Zombie-ant fungi across continents: 15 new species and new combinations within Ophiocordyceps. I. Myrmecophilous hirsutelloid species

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Abstract: Ophiocordyceps species infecting ants – the so-called zombie-ant fungi – comprise one of the most intriguing and fascinating relationships between microbes and animals. They are widespread within tropical forests worldwide, with relatively few reports from temperate ecosystems. These pathogens possess the ability to manipulate host behaviour in order to increase their own fitness. Depending on the fungal species involved the infected ants are manipulated either to leave the nest to ascend understorey shrubs, to die biting onto vegetation, or descend from the canopy to die at the base of trees. Experimental evidence has demonstrated that the behavioural change aids spore dispersal and thus increases the chances of infection, because of the existing behavioural immunity expressed inside ant colonies that limits fungal development and transmission. Despite their undoubted importance for ecosystem functioning, these fungal pathogens are still poorly documented, especially regarding their diversity, ecology and evolutionary relationships. Here, we describe 15 new species of Ophiocordyceps with hirsutella-like asexual morphs that exclusively infect ants. These form a monophyletic group that we identified in this study as myrmecophilous hirsutelloid species. We also propose new combinations for species previously described as varieties and provide for the first time important morphological and ecological information. The species proposed herein were collected in Brazil, Colombia, USA, Australia and Japan. All species could readily be separated using classic taxonomic criteria, in particular ascospore and asexual morphology.

Key words: Behaviour manipulation, Camponotini, Entomopathogenic fungi, Host association, Hypocreales, Insect pathogen, Multigene phylogeny, Ophiocordyceps, Ophiocordyceps unilateralis, Zombie-ant fungi.

Taxonomic novelties: new combinations: Ophiocordyceps dolichoderi (H.C. Evans & Samson) Araújo, H.C. Evans & D.P. Hughes, O. monacidis (H.C. Evans & Samson) Araújo, H.C. Evans & D.P. Hughes; new species: O. albacongiae Araújo, H.C. Evans & D.P. Hughes, O. blakebamesii Araújo, H.C. Evans & D.P. Hughes, O. camponoti-charlificis Araújo, H.C. Evans & D.P. Hughes, O. camponoti-femorati Araújo, H.C. Evans & D.P. Hughes, O. camponoti-flondiani Araújo, H.C. Evans & D.P. Hughes, O. camponoti-hippocrepidis Araújo, H.C. Evans & D.P. Hughes, O. camponoti-nidulantis Araújo, H.C. Evans & D.P. Hughes, O. camponoti-renngeri Araújo, H.C. Evans & D.P. Hughes, O. camponoti-saxxtutati Araújo, H.C. Evans & D.P. Hughes, O. daceti Araújo, H.C. Evans & D.P. Hughes, O. kimfilimentiariae Araújo, H.C. Evans & D.P. Hughes, O. naomipierceae Araújo, H.C. Evans & D.P. Hughes, O. oecophyllae Araújo, S. Abell, T. Marney, R. Shivash H.C. Evans & D.P. Hughes, O. ootaki Araújo, H.C. Evans & D.P. Hughes., O. satoi Araújo, H.C. Evans & D.P. Hughes.

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INTRODUCTION

Fungi associated with insects are one of the most spectacular and diverse interactions found in nature. There is an enormous variety to consider: mutualistic symbionts (Suh et al. 2005); fungi serving as an obligate food source, such as those found in fungus-gardening ants (Currie et al. 2003); sexually- and behaviourally-transmitted parasites – e.g. Laboulbeniales (De Kesel 1996); and entomopathogenic fungi that are highly virulent and are considered to have pronounced effects on host populations (Evans 1974). Despite this increasing knowledge, fungal-insect associations still remain an understudied area of fungal biodiversity and likely harbour one of the largest reservoirs of undocumented species among Fungi.

Insects, with more than a million described species (Foottit & Adler 2009) are distributed among 29 orders (Mishof et al. 2014). The fungal pathogens are able to colonize 19 of these orders, resulting in the evolution of a wide diversity of morphologies and strategies that enable infection and onward transmission using the insect body as the ecological niche (Araújo & Hughes 2016). Among these different strategies, one of the most impressive and sophisticated interactions between insects and entomopathogenic fungi is that involving ants and species of fungi in the genus Ophiocordyceps (Andersen et al. 2009). The genus is estimated to have arisen about 100 million years ago (Sung et al. 2008) and since then has colonized 10 orders of insects (Sanjuan et al. 2015, Araújo & Hughes 2016), comprising about 200 species of entomopathogens (Crous et al. 2004). Although ants account for less than 2 % of insect species, they contribute as much as 50 % of animal biomass in tropical forests (Hollódiobler et al. 2009). Ants occupy a wide range of habitats, from high canopy to the leaf litter, forming colonies comprising from a few dozen (Jahyny et al. 2002) to millions of individuals (Currie et al. 2003), especially in tropical forests. As such dominant members of most terrestrial biomes, ants are the most commonly encountered hosts for species in the genus Ophiocordyceps in tropical forests worldwide.

The genus Ophiocordyceps was erected by Petch (1931) to accommodate species of Cordyceps that exhibited clavate thick-
walled asci and ascospores that do not disarticulate into parts.
Kobayasi (1941) used the term as a subgeneric classification, based solely on ascospore morphology. However, in more recent years, Sung et al. (2007) proposed separation of the family Clavicipitaceae into three monophyletic families: Clavicipitaceae, Cordycipitaceae and Ophiocordycepaceae, based on well-supported molecular data. The same study also proposed the re-establishment of Ophiocordyceps as a genus. All species forming a sister clade with Tolypocladium (former Elaphocordyclops, see Quandt et al. 2014) were transferred from Cordycydes s.l. to Ophiocordyceps, including all species infecting ants. Included within that is the relatively well-known and iconic species O. unilateralis, which infects ants and dramatically alters their behaviour as a developmental necessity (Evans et al. 2018).

From the time when O. unilateralis sensu stricto was originally published – as Torrubia unilateralis (Tulasne & Tulasne 1865) – few species have been described belonging to this group, despite their diversity being estimated at about 580 species worldwide (Araújo & Hughes, unpublished data), and separation was considered to be premature – due to lack of data – or, at the best, at the varietal level (Evans & Samson 1984). Species delimitation in this group started to be investigated based on fresh specimens, where ascospore morphology and the germination process could be studied in depth (Evans et al. 2011). In that study, four new species were described from Atlantic rainforest in Brazil, in which it was posited that each species within the tribe Camponotini could host a different species of Ophiocordyceps. Subsequently, with the support of molecular data, six species were described from Thailand (Luangsa-ard et al. 2011, Kobmoo et al. 2012), one from Japan (Kepler et al. 2011) and three from the Brazilian Amazon (Araújo et al. 2015). Consequently, there is increasing support for the “one ant-one Ophiocordyceps species” hypothesis as proposed by Evans et al. (2011). The present paper builds on the hypothesis.

Asexual morphs associated with Ophiocordyceps, include Sorospora, Syngliocladium, Parasaria, Stilbella, Hymenostilbe and Hirsutella (Quandt et al. 2014). With the exception of Sorospora and Syngliocladium, all are recorded to be associated with ants. The asexual morphs Hymenostilbe and Hirsutella are commonly found associated with Ophiocordyceps infecting ants (myrmecophilous species) (Evans & Samson 1982, 1984, Araújo et al. 2015, Araújo & Hughes 2017). The two distinct clades that these asexual morphs form include the vast majority of myrmecophilous species within Ophiocordyceps: 1) O. unilateralis clade: O. unilateralis core clade + O. knipholoides sub-clade, classified here as myrmecophilous hirsutelloids; 2) Species within Ophiocordyceps subg. Neocordycodes or “sphenocorhapha clade” sensu Sung et al. (2007), classified here as myrmecophilous hymenostilboids (see Araújo & Hughes 2017). This study focuses exclusively on myrmecophilous hirsutelloids.

Both the O. unilateralis core clade and O. knipholoides sub-clade can easily be distinguished in the field, based on macro-morphological and ecological characters. For instance, the typically orange ascoma produced by species within O. knipholoides sub-clade develops on a stroma that emerges laterally from the host’s thorax with the fertile part covering 360° of the stalk. The hosts often die among the moss carpets at the base of large trees in the Amazon rainforest. Conversely, the stroma of species within O. unilateralis core clade consistently arises from the dorsal pronotum and produces a brown to black ascoma, attached laterally on the stalk (hence the “unilateralis” epithet). The hosts are exclusively Camponotini species (i.e. Camponotus, Colobopsis, Dinomyrmex and Polyrhachis) that once killed by the fungus, always die biting onto the substrate. Other characters such as ascospore morphology and germination, asexual morphs and differences in behaviour manipulation are also important criteria used to separate the species within these clades, discussed in detail below.

The 15 new species proposed herein were collected during field surveys in five countries across four continents: South America (Brazil, Colombia), North America (USA), Oceania (Australia) and Asia (Japan). Based on macro-morphological characters, most species were readily identified as being part of the O. unilateralis core clade, with just one new species belonging to the O. knipholoides sub-clade. The present work extends our understanding of this unique group of entomopathogens, providing novel insights into their morphology, ecology and evolution.

MATERIAL AND METHODS

Sampling

Surveys were undertaken in the central Amazonian region of Brazil (Reserva Ducke, Manaus, Amazonas), Colombia (Canyon Rio Claro, Antioquia), USA (South Carolina, Florida and Missouri), Japan (Matsuyama and ArimaFuji Park, Kyoto) and Australia (Licuala State Forest and Kuranda, Queensland). Reserva Ducke (Brazil) comprises ca. 10 000 ha of terra-firme forest with plateaus, lowlands and campaninarana vegetation, characterized by areas of sandy soil across the Rio Negro basin. Canyon Rio Claro Reserve (Colombia) encompasses 450 ha of tropical forest and canyons along the Magdalena River with marble caves and a rich diversity of plants and animals. The Japanese species were collected at Matsuyama (Mt. Matsu), a mountainous area with up to 687 m elevation, on the west side of Kyoto and Arima Fuj Park, located at the base of Mt. ArimaFuji, Sanda, Hyogo Prefecture. In Australia, two places served as collecting sites: Licuala State Forest comprising almost 900 000 ha of lowland tropical forest dominated by the native fan palm Licuala ramsayi, but also areas of eucalyptus forest, wetlands and mangrove forests. Kuranda is a tropical rainforest on the eastern edge of the Atherton Tablelands at an elevation of 380 m. The North American sites are composed of deciduous forests with snowfall and below-zero temperatures during winter (Missouri and South Carolina), which contrast with the Florida site, composed of ever-green tropical rainforest.

Our sampling protocol consisted of a careful inspection of the soil, leaf litter, shrub leaves and tree trunks, up to ca. 2 m high. Infected ants – and the substrata they were attached to – were collected in plastic containers, transported to the laboratory and, when possible, examined the same day. During longer surveys, the samples that exhibited informative taxonomic characters were air-dried overnight to prevent growth of opportunistic fungi. For molecular work, samples were placed in plastic tubes with 100–200 μL CTAB (G-Biosciences) for further DNA extraction. All specimens were photographed individually, using a Canon 7D camera equipped with EF-100 mm macro lens or MP-E 65 mm (×5) with a MT-24EX Canon macro lite flash attached.
Morphological studies

For macro-morphological characterization, specimens were examined using a stereoscopic microscope Olympus SZX16 and sorted for further macro-morphological studies. The characters investigated were: host location (e.g. leaf, spine, trunk, moss, base of trunk, soil); interaction between fungus/substrate (e.g. presence or absence of attachment structures); ascosomal size, colour, position, presence/absence and characterization of asexual morphs and perithecial insertion (e.g. immersed, semi-immersed, erumpent, superficial). For micro-morphological characterization, either free-hand or cryosectioning of the ascoma was performed using a Leica CM1950 Cryostat. Samples were mounted on a slide with lacto-fuchsin (0.1 g of acid fuchsin in 100 mL of lactic acid) for light microscopy examination using an Olympus BX61. In order to obtain naturally released ascospores, infected ants with mature ascomata were attached to the lid of a plastic Petri plate (9 cm diam) using tape, and suspended above a plate containing either distilled water agar (DWA) or potato-dextrose agar (PDA). Plates were transferred to sheltered stands installed in the forest, subject to natural temperature and light fluctuations. The plates containing the infected ants were examined twice a day for the presence of ascospores, once in the morning and again after sunset. When present, ejected ascospores form sub-hyaline halos on the agar surface. Part of the freshly deposited ascospores was removed with a sterile hypodermic needle under a stereoscopic microscope, and mounted on a slide with lacto-fuchsin for light microscopy examination (Olympus BX61). The remaining ascospores were left on the agar surface and examined over a number of days in order to follow germination events. A minimum of 50 naturally released (mature) ascospores was measured for morphological comparison (Table 1).

