









# Uncovering cryptic diversity in the enigmatic ant genus *Overbeckia* and insights into the phylogeny of Camponotini (Hymenoptera : Formicidae : Formicinae)

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## ABSTRACT

Many tropical insect species remain formally undescribed, and the validity of some rarely collected and poorly studied taxa is uncertain. *Overbeckia* Viehmeyer, 1916 is a monotypic ant genus and a rare member of the arboreal ant communities of tropical South East Asia and Australasia. *Overbeckia subclavata* Viehmeyer, 1916 was collected and described from Singapore more than a century ago and there have been few other records of these ants since. Here we compare the existing *Overbeckia* records with recent collections in Australia, New Guinea and Indonesia. We revise the taxonomic diagnosis of the genus *Overbeckia* in comparison with other genera of the diverse tribe Camponotini, redescribe *O. subclavata* and describe two new species, *Overbeckia papuana* sp. nov., and *Overbeckia jambiensis*, sp. nov. We also summarise all available ecological and distributional information of the genus, indicating that *Overbeckia* occurs in a variety of lowland vegetation types, from rainforests to secondary growth and plantations but is extremely rare. Using external morphological characters, internal proventricular structures and a molecular phylogeny of 78 Camponotini species based on five protein-coding loci, we demonstrate that *Overbeckia* is a monophyletic lineage. Molecular-clock calibrated trees using 21 fossil records indicate that the divergence between *Overbeckia* and its sister clade comprising the genera *Echinopla* Smith, 1857 and *Calomyrmex* Emery, 1895 occurred c. 21 Ma (95% highest posterior density 14–28), and that the divergence between the three *Overbeckia* species likely occurred in the late Miocene and Pliocene. In addition, we transfer one *Camponotus* Mayr, 1861 species to *Colobopsis* in the light of molecular evidence: *Colobopsis triangulata* (Klimes & McArthur, 2014) comb. nov. Our analysis represents the most comprehensive phylogeny of the tribe Camponotini in terms of coverage of extant divergent lineages, including *Overbeckia*, and suggests relatively robust phylogenetic relationships among genera that are consistent with published phylogenomic trees. Overall, our study reveals cryptic species diversity in *Overbeckia* and provides strong evidence that this is a valid and polytypic ant genus.

**Keywords:** ants, arboreal insects, canopy, evolution, integrative taxonomy, multi-locus phylogeny, novel species, tropical rain forests.

## Introduction

Ants (Formicidae) are ecologically important insects that often function as ecosystem engineers and are ubiquitous in all terrestrial biomes except the Earth's poles (Hölldobler and Wilson 1990; Lach *et al.* 2010). They form a diverse insect family with nearly 14 000 valid species, the highest number among eusocial insects (B. Bolton, AntCat, see <https://antcat.org>, accessed 9 September 2021). The endemism and morphological diversity of ants is highest in tropical rainforests, especially in areas threatened by habitat change (Guenard *et al.* 2012). Among the 17 extant ant subfamilies, Formicinae is the second most diverse (3201 species) and a great deal of this species diversity is harboured within the tribe Camponotini (1913 species, 14% of all ant species) (AntCat, see <https://antcat.org>).

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The tribe Camponotini originated after the time of angiosperm plant diversification, c. 70–40 Ma (Moreau *et al.* 2006; Magallón and Castillo 2009; Blaimer *et al.* 2015), most likely in the early Eocene in the Indomalayan region (Blaimer *et al.* 2015). Many Formicinae genera and species are associated with plants, either by foraging or nesting arboreally, often feeding on honeydew from sap-feeding trophobionts and nectar (Blüthgen *et al.* 2003; Blüthgen *et al.* 2004; Davidson *et al.* 2004). Although some Camponotini species feed omnivorously (Feldhaar *et al.* 2007; Feldhaar *et al.* 2010), most are ‘cryptic herbivores’ (Hunt 2003) and common members of the lower levels of food webs (Blüthgen *et al.* 2003; Davidson *et al.* 2003; Pfeiffer *et al.* 2014). In the tree canopy, most Camponotini species exhibit a high degree of worker polymorphism or dimorphism (genera *Colobopsis* Mayr, 1861 and *Camponotus* Mayr, 1861) (Hölldobler and Wilson 1990; Klimes and McArthur 2014) and a high morphological trait diversity (pubescence, colour, spines, head shape etc.), even among closely related species or castes (Mezger and Moreau 2016; Ward *et al.* 2016).

The morphological features of internal structures (organs) are also diverse and hold important diagnostic characters but are rarely considered in ant taxonomy and systematics (but see e.g. Booher *et al.* 2021). For example, the structure of the stomach pump (proventriculus) appears to be unique in workers and varies in the length of sepals among ant genera. In particular, much longer sepals than the bulb associated with the crop are known in workers of the genus *Camponotus* (Eisner and Wilson 1952). Although the proventricular anatomy has been suggested to be sufficiently variable to provide diagnostic characters at the genus level, this has only been examined in four of the eight extant Camponotini genera (Viehmeyer 1916; Eisner 1957).

Owing to the high morphological and ecological diversity, and relatively low nest density and difficulty of sampling, many arboreal Camponotini species in tropical rainforests are poorly studied and many species remain undescribed (Klimes and McArthur 2014; Laciny *et al.* 2018; Hartke *et al.* 2019). Eight genera are currently recognised in the Camponotini: *Camponotus* is the most diverse genus (1052 spp.), followed by *Polyrhachis* Smith, 1857 (706 spp.), *Colobopsis* (95 spp.) and *Echinopla* Smith, 1857 (36 spp.) (AntCat, see <https://antcat.org>). However, the tribe also contains the much less diversified genera *Opisthopsis* Dalla Torre, 1893 (13 spp.), *Calomyrmex* Emery, 1895 (9 spp.), and two monotypic genera, *Dinomyrmex* Ashmead, 1905 and *Overbeckia* Viehmeyer, 1916 (Ward *et al.* 2016; AntCat, see <https://antcat.org>). The biology of *Dinomyrmex* is well known and the taxonomy well established (Pfeiffer and Linsenmair 2007; Ward *et al.* 2016). By contrast, little is known about the distribution and biology of *Overbeckia* that some authors consider to be a junior synonym of *Camponotus*, although this opinion has not yet been formalised (Bolton 2003; Ward *et al.* 2016). The systematics of Camponotini has been clarified thanks to a phylogenomic analysis at the genus level based on 959 loci-based

ultraconserved elements (UCE) (Blaimer *et al.* 2015). For example, *Colobopsis* and *Dinomyrmex*, former subgenera of *Camponotus*, were confirmed to be monophyletic and divergent lineages within the tribe. However, the status and phylogenetic placement of *Overbeckia* within Camponotini remain uncertain, despite previous efforts focused on the molecular phylogenetics of ants, including the tribe Camponotini (Moreau and Bell 2013; Blaimer *et al.* 2015; Clouse *et al.* 2015). Recently, a close relationship between *Overbeckia* and *Echinopla* was suggested by Kreider *et al.* (2021), though this was based on the analysis of only one *Overbeckia* specimen and two short gene fragments, the nuclear 28S rRNA and the mitochondrial cytochrome c oxidase subunit I (COI).

The only known species of the enigmatic genus *Overbeckia*, *O. subclavata* Viehmeyer, 1916 was described more than 1 century ago (Viehmeyer 1916). In that work, the genus was named after Hans Friedrich Overbeck, who collected the only known nest series of the species in Singapore in 1913–1914 (Taylor 2014). Until recently, this type series (syntypes) of *O. subclavata* was the only *Overbeckia* material available, although this includes several workers, a queen and several males, all distributed across multiple museums (see the Materials section in the present study, and Viehmeyer 1916). In addition, a damaged specimen of *O. subclavata* was collected in the early 20th century from the Philippines but was only rediscovered and identified a decade ago (General and Alpert 2012). Finally, *O. subclavata* was recently discovered to also have been collected in Queensland, Australia, in 2002 by an amateur collector (Heterick 2019). These findings led to speculation that *Overbeckia* was introduced to tropical North Australia from Singapore (Heterick 2019). However, without further records and detailed analyses of morphological variation and phylogenetic relationships, the true species diversity and geographic distribution of *Overbeckia* remain unclear. Furthermore, *Overbeckia* is thought to be arboreal based on the original collection of the type series from tree stems (Viehmeyer 1916) but further information on the biology and ecological characteristics of the genus is lacking due to the scarcity in museum collections and samples from the field.

In this study, we revised all available records of *Overbeckia* and compared these with recently collected specimens from rainforest tree canopy in Papua New Guinea and Sumatra (Indonesia). The new records from Papua New Guinea and Indonesia were part of large-scale research projects that focused primarily on the ecology of arboreal ant communities and resulted in nearly complete species censuses in the plots of different forest types (Klimes and McArthur 2014; Klimes *et al.* 2015; Nazarreta *et al.* 2020; Kreider *et al.* 2021). Based on the integrative approach using geographical, ecological, morphological and molecular evidence, we redescribed the genus *Overbeckia* and the species *O. subclavata*, and we describe two new species. We also compared proventricular structures among Camponotini genera (Eisner and Wilson 1952; Eisner 1957) that allowed us to re-evaluate the

uniqueness of *Overbeckia* within the tribe as suggested by Viehmeyer (1916). Finally, we built a time-calibrated phylogeny of Camponotini using published and novel sequences from 78 representative species of all eight genera of Camponotini, maximising coverage of divergent lineages.

## Materials and methods

### Taxonomic and morphological study

#### Sample collection and material

Material of *Overbeckia* from Papua New Guinea was collected from a single nest found on a tree in a 0.3-ha plot of a secondary lowland forest (~10-year-old growth in a former garden; see further details in the Taxonomy section) (Klimes *et al.* 2015). Note the species described here as *O. papuana* sp. nov. (morphospecies code CAMP021) has been incorrectly identified in previous studies by A. McArthur as *Camponotus* sp. 021 aff. *janeti* Forel, 1895 (McArthur 2012; Klimes and McArthur 2014; Klimes *et al.* 2015).

Indonesian material of *Overbeckia* including that proposed as *Overbeckia jambiensis* sp. nov. was collected during a canopy fogging campaign using knockdown insecticide as part of the framework of the German-Indonesian EFForTS research project, conducted inside and around the Bukit Duabelas National Park and Harapan Rainforest in Jambi Province, Sumatra (exact locations below) (Drescher *et al.* 2016). Thirteen individuals of *O. subclavata* were sampled during a canopy fogging campaign in the dry season of 2013 (Nazarreta *et al.* 2020; referred to as *Overbeckia* sp. 01 but later re-identified as *O. subclavata*). Six additional individuals of *Overbeckia* were collected during the rainy season in 2013–2014 from the same sites, referred to as *Overbeckia* sp. 01 in Kreider *et al.* (2021) but re-identified in this study as *Overbeckia subclavata* (four specimens) and *O. jambiensis* sp. nov. (two specimens). Using the species abundance data published in Nazarreta *et al.* (2020) and partially in Kreider *et al.* (2021), we compared the occurrence of *Overbeckia* in four land-use systems sampled as part of the EFForTS project: lowland rainforest, jungle rubber and monocultures of rubber and oil palm. The same sampling effort per habitat and per season allowed for a meaningful quantitative comparison of species occurrence; see further details on sampling in Nazarreta *et al.* (2020). Given the scarcity of *Overbeckia* samples to date, the EFForTS canopy ant collection provides a first glimpse into the habitat requirements of *Overbeckia*, although the replication is too small to allow for statistical comparison.

In addition, we examined all previously collected samples of *Overbeckia* (Viehmeyer 1916; General and Alpert 2012; Heterick 2019) using the high-resolution photos available in the AntWeb database (AntWeb, ver. 8.64.2, California Academy of Science, see <https://www.antweb.org>, accessed 10 August 2021) and the photos from McArthur (2012). For sequencing, we used additional species of Camponotini

sampled in Klimes *et al.* (2015), and openly available DNA information of additional species (see Supplementary Table S1, S2 for more details on specimens examined).

