infections points to invertebrate prey as a protein-rich source of nutrition for host larvae. This is a novel result for the ants of the COCY group, as their nutrition is poorly studied (see 3.2.4.) and they have long been assumed not to consume animal prey (Davidson et al. 2016, but see Zettel et al. 2018a).

In case of the apparent morphological changes in the host ant, two scenarios have been proposed: i) The presence of the nematode may lead to hormonal disruptions during development (Molet et al. 2012). The hosts are only known to be sexuals (Czösz & Majoros 2008, O’Grady & Breen 2011, studies 2, 3) and the large parasites occupy space in the gaster, thus hampering the development of sexual organs and consequently gyne- and male specific characters. ii) Alternatively, the parasite may simply represent a “competing tissue” (sensu Nijhout & Emlen 1998) during development, leading to reduced nutrient allocation to the organs of the developing ant and thus shifting the proportions of the adult host (Czösz & Majoros 2008, S. Czösz pers. comm). Which explanation best represents the developmental scenario of the examined mermithogynes of *Lasius niger* (study 3) and the COCY group (studies 2, 5) cannot be determined at this point. However, none of them offers a satisfying reason for the observed unusually large body size and drastic elongation of appendages in the mermithized individuals treated in the present dissertation.

The variability of responses to mermithid infection in different host taxa also remains an unexplained phenomenon: While previous publications have reported a wide range of possible host phenotypes in *Myrmica* (Cz  sz & Majoros 2008), the genus *Lasius* was found to exhibit rather mild and consistent aberrations (e.g., slightly shortened, but fully formed wings; see O’Grady & Breen 2011, study 3) and the mermithogynes of the COCY group all presented as micropterous with extremely reduced gyne-like characters (see also Wheeler 1928). Thus, there seems to be a difference in developmental robustness against environmental perturbations. To uncover the details of this phenomenon and other parasite-induced morphological aberrations in the light of an EcoEvoDevo framework (Abouheif et al. 2014, Toth & Rehan 2017) will be the core subject of my planned postdoctoral studies.

Regarding the issue of host suicide, the behaviour of mermithized ants drowning themselves to release the mature nematode (Kaiser 1986, Maeyama et al. 1994) is reminiscent of other parasite-induced behaviours, as they are known from ants infested with liver flukes or entomopathogenic fungi (see Libersat et al. 2018 for a review). These phenomena have been viewed in light of the “extended phenotype” sensu Dawkins (1982), wherein the host’s altered behaviour leads to increased parasite fitness. The mechanisms involved in inciting these behaviours are manifold and have certainly arisen multiple times during evolution (Libersat et al. 2018). Studies on the host-parasite system of crickets and hairworms have revealed parasite-mediated alterations in protein production connected to the host’s response to light (Biron et al. 2006). A similar mechanism of parasite-induced positive phototaxis, which draws the host towards the reflective water surface, is also plausible for the ant-mermithid system, although hitherto unstudied.

As previously mentioned in the context of caste development, further studies aiming at uncovering the mechanisms involved in these host-parasite interactions would benefit from an experimental approach. Epigenetic, genomic and proteomic studies may show parasitic influences on the developing and adult host at a molecular level. In addition, induction of mermithogenic phenotypes in an experimental environment would facilitate studies of host development; however, previous attempts of rearing entomopathogenic mermithids under laboratory conditions were unsuccessful (O’Grady & Breen 2011).

#### **3.2.4. Ecology and evolution of the COCY group**

Although not the main focus of the present dissertation, many of the included publications (studies 2, 4, 5) are tightly linked to the project “Voluntary self-sacrifice in exploding ants: a

mechanism to defend co-evolved microbiomes?” which explores the enigmatic group of Southeast-Asian “exploding ants” in an interdisciplinary manner. Despite nearing the end of the project’s funding period (February 2014 – January 2019), many aspects of these ants’ evolution and life history are still unresolved, due to the unforeseeably complex nature of their taxonomy and ecology.

Within the project, considerable progress has been made in identifying new sources of nutrition (Davidson et al. 2016, Zettel et al. 2018a, studies 2, 4), describing new species within the group (study 4, Zettel et al. in prep) and uncovering the details of their microbiome, cuticular hydrocarbon profile, and mandibular gland chemistry (Hoenigsberger et al. 2018, Druzhinina et al. in prep, Hoenigsberger et al. in prep). However, the diversity and abundance of COCY species makes generalizations across the entire group difficult. Furthermore, the inaccessibility of their arboreal colonies, the strong intraspecific variation of minor workers, and the scarcity of available major workers, gynes, and males make taxonomy quite challenging. Certain aspects, such as the true relationship between COCY ants and their presumably parabiotic “interlopers” (*Camponotus inquilinus* group, see Zettel et al. 2018b), or the details of the evolutionary trajectory that led towards the eponymous, dramatic “exploding” behaviour of minor workers, are still very poorly understood.

