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

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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., HÖLLDOBLER & 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) (BUSHER & 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
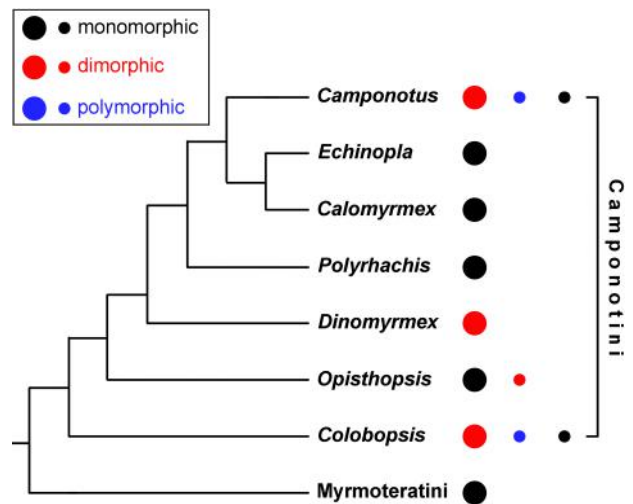

Fig. 1: Worker caste diversity in Camponotini applied to a tree by BLAIMER & al. (2015) based on 959 ultraconserved element loci. Large symbols indicate the dominant situation in the genus, small symbols deviations.

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, CSŐSZ 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, CSŐSZ 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; CSŐSZ 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") (CSŐSZ & 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 (CSŐSZ & 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
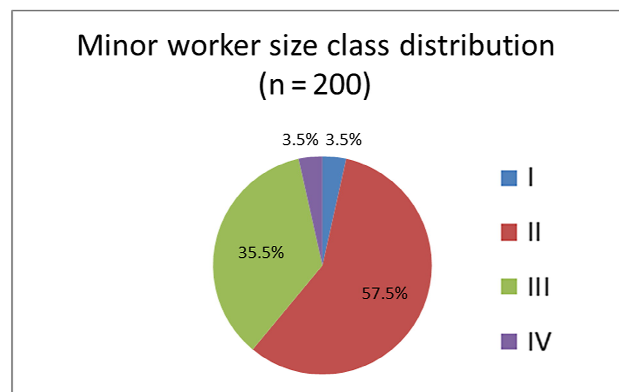

Fig. 3: Distribution of head width classes in a sample of 200 minor workers of *Colobopsis* sp. nrSA. Specimens were sorted into four size classes by head width, each group ranging 0.125 mm. Class-ranges (mm): I (< 1.375), II (1.375 - < 1.50), III (1.50 - < 1.625), IV (≥ 1.625).

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 × 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	CCACARATTTCWGAACATTG	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 glue-boards, individually numbered, and used to take measurements at magnifications from 25.6 × up to 256 ×, 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 × 100
EI   Eye Index. EL / HW × 100
EL   Eye length. Maximum diameter of compound eye, measured in lateral view.
FeI   Femur Index. FeL / HW × 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 gynes).
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 meso-scutum, 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 × 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 × 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 × 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 × 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 μm.