#### Acronyms of depositories

- CASC, California Academy of Sciences, San Francisco, CA, USA.
- DEIC, Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany.
- IECA, Czech Academy of Sciences, Institute of Entomology, Ceske Budejovice, Czech Republic.
- MCZC, Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA.
- NAIC, National Agricultural Insect Collection, National Agricultural Research Institute, Port Moresby, Papua New Guinea.
- NHMB, Naturhistorisches Museum Basel, Basel, Switzerland.
- MSNG, Natural History Museum, Genoa, Italy.
- MZB, Museum Zoologicum Bogoriense, Lembaga Ilmu Pengetahuan Indonesia (LIPI), Bogor, Indonesia.
- SAMA, South Australian Museum, Adelaide, South Australia, Australia.
- WAM, Western Australian Museum, Perth, Western Australia, Australia.
- ZMHB, Museum für Naturkunde Berlin, Germany.

#### Measurements and indices

In the case of previously collected material, we examined the high-resolution images available from AntWeb (type and paratype series of *Overbeckia subclavata* collected by H. Overbeck, and additional three specimens from Heterick 2019; see Materials examined for specimen codes and localities). In the case of new material from Indonesia, Australia and Papua New Guinea, we used an Olympus SZX7 stereo-microscope up to 105× magnification for the specimen examination. Furthermore, detailed photographs were taken of the new specimens with a Leica DFC450 camera fitted with Leica Z16 APO microscope and the pictures were stacked in Helicon Focus (ver. 5.3x64, Helicon Soft Limited, Kharkiv, Ukraine, see [www.heliconsoft.com](http://www.heliconsoft.com)). We also used a digital microscope Dino-Lite AD7013MT to take pictures from different angles using Dinocapture 2.0 (ver. 1.5.31, see <https://www.dino-lite.eu>) that allowed us to measure the morphological parameters used for the descriptions. Whenever possible (i.e. when structures were parallel to the camera focus, avoiding parallax error), we measured the same set of morphological parameters from the AntWeb images, using ImageJ (ver. 1.38x, W. S. Rasband, US National Institutes of Health, Bethesda, MD, USA, see <https://imagej.nih.gov/ij/>, accessed 29 August 2017). We measured the holotype worker, and if available up to five additional workers from each paratype series (nest), or locality (sampling event) to assess intraspecific variability. Alates were also examined and measured for each species when available (one male or queen per species). Measurements of

all morphological traits presented hereafter are in mm and rounded to two decimal places (see Supplementary Table S1 for the specimen list and complete data).

Parameters used:

- HL, head length: maximum distance measured in full face between midpoint of anterior clypeus margin to vertex, with both in the same horizontal plane
- HW, maximum head width including the eyes in full face (frontal) view
- IOD, interocular distance: maximum distance between eyes in frontal view
- CL, maximum clypeus length measured in frontal view between midpoint of anterior margin to posterior clypeus margin, with both in the same horizontal plane
- CW, maximum clypeus width measured in frontal view
- ML, maximum mandible length measured from mandibular insertion to apex of mandible
- EL, eye length measured along the maximum longitudinal diameter with head in lateral view
- EW, eye width perpendicular to EL
- FCDp, posterior frontal carinae distance: distance measured between most posterior points of carinae with sharp-edged curvature
- FCDA, anterior frontal carinae distance: distance measured at the most anterior points of carinae near clypeus
- SL, scape length from anterior to posterior margin of the scape and excluding the condylar bulb
- SWmax, maximum scape width
- SWmin, minimum scape width
- WL, Weber's length: length of mesosoma from lateral view, from angle at which pronotum meets cervix to posterior basal angle of metapleuron
- PW, maximum width of pronotum in dorsal view
- HFL, length of hind femur
- HFTmax, maximum width of hind femur
- HTL, length of hind tibia
- PetW, maximum petiolar width measured in dorsal view
- PetL, maximum petiolar length excluding posterior joint to abdomen in dorsal view

Indices based on the parameters:

- Frontal carinae distance index:

$$\text{FCDI} = \text{FCDp} \div \text{FCDA} \times 100$$

- Cephalic index:

$$\text{CI} = \text{HW} \div \text{HL} \times 100$$

- Scape index:

$$\text{SI} = \text{SL} \div \text{HW} \times 100$$

- Mandible index:

$$\text{MI} = \text{ML} \div \text{HL} \times 100$$

## Proventriculus structure

The internal structure of the proventriculus in the digestive system of ants and the shape (i.e. the stomach pump) have been proposed to be important characters for the diagnosis of *Overbeckia* (Viehmeyer 1916) and for several other ant genera and tribes, including other Camponotini (Eisner 1957; Davidson et al. 2004). We dissected the proventriculus of *Overbeckia* and three of the four Camponotini genera for which no information on proventriculus structures was available (Eisner 1957), i.e. *Echinopla*, *Calomyrmex*, *Colobopsis* (with the exception of *Dinomyrmex*, for which we lacked a specimen for dissection). A single worker of each of the four genera, preserved in ethanol, was transferred to 10% KOH, boiled for ~30 min and transferred to 50% ethanol for dissection. The proventriculus was carefully removed from an aperture cut between the first and second abdominal tergite from the gaster with sharp forceps following Eisner (1957). Photographic documentation of the structure was performed using the same procedure as for the dry ant specimens, but directly in the solution while the structure was fixed between two fine microscope slides. No stain was used. The following species and samples were dissected: *Overbeckia papuana* sp. nov. (from the paratype nest series, see details below), *Echinopla* aff. *australis* Emery, 1897 (leg. Klimes P., nest sample acc. n. HP00735), *Calomyrmex laevisissimus* (Smith, 1859) (leg. Klimes P., nest sample acc. n. HP0725) and *Colobopsis quadriceps* (Smith, 1859) (leg. Klimes P., nest sample acc. n. HP0074). All material used was collected from the same site in Papua New Guinea as the type specimens of *O. papuana* sp. nov. (see below and Klimes et al. 2015 for details).

## Phylogenetic analysis

### Molecular dataset

We obtained new molecular data from four *Overbeckia* individuals and from an additional nine specimens from Camponotini genera collected in Klimes et al. (2015) (Supplementary Table S3). Given the scarcity of *Overbeckia* specimens in research collections, we opted for a non-destructive protocol to isolate DNA for all species except *O. papuana*, for which many individuals were collected from a single colony. We used individuals stored in ethanol (BF2.2, BF3.1, HP0177) or already pinned specimens (BJ6.2), and the QIAGEN DNeasy kit following manufacturer's recommendations, except that we kept the specimens overnight in tissue lysis buffer at 56°C. All specimens were remounted for later morphological measurements. We sequenced five protein-coding loci: the mitochondrial *COI* (659 bp) and nuclear genes arginine kinase (*argK*, 832 bp, across two exons and one intron), *EF-1αF1* (359 bp), long-wavelength rhodopsin (*LWRh*, 583 bp, spanning two exons and one intron) and wingless (*WGL*, 421 bp). We followed the laboratory protocols and PCR primers described in Brady et al. (2006), Clouse et al. (2015) and Ward and Downie (2005). The Sanger sequencing reactions were outsourced to



Macrogen (Amsterdam, Netherlands). DNA sequences were manually curated and the alignments were prepared using the software Geneious Prime (ver. 2021.0.3, see <https://www.geneious.com/prime/>). In addition, we included in the phylogenetic analyses the DNA sequences of another *O. subclavata* obtained by target sequence capture, kindly provided by P. S. Ward (CASENT0872838, unpublished molecular data). All newly generated DNA sequences from 11 species and 13 specimens (including *Overbeckia*) are deposited in GenBank (see Data availability and Supplementary Table S3).

To cover all Camponotini genera, we added the sequences of 65 species from previous studies (Blaimer *et al.* 2015; Clouse *et al.* 2015; Clouse *et al.* 2016; Mezger and Moreau 2016; Wang *et al.* 2018a) and unpublished resources available in GenBank at <https://www.ncbi.nlm.nih.gov/genbank/> (accessed 22 November 2020) (Supplementary Table S3) to our molecular alignments. We limited our selection to the species that were sequenced for at least three of the five study loci and that represented the main Camponotini lineages, by subgenera and geographic regions (typically two species per lineage and region from the previous studies above). To increase the genetic coverage of the selected Camponotini species, we merged the *COI* sequences and nuclear alignments from different specimens of the same species in a few cases (8 species; Supplementary Table S3), after verifying topological congruence in preliminary single-gene phylogenies. In total, we used 82 specimens representing 78 species from the tribe Camponotini for our phylogenetic analysis (Supplementary Table S3).

### Phylogenetic inference

We inferred the phylogeny of Camponotini using the maximum-likelihood software IQ-TREE (ver. 2.0.6, <https://github.com/iqtree/iqtree2>; Minh *et al.* 2020). We allowed the program to estimate the best partitioning and substitution models (in ModelFinder, part of IQ-TREE) from 17 initial blocks comprising the three codon positions of five protein-coding loci and two intronic regions. To estimate branch supports, we carried out 1000 ultrafast bootstraps followed by NNI optimisations and 1000 repetitions of the Shimodaira–Hasegawa-like approximate likelihood ratio test (SH-aLRT; Guindon *et al.* 2010) followed by an approximate Bayes test (Anisimova *et al.* 2011).

### Divergence time estimation

The fossil record of Camponotini is rich (Perrichot 2020). A curated subset of the record has been compiled by Economo *et al.* (2018) and includes 1 taxon classified as *Polyrhachis* and 20 taxa as *Camponotus*. Rather than condensing these data into only two minimum calibration points (one for crown *Polyrhachis* and one for stem *Camponotus sensu lato*; see Ward *et al.* 2016), we preferred to use the curated 21 fossil records (Supplementary Table S4) to infer divergence times using the ‘fossilised birth–death’ (FBD) model (Heath *et al.* 2014).

We concatenated the molecular dataset with one specimen per species and estimated the best-fit partitioning strategy using PartitionFinder (ver. 2.1.1, see <https://github.com/brettc/partitionfinder>; Lanfear *et al.* 2017). We used 17 initial blocks (3 codon positions for 5 genes and 2 intronic regions), the greedy search strategy, and one linked underlying set of branch lengths. The best substitution models available in BEAST (ver. 2.6.3, see <https://github.com/CompEvol/beast2>; Bouckaert *et al.* 2014) were selected based on the BIC criterion. Similarly, we estimated the best-fit clock-partitioning scheme using ClockstaR (ver. 2, see <https://github.com/sebastianduchene/ClockstaR>; Duchêne *et al.* 2014). The analysis was run in R (ver. 4.0.0, R Foundation for Statistical Computing, see <http://www.R-project.org>). We used the partitioned dataset as suggested by PartitionFinder (ver. 2.1.1) as input for estimating patterns of among-lineage clock rate variation.

We used the *sampled-ancestors* (ver. 2.0.2, see <https://github.com/CompEvol/sampled-ancestors>) package (Gavryushkina *et al.* 2014) to incorporate the FBD tree prior into the environment of BEAST (ver. 2.6.3). We constrained the monophyly of extant and extinct sampled taxa of Camponotini and *Polyrhachis*, i.e. both crown nodes, and allowed the program to freely estimate tree topologies and divergence times within both groups. In addition, we constrained the divergence time of the most recent common ancestor of extant Camponotini lineages to a normal distribution with Mean 51 and Sigma 5 (Blaimer *et al.* 2015), i.e. the ‘CONSTRAINED’ divergence time estimations (Table 1). Note that such a constraint did not force the monophyly of extant Camponotini, therefore sampled lineages not included in the constrained group might have been inferred within the group. We also ran separate analyses without such a secondary calibration constraint (the ‘UNCONSTRAINED’ estimations). As suggested by Barido-Sottani *et al.* (2019), we took into account fossil age uncertainty by sampling from Camponotini fossils’ time intervals available at the Paleobiology Database at <https://paleobiodb.org/> (accessed 1 November 2020). We added the molecular data of *Gigantiops destructor* as an outgroup taxon and constrained the divergence from Camponotini to a normal distribution with Mean 83 and Sigma 10 (Blaimer *et al.* 2015).