DNA extraction, PCR and sequencing

All the species proposed in this study were collected in their natural habitat. The DNA templates were obtained directly from the specimens with the following protocol: samples were placed in 1.5 mL Eppendorf tubes with 100–200 μL of CTAB immediately after collection. In the lab, the samples were ground mechanically, 400 μL of CTAB were added and samples were incubated at 60 °C for 20 min and then centrifuged for 10 min at 14 000 rpm. The supernatant (approx. 400 μL) was transferred to a new 1.5 mL Eppendorf tube, mixed with 500 μL of 24:1 Chloroform: Isoamyl-alcohol (Sigma) and mixed by inverting. The mix was then centrifuged for 20 min at 14 000 rpm and the supernatant transferred to a new 1.5 mL Eppendorf tube and further cleaned using the GeneCleanIII kit (MP Biomedicals), following the recommended protocol. The only step modified was the addition of 30 μL of GlassMilk per sample, instead of the recommended 10 μL, to increase yield.

Five loci were used in the analyses, i.e. small subunit nuclear ribosomal DNA (SSU), large subunit nuclear ribosomal DNA (LSU), translation elongation factor 1α (tef) and the largest and second largest subunits of RNA polymerase II (RPB1 and RPB2 respectively) with a total read length of approximately 4 800 bp. However, for our field collected samples, RPB2 could not be successfully amplified. The primers used were, SSU: NS1 (GTAGCTATAGTTGCTTCT) and NS4 (CTTCCGTCATTTCCCTTAAAG) (White et al. 1990); LSU: LR0 (5′-ACCGCTGGAAC-TTAACG-3′) and LRS (5′-TCCTAGGAAAACCTTGC-3′) (Vigay & Sun 1994); tef: 983F (5′-GCYCCYG GHC AYGCTGAYTTAT-3′) and 2218R (5′-ATGACACCCAGGC-ATRA فأر 3′); RPB1: (5′-CCWGYTTYTCAAGAAARCT-3′) (Castlebury et al. 2004) and RPB1Cr_oph was designed specifically to address the species proposed herein (5′-CTGVCMGCRATGCCTTGCCTCAT-3′). All the RPB2 sequences were downloaded from GenBank.

Each 25 μL-PCR reaction contained 4.5 μL of Buffer E (Premix E – Epicentre), 0.5 μL of each forward and reverse primer (10 mM), 1 μL of DNA template, 0.1 Platinum Taq Polymerase (Invitrogen) and 18.4 μL of Ultra Pure Distilled Water (Gibco). The PCR reactions were placed in a Biometra T300 thermocycler under the following conditions: for SSU and LSU (1) 2 min at 94 °C, (2) 4 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min, followed by (3) 35 cycles of denaturation at 94 °C for 30 s, annealing at 50.5 °C for 1 min, and extension at 72 °C for 2 min and (4) 3 min at 72 °C. For tef and RPB1(1) 2 min at 94 °C, (2) 10 cycles of denaturation at 94 °C for 30 s, annealing at 64 °C for 1 min, and extension at 72 °C for 1 min, followed by (3) 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 1 min, and extension at 72 °C for 1 min and (4) 3 min at 72 °C. Each 25 μL PCR reaction was cleaned by adding 3.75 μL of Illustra ExoProStar enzymatic PCR clean up (1:1 mix of Exonuclease I and Alkaline Phosphatase) (GE Healthcare Life Sciences), incubated at 37 °C for 1 h and 80 °C for 15 min in the thermocycler. The clean PCR products were sequenced by Sanger DNA sequencing (Applied Biosystems 3730XL) at Genomics Core Facility service at Penn State University.

Phylogenetic analyses

The raw sequence reads (.ab1 files) were edited manually using Geneious v. 8.1.8 (Kearse et al. 2012). Individual gene alignments were generated by MUSCLE (Edgar 2004). The alignment of every gene was improved manually, annotated and concatenated into a single combined dataset using Geneious v. 8.1.8 (Kearse et al. 2012). Ambiguously aligned regions were excluded from phylogenetic analysis and gaps were treated as missing data. The final alignment length was 4598 bp: 1031 bp for SSU, 893 bp for LSU, 991 bp for tef, 641 bp for RPB1, and 1042 for RPB2. Maximum likelihood (ML) analysis was performed with RAxML v. 8.2.4 (Stamatakis 2006) on a concatenated dataset containing all five genes. The dataset consisted of 11 data partitions, these included one each for SSU and LSU, and three for each of the three codon positions of the protein coding genes, tef, RPB1 and RPB2. The GTR Gamma model of nucleotide substitution was employed during the generation of 1 000 bootstrap replicates. Bayesian analyses were performed with MrBayes v. 3.2.6 (Ronquist et al. 2012). The dataset was partitioned as in likelihood analyses. The GTR Gamma model with invariant sites was applied separately to each model. Two independent runs of five million generations were executed simultaneously, with four chains per run, and trees were sampled and printed to output every 500 generations. After the analyses had stopped, runs were checked for convergence and sampling of model parameters. The first 25 % of trees sampled were discarded as burning. The remaining trees were used to create a consensus tree using the sum function. Branches were considered strongly supported if posterior probabilities were 0.95 or higher. For this study, we generated 142 new sequences (40 for SSU, 37 for LSU, 33 for tef and 32 for RPB1), all deposited in GenBank (Table 2).
| Species                          | Host                          | Death position          | Ascospore Size (μm) | Capilliconidiophore Septation | Hirutella asexual morph A B C Paraisaria- Stibelliformis like | Source                        | Complex                        | Distribution               |
|---------------------------------|-------------------------------|-------------------------|---------------------|-----------------------------|----------------------------------------------------------------|--------------------------------|--------------------------------|---------------------------|
| Ophiocordyceps                 | Camponotus sp.                | biting epiphytes        | 80–100 × 5          | na                          | 5–6                                                              | This study                     | O. unilateralis s.l.          | Colombia                  |
| O. blakebarnesii               | Camponotus sp.                | biting inside log       | 140–160 × 4         | na                          | 6–7                                                              | This study                     |                                | USA (Missouri)             |
| O. camponoti-atricipis          | Camponotus atriceps           | biting leaf             | 80–85 × 3           | 55                          | 5 x                                                               | Araújo et al. (2015)            | Brazilian Amazon            |
| O. camponoti-balzani           | Camponotus balzani            | biting leaf             | 135–174 × 4–5       | –                           | 14–22                                                             | Evans et al. (2011)             | Brazilian Atlantic Rainforest |
| O. camponoti-bispinosi         | Camponotus bispinosus         | biting spines           | 70–75 × 4.5         | 65                          | 4–5                                                              | Araújo et al. (2015)            | Brazilian Amazon            |
| O. camponoti-chartifidis       | Camponotus chartifex          | biting leaf             | 75–85 × 5           | 75–90                        | 9–13                                                             | This study                     | Brazilian Amazon            |
| O. camponoti-femorati          | Camponotus femoratus          | biting leaf/spines      | 75–90 × 3           | 35–40                       | 5 x                                                               | This study                     | Brazilian Amazon            |
| O. camponoti-floridani         | Camponotus floridanus         | biting leaf             | 75–90 × 4–5         | na                          | 5 x                                                               | This study                     | USA (Florida)                |
| O. camponoti-hippoprepis       | Camponotus hippoprepis        | biting spines           | 75–85 × 4–5         | 45–50                       | 5 x                                                               | This study                     | Brazilian Amazon            |
| O. camponoti-indianni          | Camponotus indi anus          | biting leaf             | 75 × 4.5            | 130                         | 5 x                                                               | Araújo et al. (2015)            | Brazilian Amazon            |
| O. camponoti-leonardi          | Camponotus leonardi           | biting leaf             | 110–125 × 2–3       | –                           | 7–8                                                                | Kobmoo et al. (2012)            | Thailand                     |
| O. camponoti-melanotici        | Camponotus melanoticus        | biting leaf             | 170–210 × 4–5       | 20–25                       | 27–35                                                              | Evans et al. (2011)             | Brazilian Atlantic Rainforest |
| O. camponoti-nidulantis        | Camponotus niduland           | biting saplings         | 90–105 × 3–4        | 50–60                       | 5 x                                                               | This study                     | Brazilian Amazon            |
| O. camponoti-novogranadensis   | Camponotus novogranadensis    | biting epiphytes        | 75–95 × 2.5–3.5     | –                           | 5–10                                                              | Evans et al. (2011)             | Brazilian Atlantic Rainforest |
| O. camponoti-renggeri          | Camponotus renggeri           | biting leaf/moss        | 90–120 × 4          | –                           | 5–8                                                                | This study                     | Brazilian Amazon            |
| O. camponoti-rufipes           | Camponotus rufipes            | biting leaf             | 80–95 × 2–3         | 60–70                       | 4–7                                                                | Evans et al. (2011)             | Brazilian Atlantic Rainforest |
### Table 1. (Continued).

| Species                  | Host                | Death position      | Size (μm) | Capillliconidiophore | Septation A | B   | C   | Paraisarai-like | Stilbelliformis like | Source                          | Complex                  | Distribution                  |
|--------------------------|---------------------|---------------------|-----------|----------------------|-------------|-----|-----|-----------------|--------------------------|--------------------------------|--------------------------|-----------------------------|
| O. camponoti-saundersi   | Camponotus saundersi| biting leaf         | 75–85 × 2–3| –                    | 7–8         | x   |     |                 |                          | Kobmoo et al. (2012)        | Thailand                  |                            |
| O. camponoti-sexguttati  | Camponotus sexguttatus| biting leaf       | 120–140 × 3| 25–30                | 7           | x   |     |                 |                          | This study                    | Brazilian Amazon            |                            |
| O. halabalaensis        | Camponotus gigas    | biting leaf         | 60–75 × 3–5| –                    | 7–8         | x   |     |                 |                          | Luangsa-ard et al. (2011)   | Thailand                  |                            |
| O. kniflemingae         | Camponotus castaneus| biting twig         | 80–90 × 5  | 80–100               | 5–6         | x   | x   |                 |                          | This study                    | USA (South Carolina)        |                            |
| O. naomipierceae        | Polyrhachis cf. robsonii | biting leaf     | 75–105 × 5–6| na                   | 4–6         | x   | x   |                 |                          | This study                    | Australia                 |                            |
| O. oecophyllae          | Oecophylla smaragdina| biting leaf        | –         | –                    | –           | –   | x   |                 |                          | This study                    | Australia                 |                            |
| O. ootakii              | Polyrhachis sp.     | biting leaf        | 85–100 × 3 | na                   | 5           | x   |     |                 |                          | This study                    | Japan                     |                            |
| O. polyrhachis-funcata  | Polyrhachis f uncata| biting leaf        | 90–100 × 2–3| –                    | 0           | x   |     |                 |                          | Kobmoo et al. (2012)        | Thailand                  |                            |
| O. ramí                 | Camponotus sp.      | biting twig        | 200–215 × 2–3| –                    | 7–8         | x   |     |                 |                          | Kobmoo et al. (2015)        | Thailand                  |                            |
| O. satoi                | Polyrhachis sp.     | biting twig        | 85–100 × 4  | 40–50                | 5           | x   |     |                 |                          | This study                    | Japan                     |                            |
| O. septa                | Camponotus gigas    | biting leaf        | 45–50 × 6–8 | –                    | 7–8         | x   |     |                 |                          | Kobmoo et al. (2015)        | Thailand                  |                            |
| O. sp. (Gh 41)          | Polyrhachis sp.     | biting trunk       | na        | na                   | na          |     |     |                 |                          | This study                    | Ghana                     |                            |
| O. monacidis            | Dolichoderus bispinosus| base of trunk (moss)| 95–110×? | –                    | 3–4         |     |     |                 | x                         | Evans & Samson (1982)/This study | Brazilian Amazon, Colombia |                            |
| O. dacei                | Daceton armigerum    | leaf (not biting)  | –         | –                    | –           | –   | x   |                 |                          | This study                    | Brazilian Amazon, Colombia |                            |
| O. kniphofoioides       | Cephalotes atratus  | base of trunk      | 110–150 × 1.5–3| –                    | 3–5         | x   |     |                 | Evans & Samson (1982)       | Brazilian Amazon, Colombia |                            |
| O. ponerinarum          | Paraponera clavata  | base of trunk      | –         | –                    | –           | –   | x   |                 | Evans & Samson (1982)       | Brazilian Amazon, Colombia |                            |