The same limitations also apply to the core issues of the present dissertation connected to the project: Characterizing caste morphology and linking the uncovered patterns to function and task allocation becomes even more difficult, if entire castes of certain species are unavailable and / or their behavioural repertoire is largely unknown. Attempts to include assessments of internal anatomy (e.g., microCT imaging of mandibular gland reservoirs) into the studies at hand were also limited by the amount and condition of available specimens. In many cases, these issues have therefore only been addressed tentatively, until more data and material becomes available. While they certainly are exciting subjects to explore the extremes of caste-specific morphological adaptations, the members of COCY group thus remain an enigma, of which we have only just begun to scratch the surface.

### **3.2.5. Adaptive value of integrated modules**

The patterns of phenotypic integration and modularity uncovered within the present dissertation (study 5) may best be viewed as first preliminary data for the tribe Camponotini, representing a baseline for further studies on the subject. The characters chosen for the study represent a reduced set in comparison to other, more taxonomically oriented publications (e.g., studies 1,

2, 4; Zettel & Laciny 2015, Ward et al. 2016, Zettel et al. 2018b). While this was a conscious choice in order to ensure the comparability of all measurements across all taxa, the inclusion of more characters and / or species may well expand or alter our current understanding of integrated character modules in future studies.

As highlighted in section 3.1.2., the studies included in the present dissertation have uncovered several characteristic proportions and morphological modules presumably linked to caste-specific functional ecology and behaviour. However, additional lab- and field-studies are necessary to strengthen the claims and hypotheses proposed herein, which are mainly based on the results of multivariate statistical analyses (PCA, cluster analysis). Especially the lack of data on behavioural ecology and task allocation in many of the examined species (particularly the COCY group) make a functional interpretation of the discovered modular patterns difficult. Detailed studies of the species' caste-specific behavioural repertoires should thus be an integral part of any further studies on the subject. In addition, studying the developmental control genes, which govern the ontogeny of the investigated species, may uncover the basis of their modularity on a molecular level and highlight parallels to similar patterns known from the better-studied vertebrates (Akam 1989, Nemeschkal 1999, Ronco et al. 2008).

I would therefore advocate for an integrative approach combining traditional techniques, such as classical taxonomy, morphometry, and behavioural observations, with more innovative approaches, like modern imaging techniques and developmental genetics. By utilizing such multidisciplinary methods in comparative studies across taxa, we may uncover the evolutionary constraints and developmental mechanisms underlying phenotypic integration and modularity, as well as their functional implications across a wide range of different organisms.

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## Summary

The present dissertation describes caste-specific morphological characters and proportions in the ant genera *Camponotus* and *Colobopsis*. These taxa are known for their tremendous evolutionary success, having brought forth remarkable adaptations, such as phragmosis (closing of nest entrances with plug-shaped heads) or suicidal “exploding”. Their complex caste-systems – ranging from monomorphic to strictly dimorphic workers – also make them ideal subjects to investigate caste-specific phenotypic integration – i.e., the propensity of structures to form strongly correlated “modules” linked through common development and function.

The five papers presented herein mainly focus on multivariate statistical analyses of morphometric data, but support this approach by application of multidisciplinary methods: Implementing assessments of behaviour, ecology, parasitology, DNA-barcoding, microCT imaging, and comparison to an outgroup taxon broadens the scope and facilitates interpretations of the obtained results.

In summary, the included studies show that camponotine castes can be reliably characterized using simple, classical methods of morphometry. Consistently across all studies, minor workers show elongated appendages (antennae, legs, mouthparts) and the highest intraspecific size variation of all castes. Major workers possess large, stout bodies, enlarged heads (phragmotic in *Colobopsis*) and extremely short, thick appendages. Gynes may be characterized by the presence of the flight apparatus (wings and large mesothorax), ocelli, a head shape similar to conspecific major workers, and large eyes. These caste-specific character complexes are partially shown to represent morphological modules linked to common development and function. The investigation of several mermithogynes (gynes infected by pathogenic mermithid nematodes) of the genus *Colobopsis* and the outgroup species *Lasius niger* showed striking morphological alterations: Ant hosts exhibited alterations in colour and overall proportions, elongation of extremities and drastic reduction of gyne-specific characters.

The combined results are mainly interpreted within a theoretical framework: Morphological modularity and phenotypic integration, and ecological evolutionary developmental biology (EcoEvoDevo) are discussed, as well as their relevance for behavioural ecology and division of labour within the colony. The morphological characters and integrated character-sets unique to each caste may be interpreted as adaptations to their specialized functions within the colony: Foraging, brood care, and nest maintenance in minor workers, nest defence in major workers, and dispersal and reproduction in gynes. While these are the first results of this kind obtained

from camponotine ants, similar correlation patterns have previously been reported from a diverse range of organisms, including vertebrates. The analysis of mermithogenic phenotypes gives additional insight into phenotypic plasticity under developmental stress, as well as nutritional sources of the hosts.