The prior distributions were set as default, except for the mean rate under the uncorrelated log-normal relaxed clock (Gamma distribution,  $\alpha = 0.001$ ,  $\beta = 1.0$ ) and the FBD process conditioned on the root, because all Camponotini fossil taxa are descendants of the most recent common ancestor of extant samples in the phylogeny (i.e. Camponotini + *Gigantiops destructor*). We set the chain length to 210 million, sampling log and trees every 30 000 cycles, and ran the analysis five independent times. We applied a 10% burn-in, thinned the sampled parameters to one-third, and merged the five posterior distributions using LogCombiner (ver. 2.6.3, part of the BEAST package). Convergence and mixing of chains were checked in Tracer (ver. 1.7.1, see <https://github.com/beast-dev/tracer>; Rambaut *et al.* 2018).

**Table 1.** Estimated crown group divergence ages of the tribe Camponotini and the seven non-monotypic genera.

Genus	Posterior probability	Crown group divergence (Ma): CONSTRAINED	Crown group divergence (Ma): UNCONSTRAINED
Camponotini	1	56.9 [48.5–65.4]	79.3 [59.2–99.9]
<i>Calomyrmex</i>	1	13.2 [7.7–19.6]	18.8 [9.9–29.4]
<i>Camponotus</i>	0.99	33.2 [25.6–41.6]	46.8 [32.4–62.3]
<i>Colobopsis</i>	1	38.6 [28.6–48.4]	53.3 [36.2–71.2]
<i>Dinomyrmex</i>	–	–	–
<i>Echinopla</i>	0.91	15.4 [10.1–21.9]	21.9 [12.3–32.9]
<i>Opisthopsis</i>	1	13.3 [7.0–21.0]	18.9 [8.9–32.1]
<i>Overbeckia</i>	1	8.1 [3.7–13.9]	11.8 [5.1–21.3]
<i>Polyrhachis</i>	1	23.2 [16.9–29.9]	32.8 [21.5–45.7]

We compared two calibration strategies, CONSTRAINED and UNCONSTRAINED (see the methods for details), using the fossilised birth–death model and 21 vetted fossil records (Supplementary Table S4). The shown divergence ages represent the Median and the 95% highest posterior density (in brackets). Complete trees with divergence ages for all lineages are given in Fig. 9 and Supplementary Fig. S2.

A maximum clade credibility (MCC) tree based on a Bayesian interference, with node heights summarised with median values, was estimated using TreeAnnotator (ver. 2.6.3, part of the BEAST package).

Tree topology tests

Despite the general congruence in the inferred phylogenetic relationships between the maximum-likelihood method implemented in IQ-TREE and Bayesian inference in BEAST, two nodes had disparate support: (i) the divergences of *Opisthopsis* and *Dinomyrmex* within Camponotini, and (ii) the relationships among the three *Overbeckia* species (see Results). Therefore, we performed likelihood-based tree topology tests to evaluate six models, wherein *Opisthopsis* or *Dinomyrmex* splits earlier in the evolution of Camponotini, and the sister-species relationships change among three *Overbeckia* species: *O. papuana*, *O. subclavata* and *O. jambiensis* (Supplementary Table S5). We inferred phylogenies with the nodes under constrained investigation using IQ-TREE and the settings as described above. We used the bootstrap proportions by resampling the estimated likelihoods of sites (bp-RELL) (Kishino et al. 1990), the expected likelihood weights (ELW) (Strimmer and Rambaut 2002) and the approximately unbiased test (AU) (Shimodaira 2002) 100 000 times.

Taxonomy

Genus *Overbeckia* Viehmeyer

(Fig. 1–6)

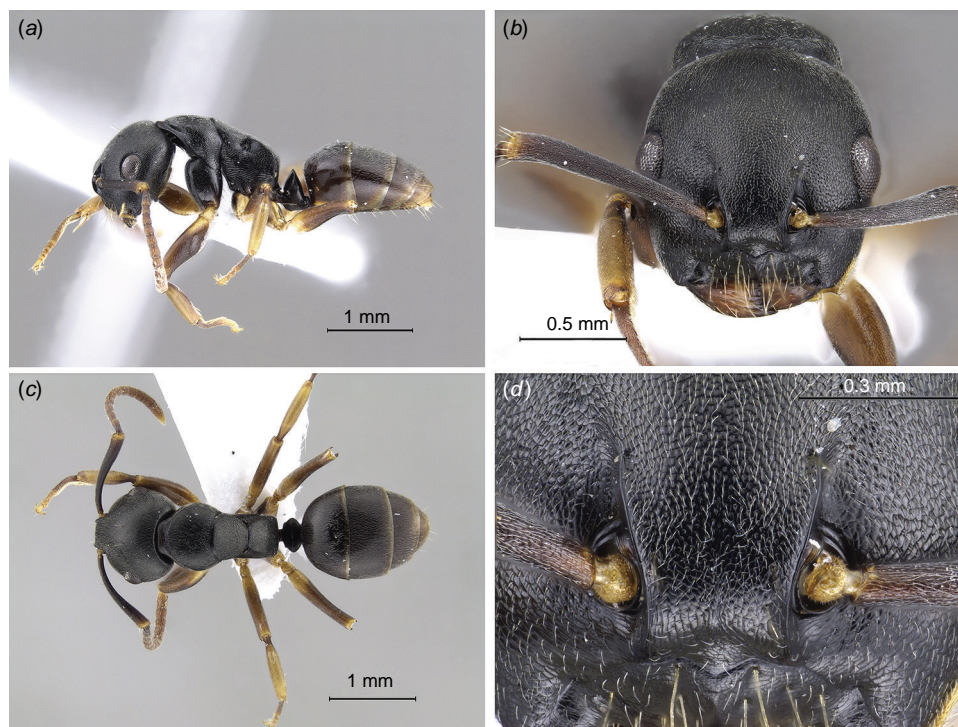
The diagnosis based on workers (for males and queens, see descriptions).

External morphology

*Body length.* 4–5 mm (min. to max. WL = 1.24–2.02, WL mean = 1.68 mm). No dimorphism or apparent polymorphism in workers.

*Head.* 12-segmented antennae including scape, without distinct club but with funicular segments continuously enlarged towards the tip, with distal flagellomere approximately twice as wide as the basal flagellomere (Fig. 2h); scape curved and widened apically (Fig. 1b, 2h). Antennal socket relatively closer to the posterior margin of clypeus than in other Camponotini. Palp formula 6: 4, with apical segment of maxillary palp 15–20% shorter than the subapical segment (Fig. 5a). Head in frontal view oval, with HL similar to HW (min. to max. CI = 94–105, CI mean = 101) (Fig. 1b). Clypeus slightly convex anteriorly and posterior clypeal margin incised in the middle by a developed suture, forming the frontal triangle; but the posterior suture of the triangle below carinae not developed, rather only outlined by bent cuticle (Fig. 1d). Frontal carinae well developed, going from clypeal margin up to the middle of the head (Fig. 1d) and forming a convex lifted ridge between each carina and the antennal socket apparent at lateral view (Fig. 1a); vertical groove in the middle of frons between carinae missing (Fig. 1d) or only outlined by a glossy smooth line (Fig. 5d). Mandibles triangular with five teeth that decrease in size from apical to basal teeth. Eyes relatively large (min. to max. EL = 0.25–0.40 mm, mean EL = 0.32) and placed vertically in the middle of the head, surpassing the head margins in frontal view, with ~20 ommatidia in the longest axis (Fig. 1b). Workers without ocelli.

*Mesosoma.* Mesosoma shape resembles that of the genus *Calomyrmex*, with a well developed promesonotal suture. Metanotal groove limited at most of the length to the dorsum of thorax and ending laterally next to the metathoracic



**Fig. 1.** *Overbeckia subclavata*, non-type. Worker from Indonesia (specimen HJ.3.1) displaying the lateral (a), frontal (b) and dorsal (c) views. Detail of head sculpture and shape of frontal carinae (d). Note hind leg tibia and tarsi are missing (damaged).

spiracle, with a depression and smooth area around the spiracle (Fig. 1a). The depression next to metathoracic spiracle region is also well visible in dorsal view, with propodeum laterally constricted compared to relatively broad pronotum (Fig. 1c).

**Metasoma.** Petiole squamiform and triangular, with sharp apex in lateral view and of relatively broad lentil-shape in dorsal view (Fig. 1c); a characteristic subpetiolar process present and divided from petiole node by a ridge (Fig. 1a, c). Petiolar and propodeal spiracle with light-coloured margins. Metapleural gland vestigial, probably not functional according to Heterick (2019).

**Sculpture.** On whole body, cuticle relatively smooth and glossy, with only fine punctation or soft lineation. No spines on thorax and petiole, and no deep wrinkles and pits present.

**Pilosity.** Rather short, appressed pilosity; a few longer erect hairs present however at least at the scape apex, on ventral head, abdomen and clypeus (hair length  $\leq$  EL); in contrast to *Calomyrmex* lacking a dense cover of long, erect hairs over thorax and legs.

**Colouration.** Head, mesosoma and petiole dark brown to blackish colour, abdomen and appendages rather lighter (brown to light brown) and with yellowish coxae and trochanters; except frontal coxa that are darker, similar in colour to thorax (Fig. 1a, 2g, 4a, 5a).

### Internal morphology: proventriculus in *Overbeckia* v. other Camponotini genera

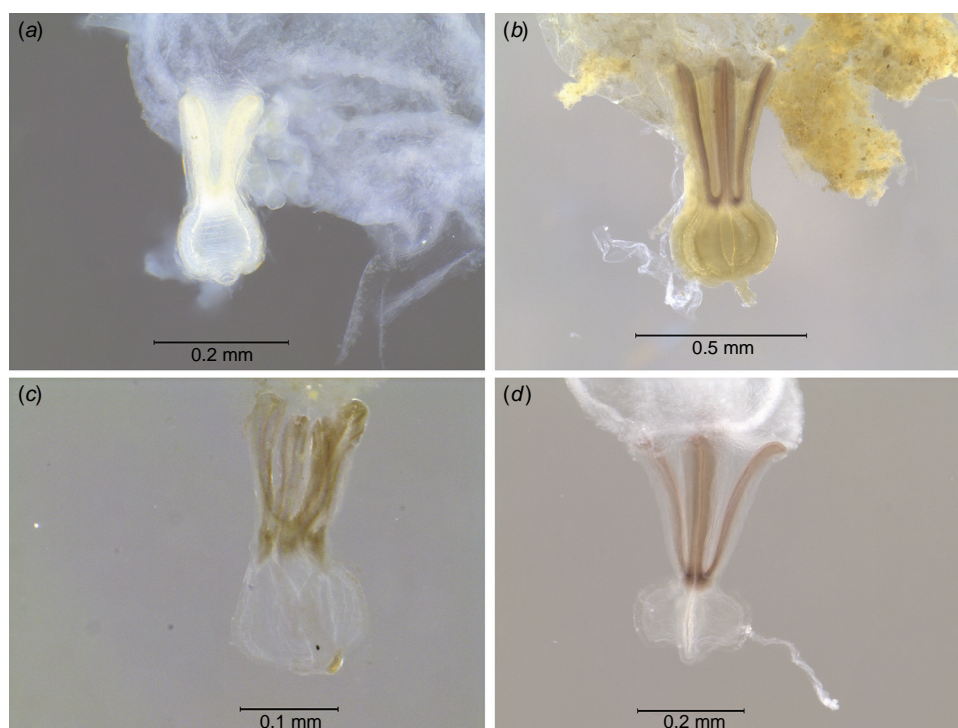
Viehmeyer (1916) provided a drawing of the proventriculus in the original genus description, in which rather short sepals were described, being slightly bent anteriorly and of a similar length to the vertical length of the valve (i.e. ‘the stomach pump’), resembling in this respect the genus *Paraparatrechina*. These short sepals have been noted as a main feature to distinguish *Overbeckia* from other SE Asian Formicinae genera (Bolton 1994). Our dissection conforms to the original drawing of *Overbeckia*’s proventriculus by Viehmeyer (1916), showing a similar proportion of sepals compared to the valve. Furthermore, we observed that the valve has relatively thicker walls of whitish colour in *Overbeckia*, when compared to the other examined genera (Fig. 3a). Our dissections of *Echinopla* also show sepals not much longer than the valve (Fig. 3c), resembling the proventriculus of *Overbeckia*, but with softer and thinner walls (brittle structure). In contrast to *Overbeckia* and *Echinopla*, *Calomyrmex* has sepals approximately twice as long as the valve, similar to *Opisthopsis* (i.e. ‘Formica’ type in Eisner 1957; Fig. 3b). *Colobopsis* resembles *Camponotus* in the long sepals, more than  $2.5\times$  longer than the valve (i.e. ‘Camponotus’ type that also occurs in *Polyrhachis* (Eisner 1957; Fig. 3d).