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 µl 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 $MgCl_2$ (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 HLPC 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 × 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 dorsolaterally, in smallest workers almost reaching outline of head. Ocelli lacking, in some larger specimens their posi-
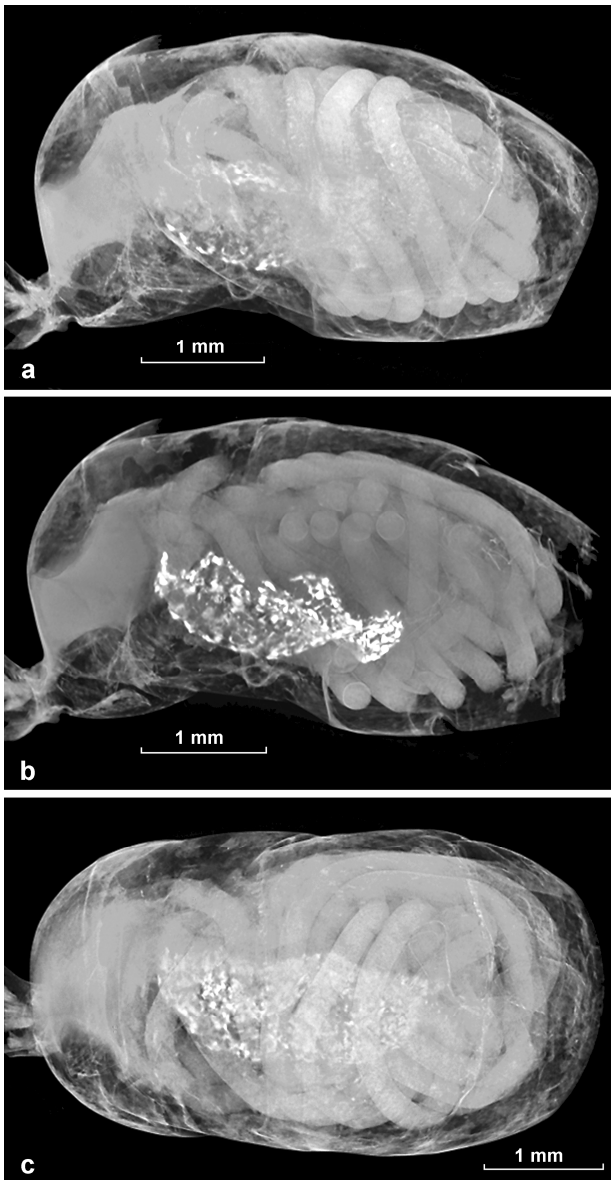
Fig. 4: X-ray microtomographic images illustrating the gaster of the mermithogyne #1137 in (a) lateral, (b) sagittal cutaway, and (c) dorsal aspect.

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 / µl] and 260 / 280 ratio of extracted DNA.