Bold represents species described in this study.
| Species                     | Voucher information | SSU       | LSU       | TEF       | RPB1      | RPB2      | Host                      | Location        |
|-----------------------------|---------------------|-----------|-----------|-----------|-----------|-----------|---------------------------|-----------------|
| Hirsutella sp.              | NHU 12525           | EF469125  | EF469078  | EF469063  | EF469092  | EF469111  | n/a                       | n/a             |
| OSC 128575                  | EF469126            | EF469079  | EF469064  | EF469093  | EF469110  | n/a       | n/a                       | n/a             |
| Ophiocordyceps acicularis   | OSC 128580          | DQ522543  | DQ518757  | DQ522326  | DQ522371  | DQ522423  | Coleoptera                | USA             |
| OSC 110967                  | EF468950            | EF468805  | EF468744  | EF468852  | n/a       | Coleoptera | USA                       |
| OSC 110988                  | EF468951            | EF468804  | EF468745  | EF468853  | n/a       | Coleoptera | USA                       |
| ARSEF 5692                  | DQ522540            | DQ518754  | DQ522322  | DQ522388  | DQ522418  | Coleoptera | Korea                      |
| O. albacongiuae             | RC20                | KX713633  | n/a       | KX713670  | n/a       | Hymenoptera (Camponotus sp.) | Colombia        |
| O. amazonica                | HUA 186143          | KJ917562  | KJ917571  | KM411989  | KP212902  | KM411982  | Orthoptera                | Colombia        |
| HUA 186113                  | KJ917566            | KJ917571  | n/a       | KP212903  | KM411982  | Orthoptera | Colombia                |
| O. annulata                 | CEM 303             | KJ878915  | KJ878881  | KJ878962  | KJ878995  | n/a       | Coleoptera                | Japan           |
| O. aphodii                  | ARSEF 5498          | DQ522541  | DQ518755  | DQ522323  | n/a       | DQ522419  | Coleoptera                | n/a             |
| O. australis                | HUA 186147          | KC610784  | KC610764  | KC610734  | KF658678  | n/a       | Hymenoptera                | Colombia        |
| HUA 186097                  | KC610786            | KC610765  | KC610735  | KF658662  | n/a       | Hymenoptera | Colombia                |
| O. blakebarnesii            | MISSOU5             | KX713641  | KX713610  | KX713688  | KX713716  | n/a       | Hymenoptera (Camponotus sp.) | USA (Missouri) |
| MISSOU4                     | KX713642            | KX713609  | KX713885  | KX713715  | n/a       | Hymenoptera (Camponotus sp.) | USA (Missouri) |
| MISSOU3                     | KX713643            | KX713608  | KX713687  | KX713714  | n/a       | Hymenoptera (Camponotus sp.) | USA (Missouri) |
| MISSOU1                     | KX713644            | n/a       | KX713686  | KX713713  | n/a       | Hymenoptera (Camponotus sp.) | USA (Missouri) |
| O. monacidis                | MF74C                | KX713646  | KX713606  | n/a       | n/a       | n/a       | Hymenoptera (Dolichoderus bispinosus) | Brazil (Amazon) |
| MF74                        | KX713647            | KX713605  | KX713712  | n/a       | n/a       | n/a       | Hymenoptera (Dolichoderus bispinosus) | Brazil (Amazon) |
| O. brunnepunctata           | OSC 128576          | DQ522542  | DQ518756  | DQ522324  | DQ522369  | DQ522420  | Coleoptera                | n/a             |
| O. buquetii                 | HMAS_199613         | KJ878939  | KJ878904  | KJ878984  | KJ879019  | n/a       | Hymenoptera                | China           |
| HMAS_199617                 | KJ878940            | KJ878905  | KJ878985  | KJ879020  | n/a       | Hymenoptera | China                   |
| O. camponoti-atricipis      | ATR13               | KX713666  | n/a       | KX713677  | n/a       | Hymenoptera (Ophiocordyceps atriceps) | Brazil (Amazon) |
| O. camponoti-balzani        | G143                | KX713658  | KX713595  | KX713890  | KX713705  | n/a       | Hymenoptera (Camponotus balzani) | Brazil (Atlantic Rainforest) |
| G104                        | KX713660            | KX713593  | KX713689  | KX713703  | n/a       | Hymenoptera (Camponotus balzani) | Brazil (Atlantic Rainforest) |
| O. camponoti-bispinosi      | OBIS5               | KX713636  | KX713616  | KX713693  | KX713721  | n/a       | Hymenoptera (Camponotus bispinosus) | Brazil (Amazon) |
| OBIS4                       | KX713637            | KX713615  | KX713692  | KX713720  | n/a       | Hymenoptera (Camponotus bispinosus) | Brazil (Amazon) |
| OBIS3                       | KX713638            | KX713614  | KX713695  | n/a       | Hymenoptera (Camponotus bispinosus) | Brazil (Amazon) |
| OBIS                        | KX713639            | KX713612  | KX713694  | KX713718  | n/a       | Hymenoptera (Camponotus bispinosus) | Brazil (Amazon) |
| BISPI2                      | KX713665            | KX713588  | n/a       | KX713700  | n/a       | Hymenoptera (Camponotus bispinosus) | Brazil (Amazon) |
| OBIS2                       | n/a                 | KX713613  | KX713691  | KX713719  | n/a       | Hymenoptera (Camponotus bispinosus) | Brazil (Amazon) |
| Species                        | Voucher information | SSU          | LSU          | TEF          | RPB1   | RPB2   | Host                                      | Location                  |
|-------------------------------|---------------------|--------------|--------------|--------------|--------|--------|-------------------------------------------|---------------------------|
| O. camponoti-femorati         | FEMO2               | KX713663     | KX713590     | KX713678     | KX713702 | n/a    | Hymenoptera (Camponotus femoratus)        | Brazil (Amazon)           |
| O. camponoti-floridanis       | Flx1                | KX713661     | n/a          | n/a          | n/a    | n/a    | Hymenoptera (Camponotus floridanus)       | Brazil (Amazon)           |
| O. camponoti-hippocrepidis    | HIPPOC              | KX713655     | KX713597     | KX713673     | KX713707 | n/a    | Hymenoptera (Camponotus hippocrepidis)    | Brazil (Amazon)           |
| O. camponoti-indianus         | INDI2               | KX713654     | KX713598     | n/a          | n/a    | n/a    | Hymenoptera (Camponotus indianus)         | Brazil (Amazon)           |
| O. camponoti-nidulantis       | NIDUL2              | KX713640     | KX713611     | KX713669     | KX713717 | n/a    | Hymenoptera (Camponotus nidulans)         | Brazil (Amazon)           |
| O. camponoti-novogranadensis  | Mal63               | KX713648     | KX713603     | n/a          | n/a    | n/a    | Hymenoptera (Camponotus novogranadensis)  | Brazil (Atlantic Rainforest) |
| O. camponoti-novogranadensis  | Mal4                | KX713649     | KX713602     | n/a          | n/a    | n/a    | Hymenoptera (Camponotus novogranadensis)  | Brazil (Atlantic Rainforest) |
| O. camponoti-renegeri         | RENG2               | KX713632     | n/a          | KX713672     | n/a    | n/a    | Hymenoptera (Camponotus renegeri)         | Brazil (Amazon)           |
| O. camponoti-rufipes          | G177                | KX713657     | KX713596     | KX713680     | n/a    | n/a    | Hymenoptera (Camponotus rufipes)          | Brazil (Atlantic Rainforest) |
| O. citrina                    | TNS F18537          | n/a          | KJ878903     | KJ878983     | n/a    | KJ878954 | Hemiptera                                  | Japan                     |
| O. flavus                     | NBRC 106962         | JN941726     | n/a          | JN941415     | n/a    | JN992460 | n/a                                        | Japan                     |
| O. flavus                     | NBRC 106961         | JN941727     | n/a          | JN941414     | n/a    | JN992461 | n/a                                        | Japan                     |
| O. flavus                     | CEM1762             | KJ878916     | KJ878882     | KJ878963     | KJ878996 | n/a    | Coleoptera                                 | China                     |
| O. flavus                     | CEM1763             | KJ878883     | KJ878883     | KJ878964     | KJ878997 | n/a    | Coleoptera                                 | China                     |
| O. flavus                     | J19                 | KX713650     | KX713601     | KX713684     | KX713710 | n/a    | Hymenoptera (Polyrhachis lamellidens)     | Japan                     |
| O. flavus                     | J7                  | KX713653     | KX713599     | KX713683     | KX713711 | n/a    | Hymenoptera (Polyrhachis lamellidens)     | Japan                     |
| O. flavus                     | HMAS, 199612        | KJ878917     | KJ878884     | KJ878965     | KJ878998 | n/a    | Lepidoptera                                | China                     |
| O. flavus                     | NHU 12581           | EF468973     | EF468831     | EF468775     | n/a    | EF468930 | Coleoptera                                 | Japan                     |
| O. flavus                     | NHU 12582           | EF468975     | EF468830     | EF468771     | n/a    | EF468926 | Coleoptera                                 | Japan                     |
| O. flavus                     | OSC 151910          | KJ878918     | KJ878885     | KJ878999     | n/a    | n/a    | Coleoptera                                 | Guyana                    |
| O. dacei                      | MF01                | n/a          | KX713604     | KX713667     | n/a    | n/a    | Hymenoptera (Daceton armigerum)           | Brazil (Amazon)           |
| O. dipterigena                | OSC 151911          | KJ878919     | KJ878886     | KJ878966     | KJ879000 | n/a    | Diptera                                    | USA                       |
| O. dipterigena                | OSC 151912          | KJ878920     | KJ878887     | KJ878967     | KJ879001 | n/a    | Diptera                                    | USA                       |
| O. elongata                   | OSC 110989          | n/a          | EF468808     | EF468748     | EF468856 | n/a    | Lepidoptera                                | n/a                       |
| O. entomorrhiza               | KEW 53484           | EF468954     | EF468809     | EF468749     | EF468857 | EF468911 | Lepidoptera                                | Japan                     |
| O. entomorrhiza               | TNS 16252           | KJ878941     | KJ878906     | KJ878986     | n/a    | n/a    | Coleoptera                                 | Japan                     |
| Species               | Voucher information | SSU   | LSU   | TEF   | RPB1    | RPB2    | Host            | Location       |
|----------------------|---------------------|-------|-------|-------|---------|---------|-----------------|----------------|
| O. formicarum        | TNS 16250           | KJ878942 | n/a  | KJ878987 | KJ879021 | n/a      | Coleoptera      | Japan          |
| O. formosana         | TNS F18565          | KJ878921 | KJ878888 | KJ878968 | KJ879002 | KJ87946 | Hymenoptera     | Japan          |
| O. forquignoni       | OSC 151902          | KJ878912 | KJ878876 | n/a  | KJ878991 | KJ878945 | Coleoptera      | Taiwan         |
| O. gracilis          | EFCC 3101           | EF468955 | EF468810 | EF468750 | EF468858 | EF468913 | Lepidoptera     | n/a            |
| O. gracilissima      | Ophgrc679           | n/a             | K610768 | K610744 | K658666 | n/a      | Coleoptera      | Colombia       |
| O. heteropoda        | OSC 106404          | AY489690 | AY489722 | AY489617 | AY489651 | n/a      | Hemiptera       | Australia      |
| O. irangiensis       | OSC 128577          | DQ522546 | DQ518760 | DQ522329 | DQ522374 | DQ522427 | Hymenoptera     | n/a            |
| O. kimbemlingiae     | SC03B               | KX713619 | KX713622 | KX713699 | KX713727 | n/a      | Hymenoptera (Camponotus castaneus/americanus) | USA (South Carolina) |
| O. konnoana          | EFCC 7295           | EF468958 | n/a             | EF468862 | EF468915 | n/a      | Coleoptera      | Korea          |
| O. longissima        | TNS F18448          | KJ878925 | KJ878971 | KJ879005 | n/a      | n/a      | Hemiptera       | Japan          |
| O. longissima        | HMAS_199600         | KJ878926 | n/a             | KJ878972 | KJ879006 | KJ87949 | Hemiptera       | China          |
| O. longissima        | EFCC 6814           | n/a             | EF468817 | EF468757 | EF468865 | n/a      | Hemiptera       | Korea          |
| O. kniphooides       | Ophkno975           | K610790 | K658867 | K610739 | K658667 | K610717 | Hymenoptera     | Colombia       |
| O. konnoana          | EFCC 7295           | EF468958 | n/a             | EF468862 | EF468915 | n/a      | Coleoptera      | Korea          |
| O. lloydi            | TNS 16250           | KJ878942 | KJ878987 | KJ879021 | KJ879021 | KJ87946 | Hymenoptera     | Japan          |
| O. longissima        | TNS 16250           | KJ878942 | KJ878987 | KJ879021 | KJ879021 | KJ87946 | Hymenoptera     | Japan          |
| O. myrmecophila      | HMAS_199620         | KJ878927 | KJ878973 | KJ879007 | n/a      | n/a      | Hymenoptera     | China          |
| O. myrmecophila      | CEM1710             | KJ878928 | KJ878944 | KJ878974 | KJ879008 | n/a      | Hymenoptera     | China          |
| O. myrmecophila      | TNS 27120           | KJ878929 | KJ878985 | KJ879009 | n/a      | n/a      | Hymenoptera     | Japan          |
| Species             | Voucher information | SSU        | LSU        | TEF      | RPB1     | RPB2     | Host                      | Location                  |
|---------------------|---------------------|------------|------------|----------|----------|----------|---------------------------|---------------------------|
| O. naomipierceae    | DAWKSANT            | KX713664   | KX713589   | n/a      | KX713701 | n/a      | Hymenoptera (Polyrhachis cf. robsonii) | n/a                       |
| O. neovolkiana      | OSC 151903          | KJ878930   | KJ878966   | KJ878976 | KJ879010 | n/a      | Coleoptera                | Japan                     |
| O. nigrella         | EFCC 9247           | EF468963   | EF468818   | EF468758 | EF468666 | EF468920 | Korea                      |                           |
| O. nutans           | OSC 110994          | DG522549   | DG518763   | DG522333 | DG522378 | n/a      | Hemiptera                 | n/a                       |
| O. odonatae         | TNS F18563          | n/a        | KJ878677   | n/a      | KJ878922 | n/a      | Odontata                  | Japan                     |
|                     | TNS 27117           | n/a        | KJ878678   | n/a      | n/a      | n/a      | Odontata                  | Japan                     |
| O. oecophyllae      | OECO1               | KX713635   | n/a        | n/a      | n/a      | n/a      | Hymenoptera (Oecophylla smaragdina) | Australia                 |
| O. ootakii          | J14                 | KX713651   | n/a        | KX713882 | KX713709 | n/a      | Hymenoptera (Polyrhachis moesta) | Japan                     |
|                     | J13                 | KX713652   | KX713600   | KX713681 | KX713708 | n/a      | Hymenoptera (Polyrhachis moesta) | Japan                     |
| O. ponerinarum      | HUA 186140          | KX61079    | KX610767   | KX610740 | KX658668 | n/a      | Hymenoptera (Paraponera clavata) | Brazil, Colombia, Ecuador |
| O. pulvinata        | TNS-F 30044         | GU904208   | GU904209   | GU904210 | n/a      | n/a      | Hymenoptera (Camponotus excavatus) | Japan                     |
| O. purpureostromata | TNS F18430          | KJ878931   | KJ878977   | KJ879011 | n/a      | n/a      | Coleoptera                | Japan                     |
| O. ravenelli        | OSC 110995          | DG522550   | DG518764   | DG522334 | DG522379 | DG522430 | Coleoptera                | n/a                       |
| O. rhenizoa         | NHU 12529           | EF468969   | EF468824   | EF468765 | EF468672 | EF468922 | Coleoptera                | n/a                       |
| O. sinensis         | EFCC 7287           | EF468971   | EF468827   | EF468767 | EF468874 | EF468924 | Lepidoptera               | n/a                       |
| O. sobolifera       | KEW 78842           | EF468972   | EF468828   | n/a      | EF468875 | EF468925 | Hemiptera                 | n/a                       |
|                     | TNS F18521          | KJ878933   | KJ878979   | KJ879013 | n/a      | n/a      | Hemiptera                 | Japan                     |
| O. sp.              | GH41                | KX713656   | KX713688   | KX713706 | KX713706 | KX713706 | Hymenoptera (Polyrhachis sp.) | Ghana (Alewa)             |
|                     | TNS F18495          | KJ878934   | KJ878989   | KJ878980 | KJ879014 | KJ879014 | Hemiptera                 | USA                       |
| O. sp.              | OSC 151904          | KJ878935   | KJ878981   | KJ879015 | KJ879051 | KJ879051 | Hemiptera                 | USA                       |
| O. sp.              | OSC 151905          | KJ878936   | KJ878900   | KJ878982 | KJ879016 | KJ879016 | Hemiptera                 | Guyana                    |
| O. sp.              | OSC 110998          | DG522551   | DG518765   | DG522336 | DG522381 | DG522432 | Hymenoptera               | n/a                       |
| O. stylophora       | OSC 111000          | DG522552   | DG518766   | DG522337 | DG522382 | DG522433 | Coleoptera                | n/a                       |
| O. tricentri        | CEM 160             | AB027330   | AB027376   | n/a      | n/a      | n/a      | Hemiptera                 | n/a                       |
| O. unilateralis     | OSC 128574          | DG522554   | DG518768   | DG522339 | DG522385 | DG522436 | Hymenoptera               | Thailand                  |
|                     | SERI2               | KX713627   | KX713676   | KX713731 | n/a      | n/a      | Hymenoptera (Camponotus sericeiventris) | n/a                       |
|                     | SERI1               | KX713628   | KX713626   | KX713675 | KX713730 | n/a      | Hymenoptera (Camponotus sericeiventris) | Brazil (Atlantic Rainforest) |
| O. variabilis       | ARSEF 5365          | DG522555   | DG518769   | DG522340 | DG522386 | DG522437 | Diptera                    | n/a                       |
| O. yakusimensis     | OSC 111003          | EF468985   | EF468839   | EF468779 | EF468856 | EF468933 | Hemiptera                 | USA                       |
|                     | HMAS_199604         | KJ878938   | KJ878902   | KJ879018 | KJ878953 | KJ878953 | Hemiptera                 | China                     |