**Fig. 2.** *Overbeckia subclavata* syntypes. Alate queen (FOCOL2566; photographer: Ch. Klingenberg): lateral (a), dorsal (c) and frontal (e) views; male (CASENT0905180; photographer: W. Ericson): lateral (b), dorsal (d) and frontal (f) views; worker (CASENT0101191; photographer A. Nobile): lateral (g) and frontal (h) views. Photos retrieved from [AntWeb.org](http://AntWeb.org).





**Fig. 3.** Proventriculus of the ant genera *Overbeckia* (a), *Calomyrmex* (b), *Echinopla* (c) and *Colobopsis* (d). The darker flanges are sepals that are attached anteriorly to the tissues of the ant gut, whereas the convex light structure represents proventricular bulb that connects posteriorly to the stomodeal valve of the worker midgut. Note the posterior connection is broken here due to the removal of the structure from the gut of ant worker. The patterns reflect natural colouring of the structures.

### *Overbeckia subclavata* Viehmeier

(Fig. 1, 2)

#### Etymology

**Viehmeier (1916)** does not explain the reasoning for the species name, but this likely refers to the club-shaped anterior part of antennae, mentioned in the original description ('Geißel gegen Ende verdickt': funiculus enlarged towards the tip) (Fig. 2h).

#### Material examined

**Syntypes.** All examined from photos (AntWeb codes): CASENT0101191, CASENT0101192, workers (NHMB; F. Santschi collection); focol0130, worker (DEIC, marked as 'Syntypus'); focol2566, alate queen (marked as 'Typus'), focol2567 and focol2568, workers, focol2564 and focol2565, males (ZMHB, all marked as 'Typus'); CASENT0178502, worker (MCZC, marked as 'Paratype 30741'); CASENT0905180, male (MSGN)/coll. H. Overbeck, <1915/Singapore/Nests in dry/narrow, hollow bamboo, colony in the hollow branch of a mango tree/1°17'35.0"N, 103°51'21.0"E/det. Viehmeier (1916).

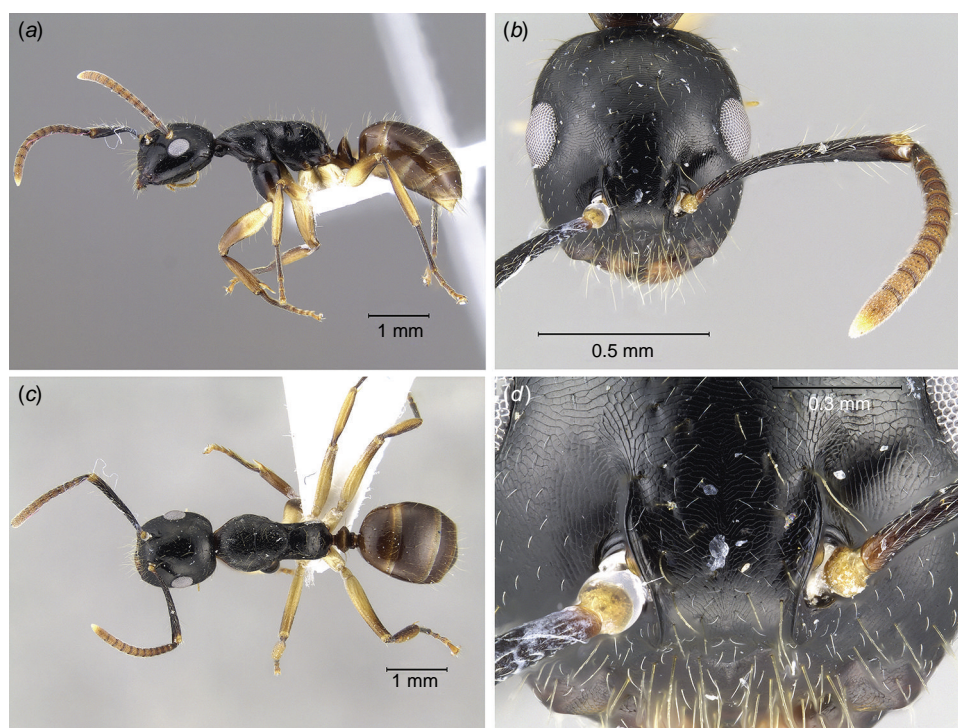
#### Additional material examined

**Indonesia:** 5 workers and 1 queen; all leg. J. Drescher, canopy fogging (det. R. Nazarreta & P. Klimes): Worker (ZMHB): HJ3.1/5.xi.

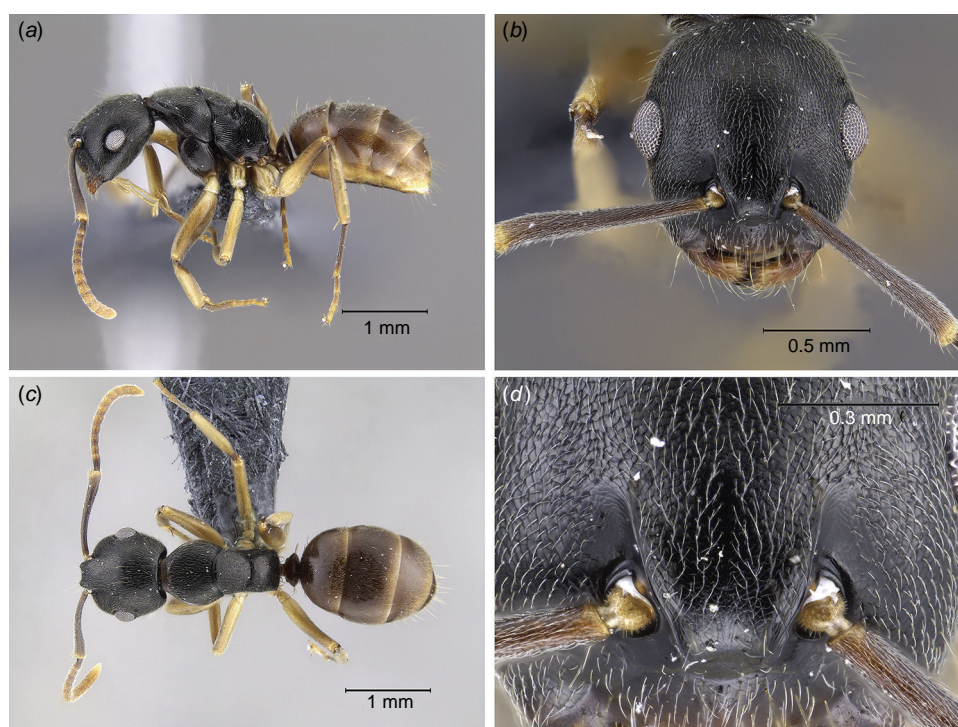
2013/Harapan Rainforest/jungle rubber/dry season/01°50'57.7"S, 103°17'59.7"E. Worker (MZB): BJ6.2/1.xii.2013/Bukit Duabelas National Park/jungle rubber/rainy season/02°01'48.4"S, 102°46'14.9"E. Worker (IECA): BF3.1/5.x.2013/Bukit Duabelas National Park/jungle rubber/rainy season/01°56'34.1"S, 102°34'51.5"E. Worker (MZB): BF1.1/8.x.2013/Bukit Duabelas National Park/forest/dry season/01°59'41.4"S, 102°45'08.5"E. Worker (CASC): CASENT0872838 (AntWeb)/HymFrm285 1.xii.2013/Bukit Duabelas National Park/jungle rubber/rainy season/02°00'56.2"S, 102°45'11.5"E. Queen without wings (IECA): HF4.1/12.ix.2013/Harapan Rainforest/forest/dry season/02°11'15.4"S, 103°20'34.7"E. **Philippines** (photos): Acc. No. 3576 (MCZC)/Lot Bu.of Sci., P.I./coll. M. Ramos (further details in General and Alpert 2012).

#### Diagnosis

Compared to the other two *Overbeckia* species described here, *Overbeckia papuana* sp. nov. and *O. jambiensis* sp. nov., workers and queens in *O. subclavata* are less hairy, with a lack of long erect hairs on dorsum of petiole, on thorax and on scape except the apical margin; head integument with fine dense punctation; no erect hairs extending past head margins in frontal view; frontal carinae approaching one another less anteriorly compared to those in *O. papuana* sp. nov. (FCDI < 166; Fig. 1b, 2h). Longer erect hairs present only on clypeus and ventral abdomen. Fine straight lineation on lateral sides of thorax limited to the basal region below metanotal groove (Fig. 1a).



**Fig. 4.** *Overbeckia jambiensis* sp. nov. holotype (worker) and lateral (a), frontal (b) and dorsal (c) views. Detail of head sculpture and shape of frontal carinae (d). Note that the right antenna has been glued into the antennal socket.



**Fig. 5.** *Overbeckia papuana* sp. nov. holotype (worker) and its lateral (a), frontal (b) and dorsal (c) views. Detail of head sculpture and shape of frontal carinae (d).





**Fig. 6.** *Overbeckia papuana* sp. nov. paratypes (alates). Queen: lateral (a), dorsal (c) and frontal (e) views; male: lateral (b), dorsal (d) and frontal (f) views. Note that the petiole in queen (a) looks wider and blunter due to being bent to the right site (c).

### Description of worker

**Morphometrics.** Non type 'HJ.3.1' (six syntypes and further five non-types workers, N.A. = measure not available hereafter; total measured  $N = 11$ , min.–max): HL 1.08 (0.87–1.25), HW 1.14 (0.88–1.24), IOD 0.87 (0.67–0.92),

CL 0.16 (0.15–0.19), CW 0.49 (0.34–0.54), ML 0.45 (0.33–0.47), EL 0.31 (0.25–0.36), EW 0.22 (0.16–0.26), FCDp 0.38 (0.29–0.44), FCDa 0.28 (0.21–0.29), SL 0.99 (0.70–1.08), SWmax 0.13 (0.11–0.17), SWmin 0.06 (0.05–0.09), WL 1.62 (1.24–1.76), PW 0.79 (0.61–0.88),

HFL 1.03 (0.92–1.08), HFT 0.24 (0.20–0.25), HTL N.A. (0.82–1.07), PetW 0.40 (0.32–0.47), PetL 0.22 (0.15–0.24), FCDI 134 (126–165), CI 105 (98–105), SI 87 (80–93), MI 41 (32–42).

**Head.** General morphology as for the genus (see above). Frontal carinae approaching one another less anteriorly (FCDI = 134) and with relatively straight curvature, slightly concave near antennal sockets (Fig. 1d).