The results of this dissertation may be viewed as a first attempt to untangle the complex topic of caste-specific morphology and phenotypic integration in the ant tribe Camponotini. The remaining open questions may be addressed in future studies focussing more strongly on analyses of behaviour, ecology, or developmental biology. Nevertheless, the work presented herein includes several novel findings, which may contribute to our understanding of caste-specific phenotypes, their adaptive value, and potential evolutionary origins.

## Zusammenfassung

Die vorliegende Dissertation beschreibt kastenspezifische morphologische Merkmale und Proportionen innerhalb der Ameisengattungen *Camponotus* und *Colobopsis*. Diese Taxa sind für ihren bemerkenswerten evolutionären Erfolg und ihre spezialisierten Anpassungen bekannt – darunter etwa stöpselförmige Köpfe zum phragmotischen Verschließen der Nesteingänge oder selbstaufopferndes „Explodieren“. Ihre komplexen Kastensysteme, die von monomorphen bis hin zu streng dimorphen Arbeiterinnen reichen, machen sie zu idealen Forschungsobjekten zur Untersuchung kastenspezifischer phänotypischer Integration – d.h., der Neigung morphologischer Strukturen, stark korrelierte, durch Entwicklung und Funktion verbundene „Module“ zu bilden.

Der Schwerpunkt der fünf hier vorgestellten Arbeiten liegt auf der multivariaten statistischen Analyse morphometrischer Daten und wird zusätzlich durch die Anwendung multidisziplinärer Methoden unterstützt: Analysen aus den Bereichen der Verhaltensbiologie, Ökologie und Parasitologie, sowie DNA-Barcoding, microCT und der Vergleich mit einem Außengruppen-Taxon erweitern den thematischen Umfang und erleichtern die Interpretation der Ergebnisse.

Zusammenfassend zeigen die Studien, dass Kasten der Camponotini zuverlässig mit einfachen Methoden der klassischen Morphometrie charakterisierbar sind. In allen Arbeiten weisen Minor-Arbeiterinnen die proportional längsten Extremitäten (Antennen, Beine, Mundwerkzeuge) sowie die stärkste Größenvarianz aller Kasten auf. Major-Arbeiterinnen hingegen haben große, stämmige Körper, vergrößerte Köpfe (phragmotisch bei *Colobopsis*) und sehr kurze, dicke Extremitäten. Gynen können durch das Vorhandensein eines Flugapparats (Flügel, vergrößerter Mesothorax), eine der Major-Arbeiterin ähnliche Kopfform, Ocellen und große Augen charakterisiert werden. Diese kastenspezifischen Merkmalskomplexe entsprechen zumindest teilweise morphologischen Modulen, die von Strukturen mit gemeinsamer Entwicklung und Funktion gebildet werden. Untersuchungen an Mermithogynen (mit parasitischen Nematoden der Familie Mermithidae infizierte Gynen) der Gattung *Colobopsis* und der Außengruppe *Lasius niger* zeigen auffällige morphologische Veränderungen: Wirtstiere weisen Abweichungen in Farbe und Proportionen sowie verlängerte Extremitäten und reduzierte gynenspezifische Merkmale auf.

Die zusammengefassten Ergebnisse werden vorrangig im Rahmen theoretisch-biologischer Konzepte interpretiert: Morphologische Modularität, phänotypische Integration und ökologisch-evolutionäre Entwicklungsbiologie (EcoEvoDevo) werden ebenso diskutiert wie

ihre Relevanz für Verhaltensökologie und Arbeitsteilung innerhalb der Kolonie. Kastenspezifische morphologischen Merkmale und Merkmalskomplexe können großteils als Anpassungen an die jeweiligen Funktionen und Aufgaben innerhalb der Kolonie interpretiert werden: Nahrungssuche, Nest- und Brutpflege bei Minor-Arbeiterinnen, Verteidigung bei Major-Arbeiterinnen, und Verbreitung und Fortpflanzung bei Gynen. Diese ersten derartigen Ergebnisse für die Tribus Camponotini weisen Parallelen zu bereits publizierten Korrelationsmustern anderer Organismen, wie etwa Wirbeltieren, auf. Die Analyse von Mermithogynen ermöglicht zudem neue Erkenntnisse im Bereich der phänotypischen Plastizität unter Stress während der Entwicklung und gibt Hinweise auf die Nahrungsquellen der Wirte.

Die Ergebnisse dieser Dissertation stellen einen ersten Versuch dar, die komplexe Thematik kastenspezifischer Morphologie und phänotypischer Integration innerhalb der Camponotini zu ergründen. Einige offen verbliebene Fragen können möglicherweise in zukünftigen Studien mit stärkerem Schwerpunkt auf Verhalten, Ökologie oder Entwicklungsbiologie aussagekräftiger bearbeitet werden. Dennoch liefern die hier vorgestellten Arbeiten zahlreiche bisher unbekannte Ergebnisse, welche einen wertvollen Beitrag zum besseren Verständnis kastenspezifischer Phänotypen, ihres adaptiven Werts und ihrer möglichen evolutionären Ursprünge leisten können.