**Table 2.** (Continued.)
RESULTS

DNA sequencing

We used a BLAST search in the GenBank nucleotide database to ensure the quality of the sequences generated in this study. Sequences that were identified as species not closely related to the species treated in this study were discarded and interpreted to be from a contaminant. All the sequences included here passed the above quality control checks.

Phylogenetic relationships

Phylogenetic analyses recovered the topology presented by Sung et al. (2007) and Quandt et al. (2014) with bootstrap proportions (BP=) of 99 % for family level, i.e. Ophiocordycipitaceae and 81 % for generic level, i.e. Ophiocordycips. The O. unilateralis clade was resolved as a monophyletic group of 23 species with bs = 100 % and was resolved as a monophyletic group of 23 species with bs = 77 %. We refer to the O. unilateralis core clade as the clade formed by the following species: O. kimflemmingiae sp. nov., O. camponoti-hippocrepidis sp. nov., O. camponoti-remgerri sp. nov., O. albacoangiae sp. nov., O. camponoti-nidulantis sp. nov., O. camponoti-atricularis, O. camponoti-floridans sp. nov., O. camponoti-balzani, O. camponoti-rufepedis, O. camponoti-femorati sp. nov., O. camponoti-chaetcladis sp. nov., O. camponoti-bispinis, O. pulvinata, O. blakebarnesii sp. nov., O. rami, O. naomiipergae sp. nov., O. ootaki sp. nov., O. halabalaensis, O. camponoti-saunderisi, O. satoi nom. et. stat. nov., O. polyrhachis-furcata and O. camponoti-leonardi. Thre are three other species described previously that also belong to this clade but were not included in this study due to lack of molecular data: O. camponoti-melanoticus, O. camponoti-indianus and O. camponoti-novogranadensis. Future field surveys will address the recollection of these species for molecular studies.

The O. unilateralis clade is strongly supported (BP = 100), with an emergent internal structure. There is a strongly supported clade of Old World species (BP = 90), as well as a poorly supported node splitting species comprised largely of New World taxa, but also including O. pulvinata, a species known only from Japan. The species within the O. kniphofioides sub-clade share a broad range of morphological and ecological traits, which reflects in their phylogenetic placement as a monophyletic group.

TAXONOMIC TREATMENT

Ophiocordycips daceti Araújo, H.C. Evans & D.P. Hughes, sp. nov. MycoBank MB822289. Fig. 1.

Etymology: Named after the ant host genus, Dactoton.

Specimens examined: Brazil: Reserve Adolpho Ducke, Manaus, Amazonas, on Dactoton armigerum (Latreille) (Myrmicinae: Dactotini), 15 Jan. 2016, J.P.M. Araújo, holotype INPA 274561.

External mycelium scarce, ginger brown. Single synnema arising from the dorsal pronotum, 1.2 cm in length, cylindrical, velvety, ginger brown, covered with Hostutella-like phialides. No sexual morph observed.

Asexual morph: Hirsutella-like phialides; cylindrical to lageniform, averaging 16–18 × 4 μm, tapering to a long neck 4–6 μm in length; verrucose. Conidia cylindrical, smooth, 7–10 × 3 μm.

Habitat: Brazilian Central Amazon rainforest. Host found attached to a leaf in the leaf litter. It was assumed the ant had died attached to the leaf when it was still on the plant. This is because other samples (n = 5) were found attached to the petiole or abaxial surface of leaves in the understorey vegetation (<1.5 m). No biting behaviour was observed, but attachment to the substrate was by the host's legs. The highly distinctive trap-jawed ant is strictly arboreal (Dejean et al., 2012), to such an extent that when an ant falls from the canopy it glides and can direct its descent enabling it to land on tree trunks (Yano et al., 2005). The fact that diseased ants are found in the litter and understorey layers indicates a dramatic behavioural change following infection.

Additional specimens examined: Paratypes: Brazil, Reserva Adolpho Ducke: locality as above, 22 Jan. 2016, J.P.M. Araújo, U04 (INPA 274562).

Ophiocordycips oecophyllae Araújo, S. Abell, T. Marney, R. Shivas, H.C. Evans & D.P. Hughes, sp. nov. MycoBank MB822290. Fig. 2.

Etymology: Named after the host ant genus, Oecophylla.

Specimens examined: Australia, Lutilca State Forest, Wongaling Beach, Queensland, on Oecophylla smaragdina (Formicinae: Oecophyllini), 8 Jun. 2015, S. Abell, T.S. Marney, R.G. Shivis, holotype BRIP 62635.

Mycelium emerging from leg joints and fissures, superficial on exoskeleton, white at early stages becoming brown with age. Conidiophores initially sterigmatic, integrated (not on synnema), ampulliform, 3–8 × 1–2 μm, with an apical sterigmate-like appendage up to 10 μm, at maturity phialidial, integrated in hyphae, gradually tapering 30–80 μm long, 5–7 μm at base, septate, pale brown at base becoming subhyaline at apex, straight or slightly curved, occasionally branched one or more times forming more complex structures. Conidiophores cells 30–50 μm, 3–4 μm at base, tapering evenly to 1–1.5 μm at apex, terminal, subhyaline. Conidia ovoid to cylindrical, 5.5–10 × 1.5–3 μm, hyaline, smooth, rounded at apex, truncate at base, slightly darkened periclinally at base.

No sexual morph observed in any of the infected Oecophylla smaragdina collected.

Habitat: Tropical Australia, rainforest. Found biting leaves at elevated positions on understorey shrubs in coastal forest; common, associated with epizootics, and characterized by the absence of the abdomen, whole or part legs, antennae (see Fig. 2A), or with only the head remaining. We suggest that the activity of other Oecophylla ants resulted in the loss of body parts. This may be because of an anti-parasite behaviour by the uninfected colony members or it may be a parasite strategy where onward infection requires close contact between susceptible ants and cadavers. Whatever possible explanation, it is likely that onward transmission requires contact and this contact is aggressive behaviour by the healthy ants leading to extensive cadaver damage. Such behaviour may explain the absence of the sexual morph and the dominance of the asexual morph due to insufficient nutrients. Typically, the abdomen of infected ants is packed with lipid-filled hyphal bodies providing the resources for stromatal development.
Ophiocordyceps daceti. A. Infected Daceton armigerum on the leaf litter. B. Cross-section of the synnema. C. Close-up of synnema showing the Hirsutella hymenium. D–F. Verrucose phialides. G. Phialides at early developmental stage. H. Close-up of the hymenium of verrucose Hirsutella phialides. Scale bars: A = 5 mm, B = 200 μm, C = 50 μm, D–H = 10 μm.
Fig. 2. A. Oecophylla smaragdina infected and biting the main vein of a leaf. B. Leg joints with phialides. C–D. Phialides. E. Phialides and conidia. Scale bars: A = 1 mm, B = 0.4 mm, C–E = 10 μm.
**Ophiocordyceps camponoti-sexguttati** Araújo, H.C. Evans & D.P. Hughes, **sp. nov.** MycoBank MB822291. Fig. 3.