**Mesosoma and metasoma.** As for the genus (see above).

**Pilosity.** Numerous tiny appressed hairs (approximately twice the length of 1 ommatidium or shorter) over scape, head and most of the thorax and abdomen; relatively long erect hairs (length  $\geq$  SW) present only on clypeus, head and ventral abdomen and one pair of hairs present on frons in the middle above the eyes (Fig. 1a); these longer hairs missing on genae and the occipital corners of the head including region above the eyes (in frontal view none of the longer hairs surpass the head margin); scape also without erect hairs except a tuft of  $\sim 8$  setae growing from the apical tip (Fig. 1b). No long erect hairs at the apex of petiole.

**Sculpture.** Fine punctation on dorsal surface of the head, in particular in the area between frontal carinae and above the clypeus (Fig. 1b, d) and on mesosoma dorsum and lateral sites of thorax above metathoracic spiracle and dorsolaterally on propodeum (Fig. 1a); this punctuation changes to the sculpturing rather forming a cell structure than individual pits posteriorly towards the occipital region; soft lineation on lateral sites of mesosoma, below the metathoracic spiracle and on propodeum between propodeal spiracle and hind coxae; same cuticular lineation also present on frontal coxae, petiole and anteriorly on the head near clypeus, but here much softer than on thorax (Fig. 1b, c).

**Colouration.** Black head, thorax and petiole; dark brown scape except at the apex where light brown; brown abdomen and femurs; tarsi, funiculus and palps light brown to yellow; femurs brown in the middle but lighter near the joints (Fig. 1a, c).

## Description of queen

**Morphometrics.** Syntype 'focol2566': HL 1.43, HW 1.45, IOD 1.08, CL 0.30, CW 0.60, ML 0.67, EL 0.53, EW 0.35, FCDp 0.47, FCDa 0.33, SL 1.17, SWmax 0.16, SWmin 0.08, WL 2.56, PW 1.14, HFL 1.08, HFT 0.36, HTL 1.34, PetW 0.58, PetL 0.28, FCDI 142, CI 101, SI 81, MI 47.

**Head (Fig. 2e).** Eyes almost twice as large as in worker. Frontal carinae approaching each other anteriorly above the posterior margin of clypeus relatively less when compared to queen of *O. papuana* (FCDI < 160). Mandibles with four teeth; apical and subapical teeth approximately twice as long as and sharper than the other three teeth. Three ocelli well developed.

**Mesosoma (Fig. 2a, c).** In lateral view, thorax dorsum flat at mesothorax, otherwise evenly rounded with convex prothoracic and propodeal angle. Overall morphology of

thoracic segments and wings as in *Camponotus* queens, except apical part of the forewing being relatively broader with evenly convex margins laterally towards the tip.

**Metasoma.** Petiole similar to worker but relatively thicker and with less sharp apex. Abdomen as in workers but larger.

**Pilosity.** Numerous tiny appressed ( $\geq 1/2$  length of ocellus) hairs over scape; erect relatively long hairs (length  $> 1$  ocellus) present only on clypeus, ventral head, near ocelli, on mesothorax dorsum and on 3rd to 5th abdominal tergites (Fig. 2a); these longer hairs missing on genae and the occipital corners of the head including region above the eyes (in frontal view no longer hairs surpass the head margins); scape also lacks erect hairs except five to six setae at the apical tip (Fig. 2e). No long erect hairs on apex of petiole.

**Sculpture.** Fine punctation on the dorsal surface of the head, and on mesosoma dorsum and propodeum; soft lineation on lateral sites of mesosoma, similar to workers.

**Colouration.** Brown head, thorax, scape (except the tip that is lighter) and petiole; light brown abdomen; legs and funiculus yellow (Fig. 2a, c).

## Notes

The generally lighter colouring may also reflect either the old age of the museum specimen ( $> 100$  years) or a relatively young queen collected from inside nest (alate).

## Description of male

**Morphometrics.** Syntype 'CASENT0905180': HL 0.90, HW 1.08, IOD 0.69, CL 0.17, CW 0.40, ML 0.34, EL 0.46, EW 0.32, FCDp 0.32, FCDa 0.14, SL 0.80, SWmax 0.09, SWmin 0.07, WL 1.95, PW 1.08, HFL 1.31, HFT 0.18, HTL 1.30, PetW N.A., PetL N.A., FCDI 229, CI 120, SI 74, MI 38.

**Head (Fig. 2f).** Eyes largely surpass the head margins in frontal view, with EL  $\sim 1/2$  of HL, placed at  $\sim 1/2$  of HL posteriorly. Frontal carinae approaching relatively less one another anteriorly above the posterior margin of clypeus compared to those in male of *O. papuana* (FCDI = 229), although this variance is not as distinct as among workers and among queens of the two species. Mandibles blunt without teeth. Three ocelli well developed and relatively larger than in queens (diameter as SWmax). Antennae with 13 segments not broadened towards the tip of funiculus like in the workers and queen (see frontal image of another male from the type series 'focol2564' with intact antennae) (AntWeb, see <https://www.antweb.org>). Scape long, at half of the length passing the head margin in frontal view.

**Mesosoma.** Thorax dorsum flat at mesoscutum, otherwise evenly rounded in lateral view with convex prothoracic and propodeal angle, except the mesoscutellar disc that is placed higher and of more convex shape than the other thoracic parts in lateral view (Fig. 2b). Suture between



pronotum and mesoscutum not visible in dorsal view due to highly convex and enlarged mesoscutum compared to pronotum (Fig. 2d).

**Metasoma.** Petiole triangular but relatively thicker and blunter at the apex.

**Pilosity.** Tiny appressed hairs present on scape and thorax but less numerous over the head compared to queen and workers; long erect hairs (length = SWmax) present only on clypeus, genae, ventral head, near ocelli (one long seta et each) and on 3rd to 5th abdominal tergites (Fig. 2b); these longer hairs missing completely on scape, petiole and at the occipital corners of the head including region above the eyes (in frontal view, no long hairs surpass the head margin).

**Sculpture.** Fine punctation on dorsal surface of the head, and on mesosoma dorsum and propodeum.

**Colouration.** Light brown to yellowish body, except brown head, thorax dorsum and antennae. Mandibles and appendages pale.

## Remarks

The specimens of *O. subclavata* from Indonesia that were examined have the posterior parts of the frontal carinae bent slightly inwards and therefore have slightly smaller FCDI (mean 133) than the type specimens from Singapore (mean 155) (Fig. 1d, 2h). However, the difference is small and this could possibly be due to measurement error from photographs. All other morphological parameters and the general appearance of the specimens are consistent between the two regions, therefore we regard this to be only a variation within the species.

The specimen from the Philippines has a damaged left upper side of the head and a whitish dirt attached to the carinae. Therefore, we did not consider this for morphological measurements. However, absence of the longer hairs on the scape and the thorax, and the finely punctate head with numerous minute recumbent hairs, clearly fit the general appearance of *O. subclavata*.

Viehmeyer (1916) reported that *O. subclavata* nested in a hollow bamboo stem and in a hollow branch of a mango tree, therefore there is uncertainty whether the syntype series of the species (six workers, three males and one queen, available from AntWeb) belongs to a single nest. Five vials (samples?) collected in 1913 and two collected in 1914 were reported, therefore several colonies from one or more sites in Singapore might have been pooled for the syntype series (most likely two colonies from two stems, a mango and a bamboo). There is also uncertainty as to whether all specimens collected in 1913–1914 are documented online at AntWeb, therefore more material might be scattered in museums worldwide. Nevertheless, all specimens collected by H. Overbeck illustrated in the AntWeb match *O. subclavata*. The arboricolous nesting in hollow plant structures of (probably) dead tissues suggests that the species nests in tree canopies, but is rather a generalist;

that is, not using living tissues or myrmecophytic plants, as do more specialised arboreal species (Klimes and McArthur 2014). Specimens of *O. subclavata* were probably collected by H. Overbeck in a human-managed area but collections from Sumatra suggest that the species utilises a variety of habitats (see Distribution and Discussion for further information).

## *Overbeckia jambiensis* Klimes, sp. nov.

(Fig. 4)

## Etymology

Named after the Jambi Province on Sumatra Island (Indonesia) where the type specimen was collected by J. Drescher in the lowland rainforest of Bukit Duabelas National Park (EFForTS project core plot BF2, see Drescher *et al.* 2016).

## Material examined

**Holotype:** Worker (MZB): BF2.2/7.x.2013/wet season/canopy fogging/lowland forest/Bukit Duabelas National Park/01°58'54.2"S, 102°45'02.3"E/leg. J. Drescher (det. R. Nazarreta, P. Klimes)

**Paratype:** Worker (ZMHB): same data as above but marked as paratype.

## Diagnosis

*Overbeckia jambiensis* workers are the hairiest of the *Overbeckia* species, with numerous conspicuous erect hairs present on dorsum of petiole, thorax, and also dorsally and ventrally on the head, abdomen and scape; head integument without punctation and with fine lineation; cuticle covered with sparse, short, appressed hairs that are less numerous but longer than in other species and present all over the head except in the area between the eyes and around carinae; integument darker and glossy; frontal carinae less approaching one another anteriorly compared to those of *O. subclavata* but curvature evenly convex when compared to those of the other species in frontal view (Fig. 4d). Lineation present on whole lateral sides of mesosoma, including upper part of frontal coxae and the areas above metathoracic spiracle.

## Description of worker

**Morphometrics.** Holotype 'BF2.2.' (one paratype): HL 1.37 (1.29), HW 1.29 (1.30), IOD 0.94 (0.96), CL 0.24 (0.22), CW 0.53 (0.54), ML 0.55 (0.55), EL 0.40 (0.40), EW 0.30 (0.31), FCDp 0.42 (0.43), FCDa 0.31 (0.31), SL 1.30 (1.27), SWmax 0.16 (0.16), SWmin 0.08 (0.08), WL 1.94 (1.94), PW 0.96 (0.97), HFL 1.30 (N.A.), HFT 0.29 (N.A.), HTL 1.24 (N.A.), PetW 0.45 (0.44), PetL 0.24 (0.22), FCDI 137 (141), CI 94 (101), SI 101 (98), MI 40 (42).

**Head.** General morphology as for the genus (see above). Frontal carinae less approaching one another anteriorly (FCDI = 137) and with convex curvature laterally (Fig. 4d).

*Mesosoma and metasoma.* As for the genus (see above).

*Pilosity.* Tiny appressed hairs (approximately twice the length of ommatidium) present only sparsely over the head and mesosoma; numerous relatively long erect hairs (length  $\geq$  SW) present on the clypeus and head, and all over abdomen; these longer hairs also present on genae and occipital corners of the head and on frons, surpassing the head margin in frontal view (Fig. 4b); scape with relatively long hairs of both appressed and erect type, the erect hairs not limited to the apical tip but present along most of the scape (Fig. 4b). Approximately eight erect hairs growing dorsally from apical margin of petiole.

*Sculpture.* Fine shallow lines, joining each other to a cell formation over the glossy cuticle of the head (Fig. 4b, d). These lines also present dorsolaterally on mesosoma; the lineation very soft and rather curved, except the lateral sites of mesosoma below metathoracic spiracle and towards coxae and on propodeum, where lines are somewhat deeper and straight (Fig. 4a); soft lineation also present on frontal coxae and petiole.

*Colouration.* Head, thorax and scape of rather glossy metallic appearance and black, except at scape apex where lighter brown; petiole, abdomen, apical parts of femurs and tibiae brown; tarsi, apex of funiculus and palps brown; femurs pale yellow near the joints; trochanters and mid and hind coxae pale yellow (Fig. 4a, c).

## Description of male and queen

None (no material)

## Remarks

Body size is somewhat larger on average than in the other two *Overbeckia* species but due to the availability of only two specimens, assessing whether body size is significantly larger is difficult (the size is comparable to the largest workers measured from series of *O. papuana*).

