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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. albacongiuae* Araújo, H.C. Evans & D.P. Hughes, *O. blakebarnesii* Araújo, H.C. Evans & D.P. Hughes, *O. camponoti-chartificis* Araújo, H.C. Evans & D.P. Hughes, *O. camponoti-femorati* Araújo, H.C. Evans & D.P. Hughes, *O. camponoti-floridani* 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-renggeri* Araújo, H.C. Evans & D.P. Hughes, *O. camponoti-sexguttati* Araújo, H.C. Evans & D.P. Hughes, *O. daceti* Araújo, H.C. Evans & D.P. Hughes, *O. kimflemingiae* Araújo, H.C. Evans & D.P. Hughes, *O. naomipierceae* Araújo, H.C. Evans & D.P. Hughes, *O. oecophyllae* Araújo, S. Abell, T. Marney, R. Shivas H.C. Evans & D.P. Hughes, *O. ootakii* 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 (Misof *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 (Hölldobler *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-

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walled asci and ascospores that do not disarticulate into part-spores. 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 *Ophiocordycipitaceae*, 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 *Elaphocordyceps*, see Quandt *et al.* 2014) were transferred from *Cordyceps 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 *Sorosporella*, *Synglíocladium*, *Paraisaria*, *Stilbella*, *Hymenostilbe* and *Hirsutella* (Quandt *et al.* 2014). With the exception of *Sorosporella* and *Synglíocladium*, 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. kniphofioides* sub-clade, classified here as myrmecophilous hirsutelloids; 2) Species within *Ophiocordyceps* subg. *Neocordyceps* or "sphecocephala 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. kniphofioides* 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. kniphofioides* 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. kniphofioides* 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 (Matsuoyama 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 campinarana 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 Matsuoyama (Mt. Matsuo), a mountainous area with up to 687 m elevation, on the west side of Kyoto and Arima Fuji 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 micro-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); ascomatal 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 1-α (*tef*) and the largest and second largest subunits of RNA polymerase II (*RPB1* and *RPB2* respectively) with a total read length of approximately 4 600 bp. However, for our field collected samples, *RPB2* could not be successfully amplified. The primers used were, SSU: NS1 (GTAGTCATATGCTTGTCTC) and NS4 (CTTCCGTCAATTCCTTTAAG) (White *et al.* 1990); LSU: LR0R (5′-ACCCGCTGAACTTAAGC-3′) and LR5 (5′-TCCTGAGGGAAACTTCG-3′) (Vilgalys & Sun 1994); *tef*: 983F (5′-GCYCCYGGHCAYCGTGAYTTYAT-3′) and 2218R (5′-ATGACACCRACRGCRACRGTYTG-3′); *RPB1*: (5′-CCWGGYTTYATCAAGAARGT-3′) (Castlebury *et al.* 2004) and RPB1Cr_oph was designed specifically to address the species proposed herein (5′-CTGVCCMGCRATGTCGTTGTCCAT-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 GTRGAMMA 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 GTRGAMMA 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 sumt 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).

Table 1. Comparison of morphological characters, host and geographical location of myrmecophilous hirsutelloid species of *Ophiocordyceps*.