**Etymology:** Named after the host ant species, *Camponotus sexguttatus*.

Specimen examined: **Brazil**, Reserva Adolpho Ducke, Manaus, Amazonas, on *Camponotus sexguttatus* (Formicidae: Camponotini), 16 Jan. 2015, J.P.M. Araújo, holotype INPA 274563.

Mycelium produced sparsely from joints, not covering the host body, dense when touching the substrate, dark brown. Stroma single, arising from the dorsal pronotum, never branching, averaging 1.8–2 cm in length, 0.2 mm thick, dark brown at the base turning lighter brown towards the apex; fertile part consisting of a single lateral cushion, disc-shaped, chestnut-brown, averaging 1 × 1 mm. Perithecia immersed to partially erumpent, flask-shaped, (205–) 225–230 (–265) × 135 (–180) μm with short neck. Ascii 8-spored, hyaline, cylindrical, 150–160 × 8–9 μm; apical cap prominent, 6 × 3 μm. Ascospores hyaline, thin-walled, multiguttulate, cylindrical, 120–140 × 3 μm, 7-septate, straight or curved tapering to the apex.

**Asexual morph:** Hirsutella A-type associated with apical region of stroma; phialides lageniform, 5–8 × 3–4 μm, tapering to a long neck, 8–12 μm; conidia hyaline, limoniform, 5 × 2 μm.

**Germination process:** Ascospores released on agar germinated after 72 h to produce a single, straight capilliciophidium; 25–30 μm, bearing a terminal capillicioidium, hyaline, smooth-walled, guttulate, 5–9 × 2 μm, narrowing apically.

**Habitat:** Brazilian Central Amazon, rainforest. Infected ants of this ground-dwelling species found biting onto palm-tree leaves, rare.

**Ophiocordyceps camponoti-renggeri** Araújo, H.C. Evans & D.P. Hughes, **sp. nov.** MycoBank MB822292. Figs 4, 5.

**Etymology:** Named after the host ant species, *Camponotus renggeri*.

Specimen examined: **Brazil**, Reserva Adolpho Ducke, Manaus, Amazonas, on *Camponotus renggeri* (Formicidae: Camponotini), 17 Jan. 2015, J.P.M. Araújo, holotype INPA 274564.

External mycelium covering most of the host, produced from all orifices and sutures, brown at maturity. Stroma single, rarely branched, produced from dorsal pronotum, averaging 15–20 mm, up to 30 mm, cylindrical, velvety and dark brown, tapering towards the apex; Fertile region (ascoma) of lateral cushions, 1–2, hemispherical to globose, dark-brown to black, variable in size, averaging 1–1.5 × 0.8–1 mm. Perithecia immersed to partially erumpent, flask-shaped, 220–250 × 100–165 μm, with pronounced ostiole, Ascii 8-spored, hyaline, cylindrical, (110–)130–145 × 8–10 μm; with prominent cap, 7–8 × 3 μm. Ascospores hyaline, thin walled, vermiform, 90–120 × 4 μm, 5–8-septate, straight to sinuous, round to slightly tapered at apex.

**Asexual morph:** Hirsutella A-type not observed. Hirsutella C-type, produced from brown cushions (sporodochia) on leg and antennal joints; phialides subulate at base, 40–60 × 3–5 μm long, tapering to a long, hyaline neck. Conidia not observed.

**Germination process:** All the ascospores remained unchanged after five days on water-agar plates. Similar non-germination has been reported in *O. camponoti-melanotici* (Evans et al. 2011).

**Habitat:** Brazilian Central Amazon, rainforest. Consistently associated with and biting onto moss at the base of upperstorey trees; sometimes buried underneath the moss mat. This ground-nesting ant is closely related to and frequently confused with *C. rufulipes*, but infection behaviour is different with the latter species always found 1.5–2 m above the ground biting into branches and leaves of understorey shrubs (Evans et al. 2011).

**Ophiocordyceps camponoti-chartifex** Araújo, H.C. Evans & D.P. Hughes, **sp. nov.** MycoBank MB822293. Fig. 6.

**Etymology:** Named after the host ant species, *Camponotus chartifex*.

Specimen examined: **Brazil**, Reserva Adolpho Ducke, Manaus, Amazonas, on *Camponotus chartifex* (Formicidae: Camponotini), 2 Feb. 2015, J.P.M. Araújo & H.C. Evans, holotype INPA 274566.

Mycelium growing from all inter-segmental membranes, often covering the host body; initially white turning brown. Stroma single, produced from dorsal pronotum, averaging 10 mm, up to 15 mm in length, cylindrical, velvety and ginger brown, becoming cream-pinkish at the apical part; fertile region of lateral cushions, 1–2, hemispherical, chocolate brown, darkening with age, slightly variable in size, averaging 1.5 × 1 mm. Perithecia immersed to partially erumpent, globose-hemispherical shaped, 200–235 × 135–175 μm, with short neck. Ascii 8-spored, hyaline, cylindrical to clavate, 100–125 × 6 μm; with prominent cap, 6–7 × 3–4 μm. Ascospores hyaline, thin-walled, vermiform 75–85 × 5 μm, 9–13-septate, sinuous to curved, never straight at maturity; rounded to acute apex.

**Asexual morph:** Hirsutella A-type associated with apical region of stromata; phialides lageniform, 5–6 × 3 μm, tapering to a robust neck, 4–8 μm in length; conidia fusiform to limoniform, averaging 7 × 2.6 μm.

**Germination process:** The released ascospores germinated within 24 h to produce a single, long and extremely narrow hair-like capilliciophidium; variable in length (65–75–90–95) μm; bearing a single terminal capillicioidium, hyaline, smooth-walled, uni- or biguttulate, fusoid, narrowing apically.

**Habitat:** Brazilian Central Amazon, rainforest. Biting exclusively on palm-tree parts, especially the spines and leaves. This species was relatively rare and the host is an arboreal species which weaves primitive carton nests in the canopy; found 1–1.5 m above the ground.

**Ophiocordyceps camponoti-nidulantis** Araújo, H.C. Evans & D.P. Hughes, **sp. nov.** MycoBank MB822294. Figs 7, 8.

**Etymology:** Named after the host ant species, *Camponotus nidulans*.

Specimen examined: **Brazil**, Reserva Adolpho Ducke, Manaus, Amazonas, on *Camponotus nidulans* (Formicidae: Camponotini), 20 Jan. 2015, J.P.M. Araújo, holotype INPA 274568.

External mycelium produced from all orifices and sutures; initially white, becoming ginger brown, covering the host body, notably the abdominal part. Stroma single, produced from dorsal pronotum, 10–15 × 0.2 mm, cylindrical, black, covered with ginger velvety hyphae fading away towards the apex; fertile region of lateral cushions, 1–2, disc-shaped to hemispherical, light brown, darkening with age, averaging 1.5 × 1 mm. Perithecia immersed to partially erumpent, flask-shaped, (170–)200–240 × 100–150 (–180) μm, with short, exposed neck or ostiole. Ascii 8-
Fig. 3. *Ophiocordyceps camponoti–sexguttati*. A. *Camponotus sexguttatus* biting into vegetation with the long stroma arising from its dorsal pronotum. B. Close-up of the ascma. C. Section through ascma showing the perithecial arrangement. D. Close-up of perithecium. E. Long ascospores with the straight capilliconidiophore bearing an apical capilliconidium. F. Ascus. Scale bars: A = 5 mm, B = 1 mm, C = 100 μm, D = 50 μm, E–F = 20 μm.
Fig. 4. Ophiocordyceps camponoti–renggeri. A. Camponotus renggeri, dead and attached to bryophytes on the base of trees. B. Close-up of the fertile part (ascoma). C. Section through ascoma showing the perithecial arrangement. D. Close-up of perithecium. E. Asci. F. Ascospores. Scale bars: A = 5 mm, B = 1 mm, C = 250 μm, D = 50 μm, E = 70 μm, F = 20 μm.
spored, hyaline, thin-walled, vermiform to clavate, 110–145 × 6–8 μm; cap prominent, 4 × 6 μm; Ascospores hyaline, thin-walled, vermiform, 90–105(–115) × 3–4 μm, 5-septate, gently curved, rarely straight; tapering to a round apex.

Asexual morph: Hirustella A-type associated with the apical part of stroma. Hirustella C-type, produced from light brown cushions on leg and antennal joints; phialides subulate, robust, 70–120 × 4–6(–8) μm. Conidia limoniform, averaging 8 × 3 μm.

Germination process: Ascospores germinating after 24–72 h to produce 1–3, uniformly straight, extremely narrow hair-like capilliconidiophores, 50–60 μm; bearing a single terminal capilliconidium, hyaline, smooth-walled, biguttulate, clavate, 9 × 2 μm, narrowing apically.

Habitat: Brazilian Central Amazon, rainforest. Biting sapling leaves and petioles, always at lower heights, 20–30 cm above the ground; forming local epizootics or aggregations of up to 20–30 individuals in about 10 m².

**Ophiocordyceps camponoti-femorati** Araújo, H.C. Evans & D.P. Hughes, sp. nov. MycoBank MB822295. Fig. 9.

*Etymology:* Named after the host ant species, *Camponotus femoratus*.

Specimen examined: Brazil, Reserva Adolpho Ducke, Manaus, Amazonas, on *Camponotus femoratus* (Formicidae: Camponotini), 22 Jan. 2015, J.P.M. Araújo, holotype INPA 274570.

External mycelium produced from all the orifices and sutures; initially white, becoming ginger brown, covering the host body with sparse hyphae. Stroma single, produced from dorsal pronotum, averaging 3.5 × 0.25, up to 6 mm in length, cylindrical to laterally compressed, ginger to dark-brown; fertile part terminal of lateral cushions, 1–3, disc-shaped to hemispherical, chestnut-brown, darkening with age, 1.2–2.2 × 0.8–1.4 mm.
Fig. 6. Ophiocordyceps camponoti–chartificis. A. Camponotus chartiflex biting onto a palm leaf. B. Close-up of the ascoma. C. Cross section of the ascoma showing the perithecial arrangement. D. Close-up of the perithecium. E. Ascus with ascospores arranged within. F. Non-germinated ascospore. G. Ascospore with long capilliconidia. H. Hirsutella A-type phialides on the stroma. Scale bars. A = 5 mm, B = 1 mm, C = 200 μm, D = 30 μm, E–F = 5 μm, G–H = 10 μm.
Fig. 7. *Ophiocordyces camponoti–nidulantis* (sexual morph). A. *Camponotus nidulans* infected and biting into a leaf (with fly larvae on the stroma). B. Close-up of the ascoma. C. Section through ascoma showing the perithecial arrangement. D. Close-up of perithecium. E. Asci. F. Ascospore with capilliconidium. Scale bars: A = 3 mm, B = 1 mm, C = 200 μm, D = 75 μm, E=F = 20 μm.
Perithecia immersed to partially erumpent, flask-shaped, 200–230(–250) × 135–165 μm, with short, exposed neck or ostiole. Asci 8-spored, hyaline, cylindrical to clavate, 110–130 × 8–9 μm; cap prominent, 6 × 3 μm; Ascospores hyaline, sinuous to curved, rarely straight, 75–90 × 3 μm, 5-septate; apex round to acute.

Asexual morph: Hirsutella A-type only; produced laterally on upper stroma; phialides rare, cylindrical to lageniform,
7–10 × 3–4 μm, tapering to a long neck, 10–15 μm; conidia limoniform, averaging 7–9 × 3 μm.

Germination process: Ascospores germinated in 24–48 h to produce a single, narrow capilliconidiophore, 35–40 μm long; bearing a single capilliconidium, hyaline, smooth-walled, uni–to biguttulate, clavate, 9 × 3 μm, narrowing apically.

Habitat: Brazilian Central Amazon, rainforest. Often associated with palm-trees, commonly on spines towards the tip, where droplets of dew collect. Abundant species, forming epizootics. The ant *Camponotus femoratus* is an arboreal species involved in an unusual mutualism (parabiosis) with other ants in which it constructs carton nests embedded with epiphytes that it "gardens" (Vantaux et al. 2007). This suggests that infected ants move away from their arboreal nests among the epiphytes on upper-storey trees and die biting onto palm vegetation.

**Ophiocordyceps camponoti-hippocrepidis** Araújo, H.C. Evans & D.P. Hughes, sp. nov. MycoBank MB822296. Fig. 10.

Etymology: Named after the host ant species, *Camponotus hippocrepidis*.