## *Overbeckia papuana* Klimes, sp. nov.

(Fig. 5, 6)

## Etymology

Dedicated to all Papua New Guineans, the nation of the country from which the type and nest series were collected (Papua New Guinea).

## Material examined

*Holotype.* Worker (ZMHB): Morphospecies 'CAMP021'/Acc.N. 'HP0177'/Tree number code 'WS4D0868'/Secondary forest/Wanang village/Madang province/Papua New Guinea/8.iii.2007/05°14'S, 145°11'E/leg. M. Rimandai.

*Paratypes.* Five workers (1w ZMHB, 1w IECA, 2w NAIC, 1w MZB), one male (ZMHB), one queen without wings (ZMHB): From the same nest series as holotype (the same data).

*Additional material examined.* **Papua New Guinea:** Other 30 workers and 2 males, including brood, kept in one vial with ethanol in IECA, all from the same nest as the type specimens (same data). **Australia** (photos): 1 worker (SAMA): 'Camponotus'/Cairns NQ/on tree/Parkland/2.viii.1975/leg. B.B. Lowery (frontal and lateral photo on p.115 in McArthur (2012) and A. McArthur, pers. comm. for coll. information; previously misidentified by the original author for *Camponotus janeti*). 3 workers (WAM): CASENT0872736, CASENT0872753, CASENT0872754 (AntWeb codes; 'Overbeckia wam01'): Queensland/Mossman/18.ix.2002/leg. R. Jordan/nest in dead twig in living tree behind sugar mill (thicket near Mossman R.)/det. B.E. Heterick, ii.2018. 1 worker (CASC): CASENT0887749 (AntWeb code; 'Overbeckia au01'): Queensland/Mt Peters/1.v.2014/leg. M. Collis/det. A. Andersen 30.xi.2020. **Australia** (physical specimen): 1 worker (ZMHB): n#15720/AUST Qld/Captain Billy Landing/rainforest, ex. dead hanging twig/11°38'S, 142°51'E/leg. P. Ward (the same collection series as CASENT0882197 at AntWeb; 'Overbeckia au01').

## Diagnosis

General appearance of worker and queen as in *O. subclavata*, but hairier on the head, with erect hairs on dorsum of petiole, thorax and abdomen; a few hairs also present on distal part of scape. Head integument less punctate than in *O. subclavata*, with many short-erect hairs extending past head margins in frontal view; tiny appressed hairs in high densities all over the head including genae; frontal carinae significantly approaching one another anteriorly (FCDI > 190) (Fig. 5d).

## Description of worker

*Morphometrics.* Holotype, 'HP0177' nest series (five paratypes; total  $N = 6$ , min.–max): HL 1.08 (1.04–1.41), HW 1.10 (1.05–1.43), IOD 0.82 (0.79–1.10), CL 0.17 (0.14–0.23), CW 0.41 (0.39–0.55), ML 0.44 (0.36–0.57), EL 0.28 (0.26–0.34), EW 0.21 (0.19–0.27), FCDp 0.37 (0.35–0.52), FCDa 0.18 (0.15–0.27), SL 1.05 (1.02–1.24), SWmax 0.14 (0.12–0.18), SWmin 0.06 (0.06–0.09), WL 1.58 (1.52–2.02), PW 0.77 (0.69–1.03), HFL 1.09 (0.92–1.24), HFT 0.24 (0.24–0.30), HTL 0.94 (0.82–1.05), PetW 0.39 (0.37–0.47), PetL 0.17 (0.17–0.24), FCDI 208 (193–236), CI 102 (99–105), SI 95 (87–101), MI 40 (30–43).

*Head.* General morphology as for the genus (see above). Frontal carinae relatively more approaching one another anteriorly above the clypeal margin compared to those of the other species (FCDI = 208), with rather straight carinae curvature all along (only slightly convex laterally adjacent to the antennal sockets at frontal view; Fig. 5d).

*Mesosoma and metasoma.* As for the genus (see above).

*Pilosity.* Tiny appressed hairs (approximately twice the length of ommatidium or smaller) present in high densities over the head, mesosoma and abdomen; relatively long erect hairs (length  $\geq$  SWmin) present on clypeus, head integument and all over abdomen; these longer hairs are also present on genae and occipital corners of the head and on

frons (extending past head margins from the frontal view; Fig. 5b); scape with relatively short, appressed hairs (as in *O. subclavata*), with a few erect hairs not limited to the apex but present ventrally along the anterior half of the scape. These hairs shorter and less numerous than in *O. jambiensis* (Fig. 5b). Approximately six erect hairs growing dorsally from apical margin of petiole.

**Sculpture.** Less dotted, rather covered by short lines joining each other to a cell formation with fine punctation over the glossy cuticle of the head (Fig. 5d) and mesosoma dorsolaterally, with most of these cells like pits at smaller ( $<80\times$ ) magnification (Fig. 5b, c). Lateral sites of thorax with soft lineation below mesothoracic spiracle towards mid and hind coxa. The lineation is also present on frontal coxa and petiole, but smoother.

**Colouration.** Head, mesosoma and frontal coxa black; scape dark brown except at apex, where lighter brown; funiculus of antennae brown except the lighter apex; petiole dark brown; abdomen and hind tibiae brown, remaining appendages rather light brown, with mid and hind coxae, trochanters and palps pale yellow (Fig. 5a, c).

## Description of queen

**Morphometrics.** Paratype 'HP0177' nest series: HL 1.56, HW 1.57, IOD 1.22, CL 0.32, CW 0.59, ML 0.63, EL 0.49, EW 0.37, FCDp 0.50, FCDa 0.24, SL 1.23, SWmax 0.16, SWmin 0.09, WL 2.51, PW 1.14, HFL 1.22, HFT 0.31, HTL 1.27, PetW 0.55, PetL 0.35, FCDI 208, CI 101, SI 78, MI 40.

**Head, mesosoma and metasoma.** Similar to *O. subclavata*, except frontal carinae significantly approaching one another anteriorly (FCDI = 208; Fig. 6e).

**Pilosity.** Tiny appressed hairs on scape surface, with three to four longer erect hairs on the lateral sites of the first half of the scape (hair length  $\geq 1$  ocellus); presence of many relatively long, erect hairs on the head (length  $\geq 1$  ocellus) on genae, above the eyes and over the occipital corners of the head (Fig. 6e), and on thorax dorsum, apical tip of petiole, and over both ventral and dorsal sites of all five abdominal tergites.

**Sculpture.** Fine punctation over the head, and on mesosoma dorsum and propodeum; and soft lineation on lateral sites of mesosoma (Fig. 6a).

**Colouration.** Black head, thorax, petiole and frontal coxae; dark brown abdomen and appendages except middle and hind coxae, trochanters, palps and apical tips of antennae and tarsi that are lighter brown (Fig. 6a).

## Description of male

**Morphometrics.** Paratype 'HP0177' nest series: HL 0.84, HW 1.00, IOD 0.66, CL 0.12, CW 0.35, ML 0.31, EL 0.34, EW 0.29, FCDp 0.27, FCDa 0.10, SL 0.67, SWmax 0.10, SWmin 0.06, WL 1.71, PW 0.96, HFL 1.31, HFT 0.18, HTL 0.99, PetW 0.33, PetL 0.21, FCDI 255, CI 119, SI 67, MI 37.

Structures and diagnosis (Fig. 6b, d, f). No apparent large distinction when compared to the male of *O. subclavata*, except for the following minor differences: The specimen is  $\sim 10\%$  larger than the measured male of *O. subclavata*, but morphological measures otherwise considerably overlapping between the two species' males. Frontal carinae relatively more approaching one another anteriorly than in *O. subclavata*, but this difference not as distinct as between the two species' workers (FCDI = 255 v. 229 in *O. subclavata* male). The hairs on clypeus more numerous than on clypeus of *O. subclavata*; presence of many more small hairs on frons and above ocelli (length near  $1/2$  of ocellus) over the occipital region (Fig. 6f). These small, dense hairs are also present dorsolaterally on thorax (on mesoscutum and mesoscutellar disc; Fig. 6b, d). Colouration as in *O. subclavata* male.

## Remarks

The arboreal nest of *O. papuana* sp. nov. in Papua New Guinea was collected at 16.4 m in a dead vine growing along the tree trunk of *Trichospermum pleiostigma* (F. Muell.) Kosterm. (diameter at breast height = 16.3 cm). The nest consisted of less than one hundred workers, with one wingless gyne, three males, and multiple larvae and pupae enclosed in cocoons.

The material from Australia was examined and measured mainly from photographs from the following sources: (i) the book by McArthur (2012), where a worker of *Overbeckia* was incorrectly assigned to *Camponotus janeti* (minor worker fits '*O. papuana*', whereas major worker presumably fits *C. janeti*; there is uncertainty as to whether the two individuals are from different localities and pins or are on a single pin at SAMA); (ii) the photos on AntWeb of the three specimens in Heterick (2019) (see above). The Australian specimens appear to be larger in body size based on the measures from the photos (4 workers measured from photos, WL  $> 1.95$  mm) than the Papua New Guinea (PNG) nest series (6w measured, WL  $< 1.74$ ). However, we treat these as one species because the Australian single specimen sent by P. Ward for direct examination (n#15720) matches *O. papuana* types in the body size (WL = 1.64; Supplementary Table S1) and morphology.

## Distribution of *Overbeckia*

### Geography

Our review of records of the genus *Overbeckia* revealed 17 new species occurrences at 16 sites (Supplementary Table S2): 5 sites for *O. papuana*, 1 site for *O. jambiensis* and 11 sites for *O. subclavata*. Only three of these sites were previously reported in the literature and all as *O. subclavata* (Singapore, Philippines; and Mossman in Queensland here re-identified as *O. papuana*; see Materials). *Overbeckia* is widespread in SE Asia and Australasia (Fig. 7), although the known records remain scarce and scattered (Singapore, Sumatra, Philippines,

Papua New Guinea and Queensland; Fig. 7, Supplementary Table S2), with most records collected geographically relatively close to each other in Sumatra's Jambi province (i.e. nine sites with *Overbeckia*) and near Brisbane, Australia (3 records) (Supplementary Table S2). Our records of *Overbeckia subclavata* and *O. jambiensis* sp. nov. from Sumatra indicate that both species likely occur only in tropical SE Asia. By contrast, *Overbeckia papuana* that is more closely related to *O. subclavata* than *O. jambiensis* based on morphological and molecular evidence, is restricted to the Australasian region. *Overbeckia subclavata* and *O. jambiensis* are in sympatry in Sumatra, where both species were found at one of the study sites and the same plot (Bukit Duabelas National Park/Forest/BF2.2), although in different sampling years (Supplementary Table S1).

### Local distribution

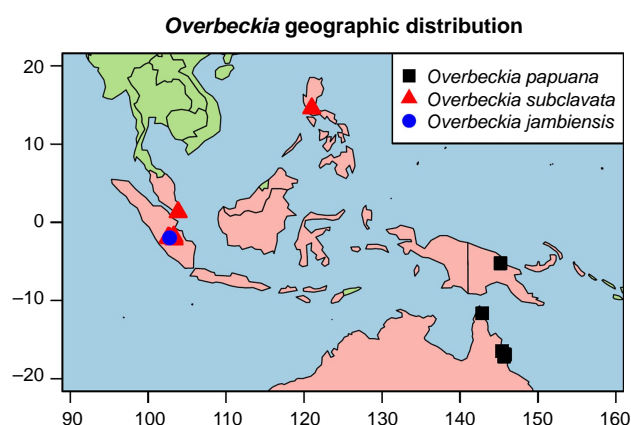
All *Overbeckia* species were collected from vegetation. Colonies appear to be rather small and difficult to detect, like species with similar nesting ecological patterns in the closely related arboreal genus *Echinopla*. The only known colony that has been fully dissected is from *O. papuana* and this was collected from a dry liana, with no trophobionts observed in the nest chamber. *Overbeckia* is one of the rarest genera in arboreal ant communities, both locally and globally (Fig. 7, 8). The sole records from Singapore and PNG are from disturbed sites such as secondary forests and shrub lands near urban areas. However, data from the forests plots in Sumatra sampled by fogging showed that the genus occurs across different forest types, from lowland rainforests to oil palm plantations, although *O. jambiensis* sp. nov. was found more frequently in lowland rainforest and in the dry season (Fig. 8).