Preservation before extraction	TUCIM No. (Sample ID)	ng / µl	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.
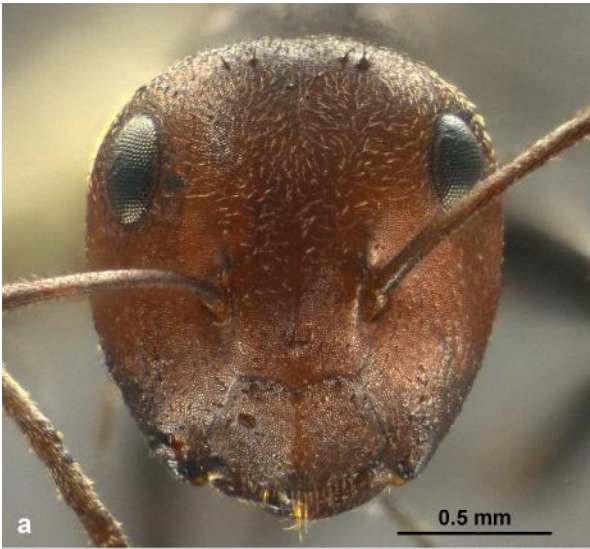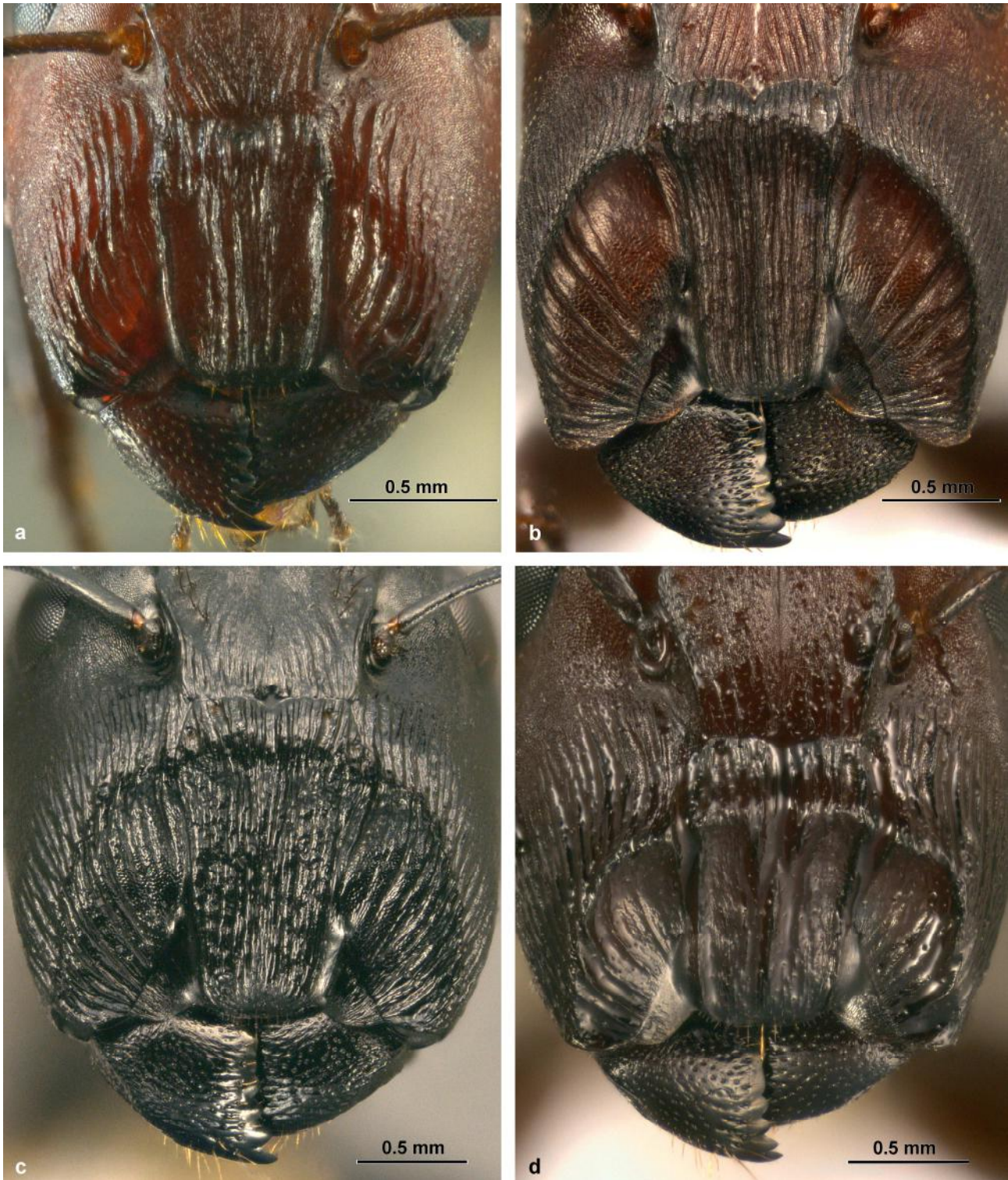
Fig. 6: Head shields, perpendicular view, in specimens of *Colobopsis* sp. nrSA. (a) Intercaste specimen; (b) phragmotic major worker; (c) mermithogyne; (d) alate gyne.