Specimen examined: Brazil, Reserva Adolpho Ducke, Manaus, Amazonas, on Camponotus (Myrmorhachis) hippocrepidis (Formicidae: Camponotini), 22 Jan. 2015, J.P.M. Araújo, holotype INPA 274572.

External mycelium produced from all the orifices and sutures; initially white, becoming ginger brown, covering the host body with

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**Fig. 9. Ophiocordyceps camponoti–femorati.** A. Camponotus femoratus biting a palm leaf. B. Close-up of the ascoma. C. Cross section showing the perithecial arrangement. D. Ascospore with capilliconidia. E. Hirsutella A-type phialide on the stroma. Scale bars: A = 1 mm, B = 0.3 mm, C = 200 μm, D = 20 μm, E = 5 μm.
Fig. 10. Ophiocordyceps camponoti-hippocrepidis. **A.** Minute Camponotus hippocrepis (ca. 1.5 mm) biting onto a palm spine. **B.** Close-up of the ascoma. **C.** Cross-section of the ascoma showing the perithecial arrangement. **D.** Ascospore with capilliconidiophore with verrucose apical portion. **E.** Ascus. **F.** Ascospores just after being released. Scale bars: A = 1 mm, B = 0.5 mm, C = 100 μm, D–E = 20 μm, F = 20 μm.
Fig. 11. Ophiocordyceps albacongiuae. A. Camponotus sp. with two fruiting bodies emerging from its dorsal pronotum and petiole. B. Sterile synnema with its hairy surface. C. Cross-section of the ascoma. D. Ascus. E. Ascospore. F. Close-up of the perithecium. Scale bars: A = 0.2 mm, B = 100 μm, C = 200 μm, D = 20 μm, E = 10 μm, F = 30 μm.
sparse hyphae. Stroma single, produced from dorsal pronotum, 5–7 × 0.15 mm, cylindrical ginger to dark-brown, characteristically swollen terminal part, clavate: ascomatal plate consistently produced at the middle part of stroma, laterally attached, circular, chestnut brown, darkening with age, averaging 2–2.5 × 0.25–0.45 mm; Perithecia immersed to partially erumpent, flask-shaped, averaging 225–250 × 135–165 μm, with short, exposed neck or ostiole. Asci 8-spored, cylindrical to clavate, 115–135 × 7–10 μm, cap prominent, 6–7 × 4 μm; Ascospores hyaline, cylindrical, robust, straight to gently curved, 75–85 × 4–5 μm, 5-septate, tapering to a round or slightly acute apex.

Asexual morph: Hirsutella A-type only; produced on the clavate part of upper stroma; phialides cylindrical to lageniform, 8–9 × 4 μm, tapering to a long neck 9–10 μm in length; conidia limoniform, averaging 5 × 2 μm.

Germination process: Ascospores germinated within 24–48 h to produce a straight, robust capilliconidiophore, verrucose near the apex, 45–50 μm long; bearing a single capilliconidium, hyaline, smooth-walled, guttulate, 10–11 × 4 μm, truncate at base, narrowing apically.

Habitat: Brazilian Central Amazon, rainforest. Predominantly associated with spiny palms. Found often at the tip of spines, where drops of dew accumulate and surround the whole ant. Abundant species, 10–20 infected ants commonly found on a single palm tree.

Ophiocordyceps albacongiuae Araújo, H.C. Evans & D.P. Hughes, sp. nov. MycoBank MB822297. Fig. 11.

Etymology: Named after Alba Congiu, wife of David Hughes, who has contributed so much to our understanding of Ophiocordyceps by facilitating the extensive travels of the senior author (Hughes) in SE Asia, Australia and South America in search of behaviourally-manipulated ants.

Specimen examined: Colombia, Rio Claro, Reserva Nacional Canyon Rio Claro, Antioquia, on Camponotus sp., 14 Nov. 2014, J.P.M. Araújo & T.I. Sanjuán, holotype HUA 186117.

External mycelium scarce, producing one or two stromata, never branching, dark brown, basal part velvety, tapering towards the apex; producing single ascoma laterally attached, disc-shaped, dark brown; Perithecia semi-immersed, flask-shaped, 240–290 × 105–135 μm, with prominent, exposed neck or ostiole. Asci 8-spored, cylindrical to clavate, 130–160 × 8–11 μm. Ascospore hyaline, cylindrical, slightly curved in “S”, 80–100 × 5 μm, 5–6-septate, tapering towards the apex.

Asexual morph: No phialides or conidia observed.

Germination process: No ascospores naturally released from dried herbarium material.

Habitat: In tropical lowland forest along Rio Claro. Typically found biting on epiphytes on tree trunks at elevated positions, ranging from 0.5 m up to 2 m in height.

Ophiocordyceps camponoti-floridanus Araújo, H.C. Evans & D.P. Hughes, sp. nov. MycoBank MB822299. Fig. 12.

Etymology: Named after the host ant species, Camponotus floridanus.

Specimen examined: USA, Broward County, Florida, on Camponotus floridanus (Formicidae: Camponotini), 15 Nov. 2015, Colbie Reed, holotype INPA 274575.

Abundant external mycelium produced from the sutures and joints. Stroma single, never branching, ginger to light brown, basal part velvety, apical part cream; fertile part laterally attached, disc-shaped; chocolate brown; Perithecia immersed to partially erumpent, flask-shaped, (253–)265 × (300–)100–125 μm, with short, exposed neck or ostiole. Asci 8-spored, cylindrical to clavate, 145 × 9–10 μm; Ascospore hyaline, cylindrical, straight, 75–90 × 4–5 μm, 5-septate, tapering towards the apices.

Asexual morph: Hirsutella A-type present along the stroma. Phialides smooth, cylindrical to lageniform, averaging 8–9 × 3–4 μm, tapering to a long neck 8–12 μm in length. Conidia limoniform, bittiguttulate, 8–9 × 3 μm.

Germination process: No ascospores released from dried herbarium material.

Habitat: Florida (USA), lowland forests. A ground-dwelling ant, found biting leaves, predominantly palms. Dying in elevated position, ranging from 0.5 m up to 1.5 m in height.

Ophiocordyceps kimmealingiae Araújo, H.C. Evans & D.P. Hughes, sp. nov. MycoBank MB822300 Fig. 13.

Etymology: Named after Kim Fleming, a naturalist, who has made a significant contribution to the studies between this fungus and Camponotus species in the USA. An image posted by Kim Fleming on the photosharing site Flickr (https://www.flickr.com) alerted Hughes to the widespread occurrence of this system in temperate woods in South Carolina. Kim has subsequently taken thousands of images and recordings of the phenology of Ophiocordyceps in South Carolina.

Material examined: USA, Donalds County, South Carolina, on Camponotus castaneus (Formicinae: Camponotini), 15 Aug. 2014, J.P.M. Araújo & K. Fleming, holotype INPA 274577.

External mycelium produced mostly on the ventral part of the host and head. Sparse mycelium produced on joints. Stroma single, rarely branched, produced from dorsal pronotum, 11–17 × 0.3–0.45 mm, cylindrical, ginger to light brown, basal part velvety, apical part cream to purple; fertile part laterally attached, disc-shaped, dark-brown to black, averaging 1.5–2 × 1.3 mm; Perithecia immersed to partially erumpent, flask-shaped, 250–275 × (100–)120–160 μm, with short, exposed neck or ostiole. Asci 8-spored, cylindrical to clavate, (100–)120–150 × 10–11 μm, cap prominent; Ascospore hyaline, cylindrical, straight, 80–90 × 5 μm, 5–6-septate, tapering towards the apices.

Asexual morph: Hirsutella A-type present on the stroma, Hirsutella C-type occurring exclusively at early stages of development, produced from leg joints and dorsal pronotum.

Germination process: Ascospores germinating from the first 24 h up to the 5th day. Germination occurred in two different manners: capilliconidiophores or germination into vegetative hyphae, separately or both on the same ascospores. Capilliconidiophores 1–3, 80–100 μm long, with a terminal capilliconidium, 10–13 × 2–3 μm.

Habitat: South Carolina (USA), temperate deciduous forest. Found biting underside of twigs, never leaves. Dying in elevated position, ranging from 0.5 m up to 1.5 m in height; forming patches, or graveyards, of infected ants where the species is found.
Fig. 12. Ophiocordyceps camponoti–floridani. A. Camponotus floridanus infected, biting into a plant. B. Close-up of the disc-shape ascoma attached to the stroma. C. Cross-section of the ascoma. D. Ascus. E. Ascospore. F–G. Hirsutella phialides. H–I. Limoniform conidia. Scale bars: A = 2 mm, B = 1 mm, C = 100 μm, D–F = 10 μm, G–I = 5 μm.
Fig. 13. Ophiocordyces kimfelingiae. A. Camponotus castaneus biting a twig. B. Close-up of the stroma showing two ascomatal plates attached on it. C. Ascoma section and Perithecia arranged on its surface. D. Perithecium. E. Cluster of ascospores. F–G. Ascus. H. Ascospore. I. Ascospore after 2–5 d on agar, exhibiting a swollen section and two capilliconidiophores. Scale bars: A = 2 mm, B = 0.5 mm, C = 300 μm, D = 100 μm, E–F = 20 μm, G = 10 μm, H = 20 μm, I = 40 μm.
Ophiocordyceps blakebarnesi

Araújo, H.C. Evans & D.P. Hughes, sp. nov. MycoBank MB822301. Fig. 14.

Etymology: Named after the collector, Blake Barnes, a medical doctor and citizen scientist who discovered this species and made important observations on its ecology.

Specimen examined: USA: North of the Indian Hills Park, Missouri, on Camponotus cf. chromaioides (Formicinae, Camponotini), 15 Nov. 2015, Blake Barnes, holotype INPA 274581.

Abundant external mycelium produced from the sutures and joints. Stroma single, sinuous, never branching, dark brown, apical part lighter and velvety; fertile part laterally attached, disc-shaped to irregular, black, averaging 1.5 × 1 mm; Perithecia immersed to slightly erumpent, elongated, flask-shaped, 300–320(–350) × 105–120 μm, with short, exposed neck or ostiole. Asci 8-spored, cylindrical to clavate, 220–250 × 12–14 μm. Ascospore hyaline, cylindrical, straight, 140–160 × 4 μm, 6–7-septate, tapering towards the apices.

Asexual morph: Hirustella A-type present along the stroma. Phialides smooth, cylindrical to lageniform, 75(–90) × 3–4 μm, tapering to a very long neck. Conidia limoniform, multi guttulate, 8–9 × 3 μm.

Germination process: No germination observed because the specimens studied were dried previously.

Habitat: Missouri (USA), temperate forest. Found biting inside logs. The log-biting behaviour is highly unusual and is also found in samples from Michigan Herbaria. The host ant, Camponotus cf. chromaioides, nests in wood and the position of the ant inside logs suggests that manipulation involves nest desertion and dying in logs where spores are distributed. Although evidence is still lacking, we suggest log-biting as an adaptation to very low temperatures and exposure on twigs (which occurs in the southern species O. kimfleemingiae).

Ophiocordyceps naomipeireae

Araújo, R. Shivas, S. Abell, T. Marney, H.C. Evans & D.P. Hughes, sp. nov. MycoBank MB822302. Figs 15, 16.

Etymology: Named after Naomi Pierce, Evolutionary Biologist at Harvard University who has mentored Hughes and many other biologists to consider ant-symbiont interactions in the deep time framework provided by phylogenetic studies.

Specimen examined: Australia, Kuranda, Queensland, on Polyrhachis sp., 22 May 2010, R. Shivas, T. Marney & S. Abell, holotype BRIP 53385.

External mycelium produced mostly on the ventral part of the host, also present on joints. Stromata ginger to light-brown, commonly clavate, produced always from dorsal pronotum, frequently on leg joints, 1.5–2.25 × 0.45–0.75 mm, branching into nodules formed along the stroma, 120–150 × 35–50 μm, phialides very abundant along the whole stroma; Fertile part single, attached laterally, hemispheric to irregular shape, orange, averaging 0.75 × 0.5–0.65 mm. Perithecia immersed, flask-shaped, 260–320 × (–130)150–200 μm, with prominent neck. Asci 8-spored, hyaline, vermiciform, cylindrical, 150–180 × 7 μm. Ascospore hyaline, straight to gently curved, vermiciform, 75–105 × 5–6 μm, 4–6-septate; tapering at apex.

Asexual morph: Paraisaria-like phialides produced profusely along the whole stroma; phialides abundant, cylindrical to clavate, 15–35 × 3 μm, producing up to 10 needle-like, verrucose conidiophores, averaging 10 μm, bearing a terminal conidium, 5–7 × 3 μm.