In PNG, *O. papuana* was found in a secondary forest, but near a small remote village of Wanang, ~100 km from the coast.

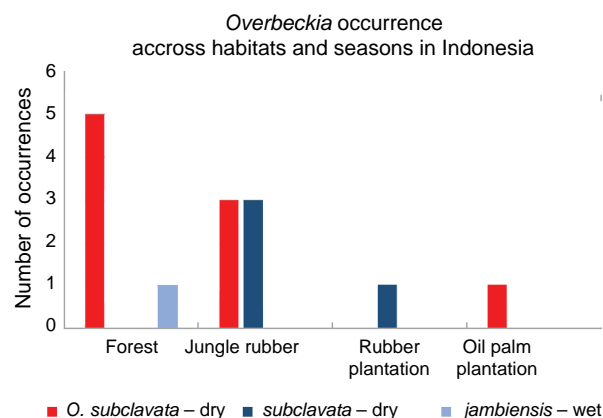
## Phylogeny of Camponotini

### *Overbeckia* placement in Camponotini and monophyly support

We sequenced at least three of the five study loci for five *Overbeckia* specimens, one *O. papuana*, one *O. jambiensis* and three individuals of *O. subclavata* from different forest plots (Supplementary Tables S1 and S3, Supplementary Fig. S1). *Overbeckia* is grouped with *Calomyrmex* and *Echinopla* with strong support (SH-aLRT = 100; aBayes = 1; ultrafast bootstrap [UfB] = 100; posterior probability [PP] = 1). The phylogenetic relationships among the three genera are not highly supported by our dataset, but *Overbeckia* is likely sister to *Calomyrmex* + *Echinopla* (SH-aLRT = 78; aBayes = 0.65; UfB = 86; PP = 0.98). The monophyly of *Overbeckia* is strongly supported (SH-aLRT = 98; aBayes = 1; UfB = 100; PP = 1) and the monophyly of *O. subclavata* is also highly supported (SH-aLRT = 89; aBayes = 0.98; UfB = 94). The phylogenetic distance among the three *O. subclavata* specimens measured as substitutions per site is low (branch lengths = 0.02), whereas the branch supporting a sister relationship between *O. subclavata* and *O. papuana* is moderately supported by our maximum-likelihood inference (SH-aLRT = 84; aBayes = 0.97; UfB = 88) but strongly supported by Bayesian inference (PP = 0.98) (Fig. 9, Supplementary Fig. S1). A likelihood-based tree topology test rejected the sister relationship between *O. papuana* and *O. jambiensis* (bp-RELL weight = 0.0318) and



**Fig. 7.** Distribution map of *Overbeckia* species records. In total, 17 records of the genus are mapped and revised to the species level of which 12 are reported here for the first time (see Results and Supplementary Table S2). Distribution by countries is coloured in pink, with the Singapore record of the types of *O. subclavata* scaled up to Malaysia. In Indonesia and Queensland, respectively nine and three sites are relatively nearby and hence appear clumped.



**Fig. 8.** Variation in *Overbeckia* species occurrence across four vegetation types and two seasons in the EForTS project (dry season, bars in red; wet season, bars in blue). Values on the y-axis show the number of cases in which the species occurred at least once in a subplot. In total, 19 individuals and 14 occurrences were found across 192 subplots sampled during dry and wet seasons (0.07% occupancy). At the level of different sites (forest plots), only 9 of 32 sampled here by canopy fogging were occupied by the genus.



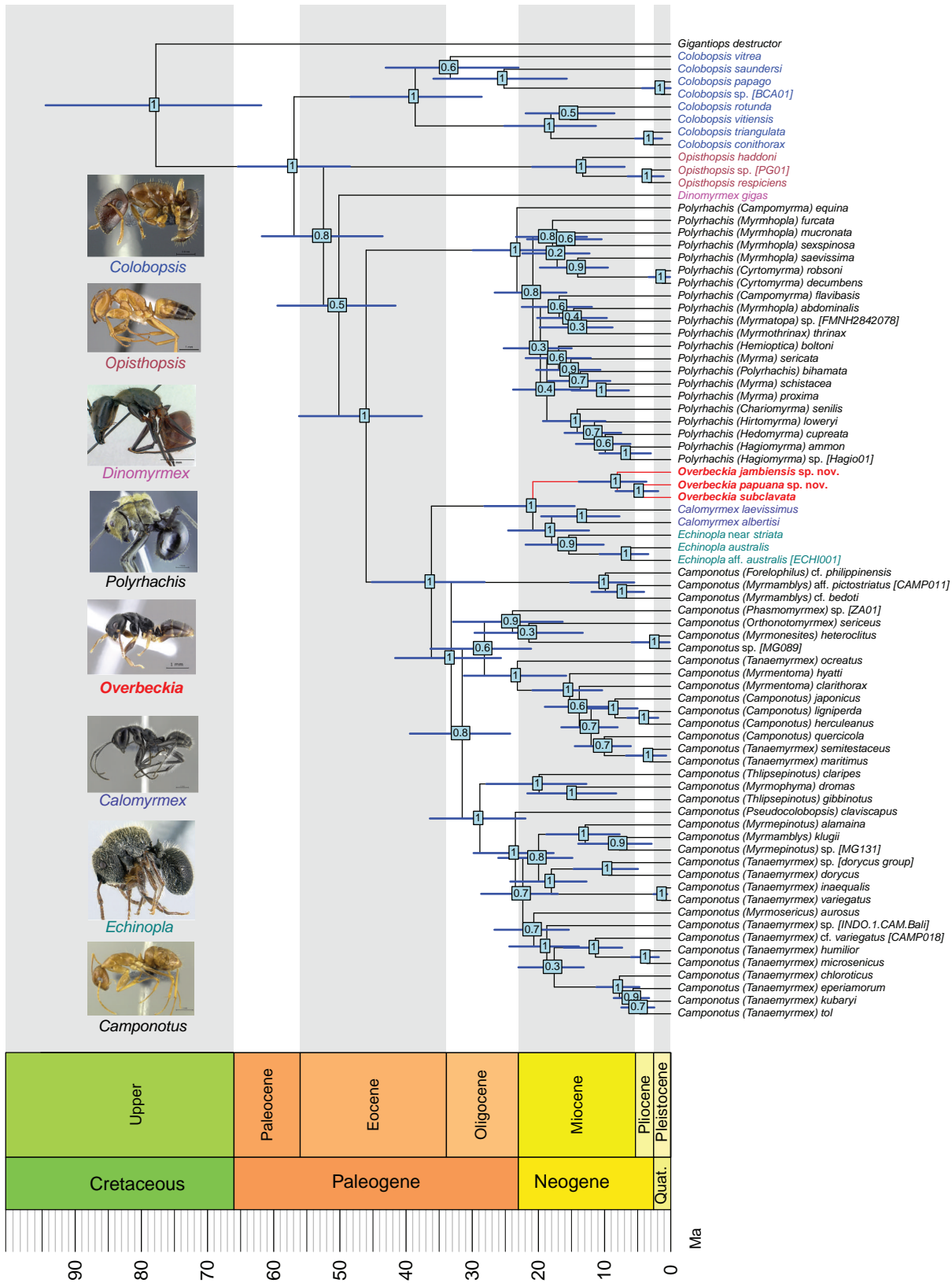


Fig. 9. (Caption on next page)

**Fig. 9.** Time-calibrated phylogeny of Camponotini including all eight extant genera. The chronogram has been inferred using the fossilised birth–death model with 21 vetted fossil records and the constrained approach (Table 1), where we used a secondary calibration point (normal distribution,  $M = 51$  and  $S = 5$ ) for the most recent common ancestor of extant Camponotini lineages. The numbers at nodes reflect the posterior probabilities (support values). The generic images placed along the tree were taken for the specimens of the representative species collected in Klimes et al. (2015) or retrieved from AntWeb (*Dinomyrmex*, *Opisthopsis*).

overall, phylogenetic relationships other than *O. papuana* and *O. subclavata* as sister species had very low  $p$  values in the AU tests, though not significant, between 0.059 and 0.067 (Supplementary Table S5). Altogether, our data suggest that *O. papuana* and *O. subclavata* are sister species, and these two are sister to *O. jambiensis*.

### Phylogeny of Camponotini and the divergence times of genera using fossils calibration

The phylogenetic relationships among Camponotini genera and for most sampled species remained similar in both the maximum-likelihood tree and maximum clade credibility (MCC) tree based on the FBD model (compare Supplementary Fig. S1, Fig. 9). At the genus level, the main difference between the two analytical approaches was the placement of *Opisthopsis* or *Dinomyrmex* as sister to the other Camponotini genera except *Colobopsis* (i.e. the clade that includes *Polyrhachis*, *Overbeckia*, *Calomyrmex*, *Echinopla* and *Camponotus*). *Opisthopsis* diverges earlier in the Bayesian inference analyses (Fig. 9) and the phylogenetic position is swapped with *Dinomyrmex* in the maximum-likelihood tree (Supplementary Fig. S1). The support values in both cases were low (PPs < 0.8 and UfBs < 64) and the tree topology tests did not reject either hypothesis (Supplementary Table S5). However, the overall genus-level phylogenetic relationships inferred using Bayesian inference, including *Opisthopsis* splitting earlier followed by *Dinomyrmex*, agree with phylogenomic trees based on hundreds of UCE loci (Blaimer et al. 2015).

The monophyly and phylogenetic relationships of other Camponotini genera were highly supported (PPs = 1; UfBs > 78), except for *Camponotus triangulatus* that was clustered within *Colobopsis*. Hence, in the light of our molecular dataset, we transfer this species to the genus *Colobopsis*: *Colobopsis triangulata* (Klimes & McArthur, 2014) comb. nov. At subgenus level, the support values within the two most speciose genera, *Polyrhachis* and *Camponotus*, ranged from strongly to weakly supported (PPs < 0.5), likely due to the lower phylogenetic resolutions of the molecular markers used. Nevertheless, some of the highly supported clades (PPs > 0.8) suggested that some subgenera are polyphyletic (e.g. *Myrmamblys*, *Campomyrma* and *Myrmhopla*) or paraphyletic (e.g. *Tanaemyrmex*).

The MCC tree topologies remained virtually the same between the UNCONSTRAINED and CONSTRAINED models. However, divergence time estimations differed between the results of the two analytical strategies using the FBD model: the UNCONSTRAINED model almost doubled the divergence

time estimations compared to the CONSTRAINED model (Supplementary Fig. S2, Fig. 9, Table 1). In the case of *Overbeckia*, our analysis suggests that extant lineages diverged during the late Miocene to the Pliocene, with the median stem age estimated to 29.8 Ma (95% highest posterior density, HPD = 18.8–42.7) and 20.8 Ma (95% HPD = 14.5–28.2). Furthermore, the median crown *Overbeckia* age was estimated at 11.8 Ma (95% HPD = 5.1–21.3) and 8.1 Ma (95% HPD = 3.7–13.9) in the UNCONSTRAINED and CONSTRAINED models respectively (Supplementary Fig. S2, Fig. 9, Table 1).