Species	Host	Death position	Ascospore			Hirsutella asexual morph				Source	Complex	Distribution
			Size (µm)	Capilliconidiophore	Septation	A	B	C	Paraisaria-like	Stilbelliformis			
Ophiocordyceps albacongiuae	***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**	**x**					**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	x		x			Evans *et al.* (2011)		Brazilian Atlantic Rainforest
O. camponoti-bispinosi	*Camponotus bispinosus*	biting spines	70–75 × 4.5	65	4–5	x					Araújo *et al.* (2015)		Brazilian Amazon
O. camponoti-chartificis	***Camponotus chartifex***	**biting leaf**	**75–85 × 5**	**75–90**	**9–13**	**x**					**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-hippocrepidis	***Camponotus hippocrepis***	**biting spines**	**75–85 × 4–5**	**45–50**	**5**	**x**					**This study**		**Brazilian Amazon**
O. camponoti-indiani	*Camponotus indianus*	biting leaf	75 × 4.5	130	5	x		x			Araújo *et al.* (2015)		Brazilian Amazon
O. camponoti-leonardi	*Camponotus leonardi*	biting leaf	110–125 × 2–3	–	7–8	x					Kobmoo *et al.* (2012)		Thailand
O. camponoti-melanotici	*Camponotus melanoticus*	biting leaf	170–210 × 4–5	20–25	27–35	x					Evans *et al.* (2011)		Brazilian Atlantic Rainforest
O. camponoti-nidulantis	***Camponotus niduland***	**biting saplings**	**90–105 (–115) × 3–4**	**50–60**	**5**	**x**		**x**			**This study**		**Brazilian Amazon**
O. camponoti-novogranadensis	*Camponotus novogranadensis*	biting epiphytes	75–95 × 2.5–3.5	–	5–10	x	x				Evans *et al.* (2011)		Brazilian Atlantic Rainforest
O. camponoti-renggeri	***Camponotus renggeri***	**biting leaf/moss**	**90–120 × 4**	–	**5–8**			**x**			**This study**		**Brazilian Amazon**
O. camponoti-rufipedis	*Camponotus rufipes*	biting leaf	80–95 × 2–3	60–70	4–7	x					Evans *et al.* (2011)		Brazilian Atlantic Rainforest

Table 1. (Continued).

Species	Host	Death position	Ascospore			Hirsutella asexual morph				Source	Complex	Distribution
			Size (µm)	Capilliconidiophore	Septation	A	B	C	Paraisaria-like	Stilbelliformis			
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. kimflemingiae	***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-furcata	*Polyrhachis furcata*	biting leaf	90–100 × 2–3	–	0	x					Kobmoo *et al.* (2012)		Thailand
O. rami	*Camponotus* sp.	biting green twigs	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* sp.	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	*O. kniphofioides s.l.*	**Brazilian Amazon**
O. daceti	***Daceton armigerum***	**leaf (not biting)**	–	–	–					**x**	**This study**		Brazilian Amazon, Colombia
O. kniphofioides	*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.

Table 2. Specimen information, GenBank accession number, host association and location. The species in bold are the new taxa presented in this study.

Species	Voucher information	SSU	LSU	*TEF*	*RPB1*	*RPB2*	Host	Location
Hirsutella sp.	NHJ 12525	EF469125	EF469078	EF469063	EF469092	EF469111	n/a	n/a
	OSC 128575	EF469126	EF469079	EF469064	EF469093	EF469110	n/a	n/a
Ophiocordyceps acicularis	OSC 128580	DQ522543	DQ518757	DQ522326	DQ522371	DQ522423	Coleoptera	USA
	OSC 110987	EF468950	EF468805	EF468744	EF468852	n/a	Coleoptera	USA
	OSC 110988	EF468951	EF468804	EF468745	EF468853	n/a	Coleoptera	USA
	ARSEF 5692	DQ522540	DQ518754	DQ522322	DQ522368	DQ522418	Coleoptera	Korea
O. albacongiuae	RC20	KX713633	n/a	KX713670	n/a	n/a	Hymenoptera (*Camponotus* sp.)	Colombia
O. amazonica	HUA 186143	KJ917562	KJ917571	KM411989	KP212902	KM411982	Orthoptera	Colombia
	HUA 186113	KJ917566	KJ917571	n/a	KP212903	KM411980	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	KX713685	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	n/a	KX713712	n/a	Hymenoptera (*Dolichoderus bispinosus*)	Brazil (Amazon)
O. brunneipunctata	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	ATRI3	KX713666	n/a	KX713677	n/a	n/a	Hymenoptera (*Ophiocordyceps atriceps*)	Brazil (Amazon)
O. camponoti-balzani	G143	KX713658	KX713595	KX713690	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	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)

Table 2. (Continued).