Germination process: No germination could be observed since the material examined was dried.

Habitat: Tropical Australia, rainforest. Found biting leaves at elevated positions on understory shrub in coastal forest; very common, associated with epizoicots.

Ophiocordyceps ootaki

Araújo, H.C. Evans & D.P. Hughes, sp. nov. MycoBank MB822303. Fig. 17.

Etymology: Named after Shigeo Ootaki, an artist and amateur mycologist who has contributed significantly to the study of entomopathogenic fungi in Japan.

Specimen examined: Japan, Matsueyama (Mt. Matsuo), Kyoto, on Polyrhachis moesta (Formicinae, Camponotini), 20 Jul. 2014, R.G. Loreto & S. Ootaki, holotype INPA 274587.

External mycelium produced from orifices and sutures; initially white, becoming light-brown with age. Stroma single or branched, produced from dorsal pronotum, averaging 6.5 × 0.3 mm, cylindrical, greyish to light brown; Fertile part produced laterally on the stroma, 1–3, disc-shaped, dark–brown, averaging 1.1 × 0.8 mm. Perithecia immersed to partially erumpent, flask-shaped, 230–260 × 120–150 μm, with short neck. Asci 8-spored, hyaline, cylindrical to clavate, 130–180 × 8–9 μm. Ascospore hyaline, vermiciform, straight to gently curved, 85–100 × 3 μm, 5-septate, tapering at both ends.

Asexual morph: Hirustella type-A only. Phialides cylindrical to lageniform, 6–8 × 3–4 μm, tapering to a long neck, 9–10 μm long, bearing a terminal conidium, averaging 5x3 μm.

Germination process: No germination could be observed since the material examined was dried.

Habitat: Japan, temperate forest. Biting evergreen plants only in a deciduous forest where leaf fall occurs. This behaviour suggests that the ants are manipulated to choose leaves that remain on the trees.

Ophiocordyceps satoi

Araújo, H.C. Evans & D.P. Hughes, nom. nov. et stat. nov. MycoBank: MB822304.

Basionym: Ophiocordyceps unilateralis var. clavata Kobayasi, Bull. Biogeogr. Soc. Japan: 272 (1939).

Etymology: Name after Takuya Sato, a Japanese biologist working on behaviour manipulation by parasites who helped enormously in the collection of specimens for this study.

Specimens examined: Japan, Honsyu, Province of Kazusa, Kitaitu-gun, Tanjiyama-mura, Myōken-zen, on Polyrhachis lamellidens (Formicinae, Camponotini), 20 Jul. 2014, R.G. Loreto & T. Sato, INPA 274589.

External mycelium scarce, produced mostly on ventral part of the host and mouth. Stromata produced from pronotum, dorsal– and laterally on both sides, clavate, 5–7.5 × 0.35–0.45 (–0.8) mm, never branching. Fertile part produced laterally on one or multiple stromata, 1–6, commonly 2 per stroma, averaging 1 × 0.8 mm,
Fig. 14. Ophiocordyceps blakebarnesii. A. Camponotus cf. chromaoides with the stroma arising from the dorsal pronotum. B. Close-up showing the biting behaviour inside the log. C. Stroma. D. Cross-section of the ascoma. E–G. Ascospores. H–K. Phialides. L. Multi guttulate conidia. Scale bars: A = 2 mm, B = 0.5 mm, C = 0.3 mm, D = 200 μm, E–G = 5 μm, H–K = 10 μm, L = 5 μm.
Fig. 15. Ophiocordyceps naomipierceae (sexual morph). A. Polyrhachis sp. biting the edge of a leaf. B. Close-up of the orange ascoma. C. Cross-section of the ascoma. D. Perithecium. E. Ascus. F. Ascospore. Scale bars: A = 0.5 mm, B = 1 mm, C = 100 μm, D = 50 μm, E–F = 20 μm.
Fig. 16. *Ophiocordyceps naomipierceae* (asexual morph). A. *Polyrhachis* sp. with stromata arising from leg joints and dorsal pronotum. B. Synnema. C. Close-up of synnema. D. Close-up phialides. E. Phialides. F. Close-up synnema showing apical phialides. G. Individual long phialide with multiple verrucose necks. Scale bars: A = 0.5 mm, B = 100 μm, C = 20 μm, D = 15 μm, E–G = 10 μm.
up to 2.5 mm in length. Perithecia immersed to partially erumpent, flask-shaped, 230–270 × 120–160 μm, with short, exposed neck. Ascii 8-spored, cylindrical to clavate, 120–160 × 8–10 μm. Ascospores hyaline, cylindrical, straight, rarely curved, 85–100 × 4 μm, 5-septate, apex rounded, tapering at base.

Asexual morph: Hirsutella type-A only. Phialides cylindrical to lageniform, averaging 12 × 7 μm, tapering to a long neck. No conidia observed.

Germination process: Ascospores germinating in 24 h to produce 1–3 hair-like capilliconidiophores, 40–50 μm long, bearing a terminal, hemispheric capilliconidium, averaging 13 × 3 μm. Some ascospores germinating directly into germ tubes and vegetative hyphae.

Habitat: Japan, temperate forest. A ground-dwelling ant species found consistently biting onto twigs in a deciduous forest where leaf fall occurs.

**DISCUSSION**

Our results support the hypothesis that species of fungi in the *Ophiocordyceps unilateralis* complex are highly specific to each ant species in the tribe Camponotini. This work significantly expands our understanding of insect pathogenic fungi and can serve as a test case against which other investigations into fungal diversity, systematics and evolution can be compared. It remains to be seen if the very high specificity we found between *Ophiocordyceps*/ant associations is mirrored in species of fungi infecting other insect groups. In the next sections, we discuss some aspects of these fungi in more detail.

**Morphology**

The species within the *O. unilateralis* clade share many macro-morphological characteristics that make them easily recognized.
in the field. Morphologically unique features include the typical single stroma arising from dorsal pronotum with at least one ascoma growing, unilaterally, from the stroma. Although there are exceptions. For example, O. satoi from Japan that usually produces three clavate stromata, with up to six ascomata attached to it (Fig. 18). Other species such as O. camponoti-indiani (North Brazilian Amazon), O. halabalaensis, O. rami (Thailand) and O. naomiipierceae (Australia) are similar to O. satoi regarding the production of multiple stalks (Luangsara-ard et al. 2011, Araújo et al. 2015). Moreover, this trait cannot be considered as a synapomorphic feature since those species are scattered along the O. unilateralis clade. Many samples of O. albacongiuae were collected exhibiting one stroma arising from the dorsal pronotum and another from the petiole (Fig. 11). All the other species within the O. unilateralis clade often produce a single stroma with the Hirsutella type-A asexual morph, with only rare occasional exceptions at the specimen level.

Furthermore, each species within the O. unilateralis clade exhibits unique micro-morphological traits. The most significant microscopic character used to split the species within this complex is the morphology of the ascospore, which includes seption, size, shape and germination process (Table 1). Other aspects such as the location where the host is attached (e.g. leaf edge, leaf middle-vein, palm spine, trunk, epiphyte), and morphology of the asexual morphs, are also valuable characters that may be used as information when distinguishing species but are less important than ascospore morphology and, of course, molecular data.

Ophiocordycipes oecophyllae and O. daceti were found producing only the asexual morph. O. oecophyllae produces the phialides directly on the host, especially from joints, while O. daceti produces a single synnema from the dorsal pronotum covered with a hymenium of verrucose hirsutella-like phialides. Both species, although lacking the sexual morph, are easily recognized as new taxa based on host association, phialide morphology and habit, which were further confirmed by the molecular data (Fig. 19). Based on morphological and ecological data, our results suggest that O. oecophyllae is a sole early divergent lineage of the O. unilateralis core clade. This means that a common ancestral form, most likely infecting ants, diversified into the hyper-diverse O. unilateralis core clade. The discovery of O. oecophyllae should help us to trace the origin of the O. unilateralis clade and to test evolutionary hypotheses regarding the factors (e.g. morphological adaptations and host association) that led them to be one of the most diverse groups of entomopathogenic fungi. However, to test this hypothesis, and to confidently propose O. oecophyllae as an early diverging lineage of the O. unilateralis core clade, we need a broader gene sampling for this species, in order to have a strong support from the morphology, ecology and molecular data.

Species of fungi within the O. kniphofiofoidei clade share several morphological and ecological characters. All species within this clade are exclusively pathogens of Neotropical ants (i.e. Cephalotes atratus, Paraponera clavata, Dolichoderus bispinosus and Daceton armigerum) (Fig. 19). The sexual morph produces vermiciform, multi-septate ascospores that do not germinate into secondary structures (e.g. capillliconidiophores) or into hyphae, despite multiple attempts. The failure of germination might indicate the need of biotic factors, possibly being triggered by contact with the host. Furthermore, the most evident morphological feature shared by all species in this clade is the Hirsutella stilbelliformis asexual morph, with its unique long verrucose phialides united into synnemata (Fig. 20). Typically, these arise from rhizoid-like outgrowths, formed on the substrate (tree bark) rather than directly on the host. This behaviour could be analogous to the “minefields” created by the germinating ascospores of O. unilateralis s.l., which produce sticky capillliconidia after landing on surrounding substrata (Araújo & Hughes 2017).

Although the topological relationship between O. unilateralis core clade and O. kniphofiofoidei sub-clade corroborates the findings of Sanjuan et al. (2015), the bootstrap value was low (BP = 47 %). With the inclusion of O. monacidis and O. daceti in the analysis, O. tiputini infecting the larval stage of Megaloptera, was supported (BP = 71 %) as a sister group of the O. unilateralis core clade + O. oecophyllae + O. kniphofiofoidei sub-clade, rather than a member of O. kniphofiofoidei sub-clade as presented by Sanjuan et al. (2015). This novel result allows us to consider the monophyly of O. unilateralis core clade + O. oecophyllae + O. kniphofiofoidei sub-clade, forming a strictly ant-pathogenic clade within Ophiocordycipes. However, this is still a working hypothesis and further more inclusive studies are needed. Thus, we currently refer to the O. kniphofiofoidei sub-clade as the clade formed by the species: O. monacidis comb. nov. et stat. nov., O. kniphofiofoidei s. s., O. ponerinarum, O. daceti sp. nov. and O. tiputini as incertae sedis regarding its position within the O. unilateralis clade.

**Ascospores**

All species belonging to the O. unilateralis and O. kniphofiofoidei clades produce ascospores that do not disarticulate into spores. No species within the O. kniphofiofoidei sub-clade has ascospores that germinate in vitro, to produce either capillliconidiophores or hyphae. Conversely, production of capillliconidiophore has been shown to be a common behaviour within the O. unilateralis core clade species (Evans et al. 2011, Araújo et al. 2015, Table 1). Unfortunately, we could not determine the germination process of some species because the specimens collected did not release spores on agar or because the samples sent by collaborators were dried (i.e. O. naomiipierceae, O. camponoti-floridani, O. blakebarnesi, O. ootakii, O. albacongiuae). In addition, the description of species from Thailand does not include any information regarding ascospore behaviour, although it is probable that they also produce capillliconidiophores.

Ophiocordycipes camponoti-indiani produces ascospores measuring 75 × 5 μm exhibiting up to three capillliconidiophores that are up to 130 μm in length, which is the longest described for the O. unilateralis group so far (Araújo et al. 2015). Interestingly, Camponotus indians is significantly bigger than other Amazonian Camponotus species infected by this group of pathogens. This could be posited to be a result of local adaptation between host and pathogen where fungal morphology matches the ant morphology/ecology in order to reach, infect and transmit the disease within that species. Future studies will test the hypothesis with capillliconidia size/shape and the ant morphology/ecology.