## Discussion

### *Overbeckia* is a valid genus with unexpected ‘cryptic’ species diversity

Using an integrative approach of morphological and molecular data, we demonstrated that *Overbeckia* is a valid ant genus in Formicinae (Camponotini). The genus is not closely related to *Camponotus* or *Colobopsis* as previously hypothesised (Bolton 2003; Ward et al. 2016) but rather to the genera *Echinopla* and *Calomyrmex*. The distinction of *Overbeckia* from other Oriental and Indo-Australian ants was established in the key by Bolton (1994) based on the proventriculus structure, whereas the relation with *Calomyrmex* was suggested by Viehmeier (1916) based on the similar thorax shape. We confirmed that the length of the sepals is shorter in *Overbeckia* than other genera of this tribe except *Echinopla* (Eisner 1957 and our study). The close relationship of *Overbeckia* and *Echinopla* agrees with Kreider et al. (2021), but these analyses also suggested that *Dinomyrmex*, *Overbeckia* and *Echinopla* rendered *Camponotus* paraphyletic (Kreider et al. 2021 Supplementary Information). By contrast, our study strongly supports *Camponotus* as a separate monophyletic lineage from the three genera and this is consistent with the analyses of Blaimer et al. (2015) on Camponotini.

Cryptic species diversity is often revealed in closely related and morphologically similar species, when an integrative framework of both genetic and morphological disparity is applied (Heethoff 2018; Struck et al. 2018). Although we measured many morphological characters and indices commonly used in ant taxonomy (e.g. Bolton 1994; Wang et al. 2018b), we found little evidence that those characters alone differed among the three species. An exception was the shape of and angle between the frontal carinae that are diagnostic morphological characters used to distinguish the three species of *Overbeckia*. Nevertheless, although the

morphometry of *Overbeckia* is rather uniform between species, other important morphological features such as pubescence and cuticular sculpturing clearly separate the species. We found no evidence that *Overbeckia* species are dimorphic like some *Camponotus* and *Colobopsis* species, or that these would exhibit head phragmosis (Klimes and McArthur 2014). The lack of worker dimorphism in *Overbeckia* may represent an evolutionary constraint, as the two sister genera, *Echinopla* and *Calomyrmex*, also lack polymorphism or dimorphism in workers (Ward *et al.* 2016; Laciny *et al.* 2017).

There are still taxa whose status within the Camponotini is unclear and that require further revision with an integrative approach, as we have done for *Overbeckia*. For example, the former genus *Phasmomyrmex* Stitz, 1910 (now considered a subgenus) was synonymised with *Camponotus* by Ward *et al.* (2016), including the three former subgenera *Phasmomyrmex*, *Myrmorhachis* and *Myrmacantha*. However, this change was based on molecular evidence from a single queen of an unknown *Phasmomyrmex* species (CASENT0217047) rather than a described species (AntWeb, see <https://www.antweb.org>). Notably, we noticed a similar appearance of the subgenus *Myrmacantha* to *Overbeckia*, particularly in the thorax and head shape, presence of subpetiolar process and a match in the colour pattern in one of the two known species, *M. wolffi* Emery, 1920 (AntWeb, see <https://www.antweb.org>). However, given the lack of molecular data for *Camponotus* (*Myrmacantha*) and the fact that these species have a disjunct distribution from *Overbeckia* (tropical Africa v. SE Asia and Australasia) and vary in other characters (presence of petiolar dorsal spines), the apparent resemblance between *Myrmacantha* and *Overbeckia* may be a case of morphological convergence of these two arboreal lineages. A similar case of phenotypic convergence has recently been revealed in some *Camponotus* and *Colobopsis* spp. (Ward and Boudinot 2021) that nest arboreally in twigs. An alternative hypothesis of the distinction of *Myrmacantha* from *Camponotus* and the possible fit to the clade comprising *Overbeckia*, *Echinopla* and *Calomyrmex*, remains open and needs to be explored through increased field collections and a multi-evidence approach.

### Advances in our understanding of distribution, ecology and rarity of *Overbeckia*

Recently, Heterick (2019) suggested that the genus *Overbeckia* has been anthropogenically introduced from the Malaysia peninsula, or alternatively, this might be an endemic group unknown to Australia. Our results highlight that both scenarios are unlikely, as we showed that *Overbeckia* is rather widespread across SE Asia and Australasia. Given all available evidence, the Australian *Overbeckia* are neither *O. subclavata* nor new Australian endemics but very likely represent a population of the more widespread species *O. papuana* sp. nov. Future studies might confirm this by

sequencing Australian *Overbeckia* to examine the genetic distances between PNG and Queensland populations.

Our revisions of data from ecological studies in Indonesia and New Guinea highlight the extreme rarity of *Overbeckia* within local ant communities, even when tree canopies are sampled at great effort. In Papua New Guinea, the genus has not been found at any other site, despite the sampling of more than 3500 trees (Klimes *et al.* 2015; Klimes 2017; Plowman *et al.* 2017; Orivel *et al.* 2018; Mottl *et al.* 2019; Plowman *et al.* 2020; Leponce *et al.* 2021). Canopy fogging across four different land-use systems in Indonesia yielded most records of the genus (Nazarreta *et al.* 2020; Kreider *et al.* 2021), including the discovery of a novel species but the species occurrences were still extremely rare (see Results). Although we assume that the inherently low density and small size of *Overbeckia* colonies within plant stems may explain the rarity, previous field sampling may possibly have overlooked or misidentified the genus (see Results and Supplementary Table S2 for the specimens from Queensland, originally all identified as *Camponotus*). We hope that our revision of the genus based on molecular and morphological characters will help to correctly identify species of *Overbeckia* from past and future collections.

The occurrence of *Overbeckia* in pristine forests and young secondary forests and plantations suggests that the rarity of the genus is not primarily caused by degradation of natural habitats, as is the case for many other canopy ant species (Fayle *et al.* 2010; Klimes 2017; Nazarreta *et al.* 2020). Nevertheless, the genus does not appear to be present in mountain forests and leaf-litter (Moses *et al.* 2021; Rizqulloh *et al.* 2021). These findings suggest a general arboreal nesting pattern and a lowland distribution of *Overbeckia* with naturally low density of nests. Such rarity is not exceptional in some of the ant genera, e.g. arboreal cryptic-nesting *Rhopalomastix* Forel, 1910, *Lasiomyrma* Terayama & Yamane, 2000 and *Rotastruma* Bolton, 1991 (Terayama and Yamane 2000; Luo and Guenard 2016; Wang *et al.* 2018b; Hosoishi *et al.* 2021).

Feeding ecology of *Overbeckia* remains unknown, although crop morphology suggests that the genus might be omnivorous. The extremely long sepals of the crop in *Camponotus* and *Colobopsis* have been hypothesised to be related to nutrient utilisation of the amino acids from a poor diet based on liquids in these ants and may play a role in maintaining the symbiotic bacteria in the gut, such as *Blochmannia* (Cook and Davidson 2006; Wernegreen *et al.* 2009). We do not know if *Overbeckia* also harbours such bacteria. However, as both *Echinopla* and *Calomyrmex* host *Blochmannia* (Wernegreen *et al.* 2009), these may similarly be present in *Overbeckia*.

### Evolution of Camponotini and *Overbeckia*

Our phylogenetic analysis based on five protein-coding loci revealed the same genus-level relationships as phylogenomic

analyses using 959 UCE loci (Blaimer et al. 2015). Importantly, our time-calibrated phylogenetic hypotheses cover all major extant lineages of Camponotini represented by 78 species and include the genus *Overbeckia* for the first time. This sampling approach and the use of multiple fossil records, while accounting for uncertainties in fossil age within the FBD model framework, have yielded robust estimates of divergence times and tree topologies that can be compared with results from previous studies (Moreau and Bell 2013; Blaimer et al. 2015; Mezger and Moreau 2016). Despite not being fully resolved by our dataset, current evidence suggests that the most likely scenario in the evolution of Camponotini for the nodes with disparate support between ML and Bayesian inference, are that *Opisthopsis* splits earlier followed by *Dinomyrmex* and that *Overbeckia papuana* is sister to *O. subclavata*, and these two species are sister to *O. jambiensis*.

Eisner (1957) used the structure of ant crop and proven-triculus to discuss the evolutionary implications in the ant subfamilies and genera, pointing at the sepalous type in the subfamily Formicinae to represent the most specialised crop type. This is different from the simple ancestral asepalous type. Here, we show that *Echinopla* and *Overbeckia* possess much shorter sepals than other Camponotini genera, while the most species-diverse genera, *Camponotus*, *Colobopsis* and *Polyrhachis*, have the longest sepals (but note that only a single species has been dissected per genus either in our study, or in Eisner 1957). There is uncertainty whether the common ancestor of the Camponotini had such very long sepals. However, as all genera except the two have much longer sepals than the valve, we assume that shorter sepals are a progression towards perhaps a less specialised diet in *Echinopla* and *Overbeckia*. This is likely as the two genera diverged more recently than the other genera except for *Calomyrmex* (Table 1).

We relied conservatively on a secondary constraint of crown extant Camponotini for our divergence time estimations. Without this constraint, our divergence times of crown genera were much older than previously reported (Blaimer et al. 2015; Economo et al. 2018), probably due to more extensive phylogenetic scope in these studies. Nevertheless, in both of our time-calibration strategies, *Overbeckia* appears to be an ancient lineage that diverged at least 14 Ma (i.e. the lowest estimated value) and radiated throughout the late Miocene to Pliocene. This suggests that *Overbeckia* dispersed across SE Asia and Australasia when a significant landmass emerged from the sea in Wallacea, during the time that other ant lineages dispersed and diversified across the region (Economo et al. 2015; Matos-Maraví et al. 2018).

While the generic relationships seem to be well supported by our and previous phylogenies of Camponotini (Blaimer et al. 2015), the relationships among species within the largest genera (*Camponotus*, *Polyrhachis*) are still not well resolved. A reclassification of subgenera in these two genera

with further expansion of molecular phylogenies in terms of taxonomic sampling is needed (but see Mezger and Moreau 2016 for the evolution of *Polyrhachis* subgenera). Given the extreme species diversity and high morphological resemblance among *Camponotus* and *Colobopsis* (Ward et al. 2016), future reclassifications of species in these genera are to be expected. Incidentally, our study documents a new placement for *Colobopsis triangulata* (Klimes & McArthur, 2014) comb. nov., formerly classified under *Camponotus* (Ward et al. 2016).

Interestingly, the allopatric species *O. subclavata* and *O. papuana* sp. nov., respectively west and east of Wallace's Line, were closely related and morphologically more similar compared to *O. jambiensis* sp. nov. This indicates that Wallace's Line has been biogeographically permeable and *Overbeckia* might have dispersed across the region during the Pliocene, in agreement with other insect groups (e.g. Tänzler et al. 2014; Condamine et al. 2015). By contrast, the overlapping distributions of *O. jambiensis* and *O. subclavata* in Sumatra may indicate differing ecological strategies facilitating evolutionary divergence. However, as only a single record is known for *O. jambiensis* and only a few records are known for the other two species, more data are needed to delineate species ranges and clarify the biogeographical history of *Overbeckia*.

## Supplementary material

Supplementary material is available online.

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**Data availability.** All data that support this study are available in the accompanying online supplementary material (Supplementary Tables S1–S4, the sequences alignment and phylogenetic TRE files). The new sequences from this study are also accessible in GenBank (Accession numbers: OK046729–OK046734, OK104496–OK104544).

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**Author contributions.** P. Klimeš conceived and designed the study. J. Drescher, D. Buchori, P. Hidayat, R. Nazarrreta and S. Scheu designed and organised the sampling and sorting of the material from Indonesia from different vegetation types and managed the collection permits. P. Klimeš and M. Rimandai collected and sorted the material from New Guinea. P. Potocký took the original images of the new ant specimens from Indonesia and Papua New Guinea and measured all morphological characters. P. Klimeš dissected and took images of the proventriculus structures. P. Matos-Maraví compiled published and novel DNA sequences, created the map and performed the phylogenetic analyses. P. Klimeš and P. Matos-Maraví drafted the manuscript with contribution from J. Drescher and all authors revised and approved the content.

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