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 striation 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.
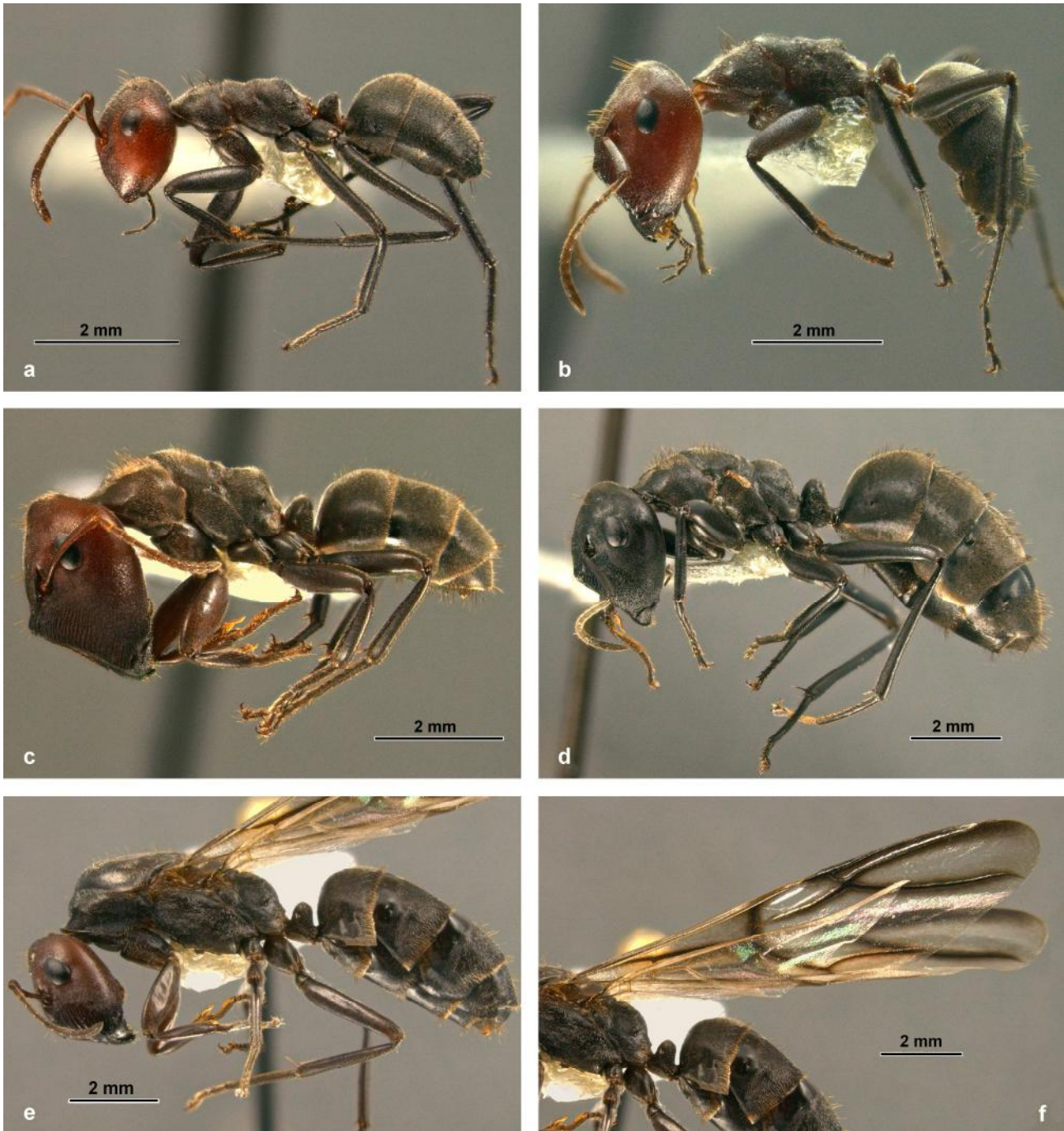
Colour: as in minor worker.

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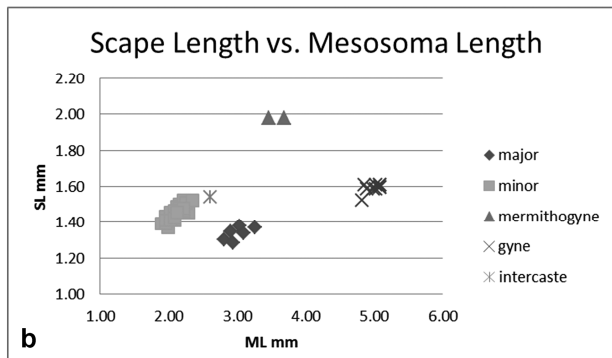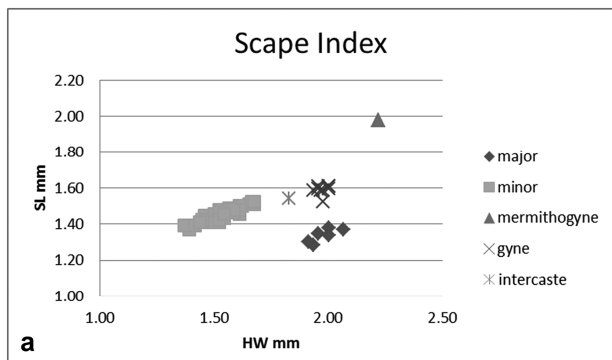
Fig. 8: Variation of scape length in female castes of *Colobopsis* sp. nrSA. (a) In relation to head width; (b) in relation to mesosoma length.
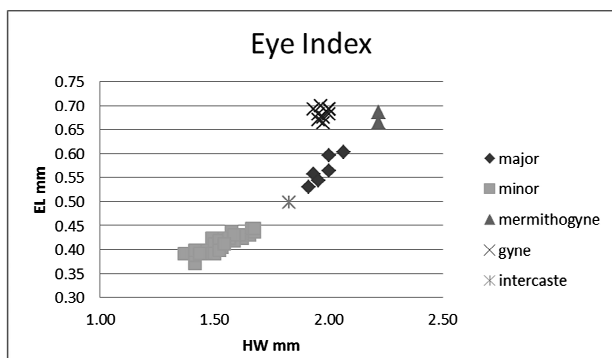