Ascospores of O. camponoti-balzani and O. camponoti-melanotici produce either a small appressorial-like structure or a single short phialide respectively, even after an extended period of incubation on agar (Evans et al. 2011). O. camponoti-sexguttati produces a large ascospore measuring 120–140 μm in length.
Fig. 18. Ophiocordyceps satoi. A. Polyrhachis lamellidens, with three stromata arising from its body. B. Close-up stroma with two ascomatal cushions. C. Cross-section of the ascoma, showing the perithecial arrangement. D. Close-up perithecium. E. Ascus. F. Ascospore germinating on agar plate after 3–5 d. G. Capilliconidium. H. Ascospore with two capillconidiophores bearing one capilliconidium at their apices. Scale bars: A = 1 mm, B = 0.5 mm, C = 100 μm, D = 40 μm, E–F = 20 μm, G = 2 μm, H = 20 μm.
length, but only small single 25–30 μm long capilliconidiophores, consistently formed in the first third of its length. *O. camponoti-hippocrepidis* and *O. camponoti-bispinosi* are very similar in size and shape, but the capilliconidiophore of *O. camponoti-bispinosi* is slightly bigger and smooth, in contrast with the terminal, verrucose capilliconidiophore produced by *O. camponoti-hippocrepidis* (Fig. 10). Only *O. satoi* and *O. kimflemingiae* germinated to form hyphae on agar; found in Japan and South Carolina (USA) respectively, both temperate forest locations. Another particular feature observed in both species was the swelling of Fig. 19. Maximum Likelihood tree of *Ophiocordyceps* obtained with a combined dataset of SSU, LSU, tef, RPB1 and RPB2 based on Bayesian/RAxML analysis with only >0.95/70 shown. Species proposed in this study are highlighted. Ant figures correspond to the ant genera infected by each clade within hirsutelloid *Ophiocordyces*. At the top right a round phylogeny showing the whole analyses with the entire dataset used in this study, which included Cordycipitaceae, Clavicipitaceae and Ophiocordycipitaceae species, with *Ophiocordyces* highlighted. (ant images from www.AntWeb.org and the photographers: Oecophylla, Camponotus, Dolichoderus, Cephalotes, Paraponera and Dacetor: April Noble, Polyrhachis: Will Ericson.}
the ascospores following germination. These traits might be related to adaptations to temperate forests since we never observed such behaviour in the ascospores of any of the tropical species.

**Asexual morphs**

**Ophiocordyceps unilateralis core clade**

There are many kinds of asexual spores or conidia produced by entomopathogenic species, from dry to mucilaginous, aseptate to septate, produced along the stroma on phialides in a palisade or hymenial layer, or on phialides arising from pulvinate structures (sporodochia). Most species within the *O. unilateralis* core clade form an asexual morph characterized by subulate phialides that bear a single conidium at their apices (Hirsutella type-A). There are also two other types of hirsutelloid morphs within *O. unilateralis* core clade species: Hirsutella type-B – found in *O. camponoti-novogranadensis* – is produced on lower joint/foot on all legs of the host, which is a solitary upright synnema with a globose head (Evans et al., 2011). Hirsutella type-C, which is produced from brown cushions (sporodochia) on leg and antennal joints, is found in *O. oecophyllae* (early divergent lineage of the *O. unilateralis* core clade), *O. camptonoti-renggeri, O. camptonoti-nidulantis, O. camptonoti-balzani* and *O. camptonoti-indiani*. *O. kimflemingiae* exhibits type-C phialides during its early stages of development, but these gradually disappear as the stroma matures. *Ophiocordyceps naomi-pierceae* from Australia has a unique asexual morph within the *O. unilateralis* core clade, formed on the surface of synnemata arising from the dorsal pronotum and leg joints. The abundant, long phialides are polyphialidic, branching sympodially to produce up to 10 pointed necks (Fig. 16). A similar paraisaria-like asexual morph is associated with the red ant (*Myrmica rubra*) in the UK, which was found to lie within the *Ophiocordyceps*, close to *O. gracilis* from which the genus *Paraisaria* was erected.

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**Fig. 20.** Comparison of the phialide morphology for the species within the *O. kniphofoioides* clade (A-phialides and conidia). A. *O. kniphofoioides* sensu stricto. B. *O. monaciidis*. C. *O. ponerinarum*. D. *O. kniphofoioides* var. gnaphoptogenyos. E. *O. dacei* sp. nov. (A–D. Evans & Samson 1984; E. This study). Scale bar = 10 μm.
Fig. 21. Ophiocordyces kniphoioides sensu stricto. A. Cephalotes atratus with the stroma arising laterally from pronotum. B. Close-up of the ascoma. C. Section of the ascoma showing the immersed perithecia. D. Ascospore. E. Ascus. F. Hirsutella-like phialides, present along the stroma (stalk). Scale bar: A = 2 mm. B = 1 mm. C = 200 μm. D–F = 20 μm.
(Samson & Brady 1983), but far from O. unilateralis core clade (Evans et al. 2010). The role of these asexual morphs is not fully understood, but we suggest that because the conidia are usually encased in mucus, they are contact spores and adhere to foraging host ants.

**Ophiocordyceps kniphooides s.s.: a strategy to persist in the environment**

This species, besides its characteristic Kniphofia-like (red-hot poker) sexual morph, is known to form four types of asexual morphs. One of these – *Hirsutella stilbelliformis* var. *stilbelliformis* – plays a remarkable role of transmission even after the host removal. Evans & Samson (1982) described a behaviour in which apparently non-infected *Cephalotes atratus* – not displaying symptoms of fungal infection – actively attempted to remove infected cadavers from the lower trunk of the so-called cemetery or graveyard tree, forming a necropolis of ant corpses just above the tree base (Fig. 21). However, this type of asexual morph serves as a perfect adaption against this behaviour displayed by the healthy workers. These asexual structures consist of prostrate rhizoid-like outgrowths from the host that creeps beneath the moss carpet and bark, giving rise to synnemata-like structures producing mucoid balls of conidia at their tips (Fig. 22 and Evans & Samson 1982 p. 436). Once the ant cadaver is removed, the fungal structures remain on the tree, serving as a persistent inoculum for future hosts that are constantly passing on the trunk on the way to their arboreal nest: a perfect hidden trap.

![Fig. 22. A. Synnemata of the asexual morph (Hirsutella type-C. arising from the moss/trunk which remain attached, even after removal of the corpse. B–C. Close-up of the infected ants with synnemata on the surrounding substrate.](image-url)
New combinations

Based on morphological, ecological and molecular data, we do not support the previous designation of varieties within the O. unilateralis and O. kniphostoides complexes and the following species, new names and new combinations are now recognized:

**Ophiocordyceps kniphostoides** (H.C. Evans & Samson) G.H. Sung et al., Stud. Mycol. 57: 44. 2007. Fig. 23.

_Basionym:_ Cordyceps kniphostoides H.C. Evans & Samson, Trans. Brit. Mycol. Soc. 79: 434. 1982, on Cephalotes atratus.

_Synonym:_ Ophiocordyceps kniphostoides var. dolichoderi H.C. Evans & Samson, Trans. Brit. Mycol. Soc. 79: 437. 1982.

**Ophiocordyceps dolichoderi** (H.C. Evans & Samson) Araújo, H.C. Evans & D.P. Hughes, comb. et stat. nov. MycoBank MB822352.

_Basionym:_ Cordyceps kniphostoides var. dolichoderi H.C. Evans & Samson, Trans. Brit. Mycol. Soc. 79: 437. 1982, on Dolichoderus attelaboides.

_Synonym:_ Ophiocordyceps kniphostoides var. monacidis (H.C. Evans & Samson) G.H. Sung et al., Stud. Mycol. 57: 44. 2007.

**Ophiocordyceps monacidis** (H.C. Evans & Samson) Araújo, H.C. Evans & D.P. Hughes, comb. nov. et stat. nov. MycoBank MB822306. Figs 24, 25.

_Basionym (replaced name):_ Cordyceps kniphostoides var. monacidis H.C. Evans & Samson, Trans. Brit. Mycol. Soc. 79: 439. 1982.

_Synonym:_ Ophiocordyceps kniphostoides var. monacidis (H.C. Evans & Samson) G.H. Sung et al., Stud. Mycol. 57: 44. 2007.

_Etymology:_ Named after the host Monacis bispinosus, currently Dolichoderus (Monacis) bispinosus.

_Type:_ Brazil, Pará, Monte Dourado, 10 Jan. 1980, H.C. Evans, RS 1540A (CBS), on Dolichoderus (Monacis) bispinosus (Dolichoderinae: Dolichoderini). Paratypes: INPA 274591, INPA 274592.

_The stroma, usually single, emerges laterally from the pronotum_ – _rarely from the gaster_ – _with a dark orange fertile terminal ascoma. The ascospores measure 95–10 μm long, with no germination in vitro observed._

_Habitat:_ Brazil, Amazonian rainforest. One of the most interesting aspects of _O. monacidis_ (Fig. 24) is the behavioural manipulation whereby the fungus consistently leads the host to die among/underneath moss, specifically _Octoblepharum albidum_ Hedwig that is commonly found in clumps at the base of trees in the Amazon forest. After host death, the fungus produces its reproductive stroma that grows through the moss carpet, before exposing its fruiting body. The resemblance of the ascomata of _O. monacidis_ and the sporophytes of _O. albidum_ is striking (Fig. 25), which makes the fungus hard to detect in situ. We hypothesize that the fungus mimics the asexual reproductive structure of this species of moss, although future studies are needed to better understand the ecological relationship between the moss, _O. monacidis_ and its host _Dolichoderus bispinosus_.

_fig. 23._ Base of the tree, with corpses of infected Cephalotes atratus removed from the trunk by the activity of other workers (Araújo & Hughes 2017).
Fig. 24. Ophiocordyceps monacidis. A. Dolichoderus (Monacis) bispinosus infected by O. monacidis. B. Cross-section of the ascoma. C. Ascomata arising from a carpet of moss. D. Ascus. E. Ascospore. Scale bars: A = 1 mm, B = 200 μm, C = 3 mm, D = 20 μm, E = 30 μm.
Behaviour manipulation

All myrmecophilous hirsutelloid species (O. unilateralis core clade + O. oecophylloae + O. kniphofioidea sub-clade) are known to alter the behaviour of their hosts. This phenomenon is called extended phenotype. The term was coined by Dawkins (1982) to describe the relationship between hosts and parasites, where the parasite genotype is expressed in any aspect of the host morphology or behaviour (phenotype).

We found that species in the O. kniphofioidea clade display a less sophisticated type of manipulation of the host compared to those in the O. unilateralis clade. O. kniphofioidea s. s., O. ponerinarum and O. monacidis that lead their hosts to die typically at the base of lower trunks of upperstorey trees and attached to the substrate by their legs, which is further reinforced with fungal structures (Hughes et al. 2016, p. 443). O. daceti is an exception in the group and dies in the leaf litter or attached to the petiole or underside of leaves (Fig. 26). In the case of the species within the O. unilateralis clade, the behaviour manipulation occurs in a much more complex manner.

Every species within Ophiocordyceps unilateralis s.l. cause the infected ants to leave the colony and to ascend to the understorey vegetation, where they bite onto branches and leaves. However, each species occupies a characteristic niche and has a clear preference for certain substrates. O. camponoti-renggeri, for example, is often found biting onto moss carpets, at the base of upperstorey trees (Fig. 27 A–C). Fungi infecting very small ants such as O. camponoti-hippocrepidis, O. camponoti-bispinosi and O. camponoti-femorati often induce the host to bite onto the tips of palm needles (Fig. 27 D–E), especially spiny palms of the genus Astrocaryum. O. camponoti-atricipis and O. camponoti-floridan, sister species in the phylogeny, bite onto...
palm leaves, specifically close to the apical edge region; whilst, *O. camponoti-leonardi* in Thailand, invariably bites onto the underside of dicot leaves, precisely on the middle vein (Andersen et al. 2009). *O. camponoti-novogranadensis* has a clear preference for epiphytes (lichens or small bromeliads) (Evans et al. 2011). *O. camponoti-nidulantis* is often found at 25–40 cm above the ground, consistently biting onto the vegetation of tree saplings with both the antennae spread, possibly to facilitate conidia transmission (Fig. 8).

**CONCLUSIONS**

Studies in biodiversity play an essential role in cataloguing and describing species, especially for understudied groups such as entomopathogenic fungi. Furthermore, by unravelling the true diversity of this group, more intriguing and complex questions will come to the light. The goal of this study is to document new taxa and help increase the knowledge necessary to answer questions related to the evolutionary history, host relationships and functional morphology of this group of pathogens. For example, which factors led to the hyper-diversity of the *O. unilateralis* clade? How did they reach the Camponotini ants and why this group of hosts is such a prolific environment for *Ophiocordyceps* radiation? Was it due to morphological adaptations such as capilliconidia? Was it due to the extremely sophisticated behavioural manipulation that arose in this group? Unfortunately, we are still unable to fully address these questions, but we hope that this study will contribute to answer these and other questions about this fascinating group of fungi.
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Fig. 27. Different behavioural manipulation within the O. unilateralis complex. A–C. Dead O. camponoti-ranggeri as they are typically found, among moss at the base of trees. D–E. Smaller ants (e.g. O. camponoti–bispinosi, O. camponoti–hiphocrepidis and O. camponoti–femorati) die often at the very tip of palm spines and epiphytes where water droplets form, providing continuous water resource.

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