Fig. 9: Variation of eye length in female castes of *Colobopsis* sp. nrSA in relation to head width.

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,
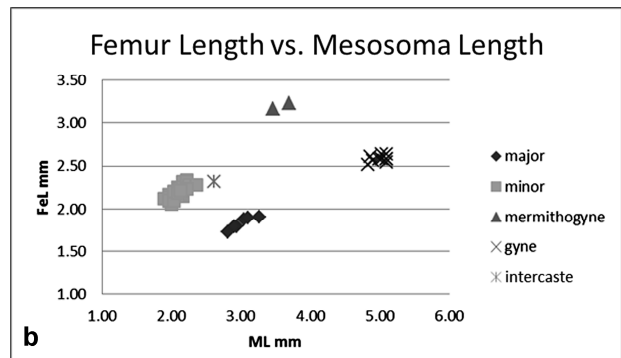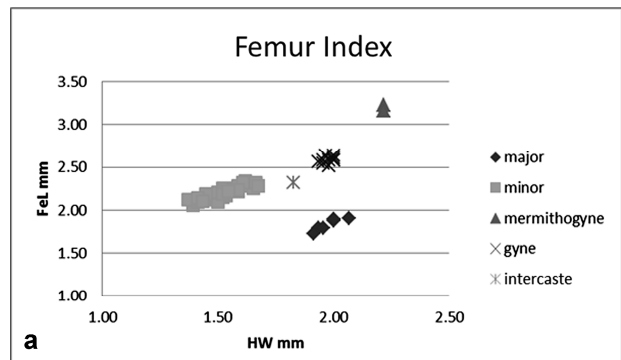

Fig. 10: Variation of metafemur length in female castes of *Colobopsis* sp. nrSA. (a) In relation to head width; (b) in relation to mesosoma length.

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 × 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.
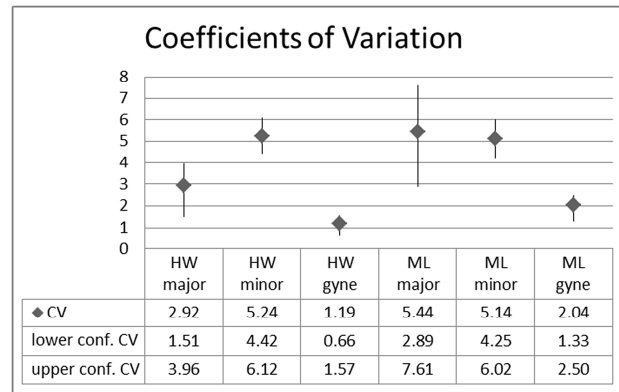

Fig. 11: Coefficients of variation and their lower and upper limits for 95% confidence intervals, for two main measures of size (HW, ML) in majors, minors, and gynes of *Colobopsis* sp. nrSA.

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 & LINSENMAIR 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. Laciny & 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 & LINSENMAIR 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 (CSŐSZ & 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.

Acknowledgements

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