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-floridani	Flx1	KX713661	n/a	n/a	n/a	n/a	Hymenoptera (*Camponotus femoratus*)	Brazil (Amazon)
	Flo4	KX713662	KX713591	n/a	n/a	n/a	Hymenoptera (*Camponotus femoratus*)	Brazil (Amazon)
	Flx2	n/a	KX713592	KX713674		n/a	Hymenoptera (*Camponotus femoratus*)	Brazil (Amazon)
O. camponoti-hippocrepidis	HIPPOC	KX713655	KX713597	KX713673	KX713707	n/a	Hymenoptera (*Camponotus hippocrepis*)	Brazil (Amazon)
O. camponoti-indiani	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)
	Mal4	KX713649	KX713602	n/a	n/a	n/a	Hymenoptera (*Camponotus novogranadensis*)	Brazil (Atlantic Rainforest)
O. camponoti-renggeri	RENG2	KX713632	n/a	KX713672	n/a	n/a	Hymenoptera (*Camponotus renggeri*)	Brazil (Amazon)
	ORENG	KX713634	KX713617	KX713671	n/a	n/a	Hymenoptera (*Camponotus renggeri*)	Brazil (Amazon)
O. camponoti-rufipedis	G177	KX713657	KX713596	KX713680	n/a	n/a	Hymenoptera (*Camponotus rufipes*)	Brazil (Atlantic Rainforest)
	G108	KX713659	KX713594	KX713679	KX713704	n/a	Hymenoptera (*Camponotus rufipes*)	Brazil (Atlantic Rainforest)
O. citrina	TNS F18537	n/a	KJ878903	KJ878983	n/a	KJ878954	Hemiptera	Japan
O. clavata	NBRC 106962	JN941726	JN941415	n/a	JN992460	n/a	n/a	n/a
	NBRC 106961	JN941727	JN941414	n/a	JN992461	n/a	n/a	n/a
	CEM1762	KJ878916	KJ878882	KJ878963	KJ878996	n/a	Coleoptera	China
	CEM1763	n/a	KJ878883	KJ878964	KJ878997	n/a	Coleoptera	China
O. satoi	J19	KX713650	KX713601	KX713684	KX713710	n/a	Hymenoptera (*Polyrhachis lamellidens*)	Japan
	J7	KX713653	KX713599	KX713683	KX713711	n/a	Hymenoptera (*Polyrhachis lamellidens*)	Japan
O. cochlidiicola	HMAS_199612	KJ878917	KJ878884	KJ878965	KJ878998	n/a	Lepidoptera	China
O. communis	NHJ 12581	EF468973	EF468831	EF468775	n/a	EF468930	Coleoptera	n/a
	NHJ 12582	EF468975	EF468830	EF468771	n/a	EF468926	Coleoptera	n/a
O. curculionum	OSC 151910	KJ878918	KJ878885	n/a	KJ878999	n/a	Coleoptera	Guyana
O. daceti	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
	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	n/a
	TNS 16252	KJ878941	KJ878906	KJ878986	n/a	n/a	Coleoptera	Japan

(*continued on next page*)

Table 2. (Continued).

Species	Voucher information	SSU	LSU	*TEF*	*RPB1*	*RPB2*	Host	Location
	TNS 16250	KJ878942	n/a	KJ878987	KJ879021	n/a	Coleoptera	Japan
O. formicarum	TNS F18565	KJ878921	KJ878888	KJ878968	KJ879002	KJ878946	Hymenoptera	Japan
O. formosana	TNM F13893	KJ878908	n/a	KJ878956	KJ878988	KJ878943	Coleoptera	Taiwan
O. forquignonii	OSC 151902	KJ878912	KJ878876	n/a	KJ878991	KJ878945	Diptera	France
	OSC 151908	KJ878922	KJ878889	n/a	KJ879003	KJ878947	Diptera	France
O. gracilis	EFCC 3101	EF468955	EF468810	EF468750	EF468858	EF468913	Lepidoptera	n/a
	EFCC 8572	EF468956	EF468811	EF468751	EF468859	EF468912	Lepidoptera	n/a
O. gracillisima	Ophgrc679	n/a	KC610768	KC610744	KF658666	n/a	Coleoptera	Colombia
O. heteropoda	OSC 106404	AY489690	AY489722	AY489617	AY489651	n/a	Hemiptera	Australia
	EFCC 10125	EF468957	EF468812	EF468752	EF468860	EF468914	Hemiptera	n/a
O. irangiensis	OSC 128577	DQ522546	DQ518760	DQ522329	DQ522374	DQ522427	Hymenoptera	n/a
	OSC 128578	DQ522556	DQ518770	DQ522345	DQ522391	DQ522445	Hymenoptera	n/a
	OSC 128579	EF469123	EF469076	EF469060	EF469089	EF469107	Hymenoptera	n/a
O. kimflemingiae	SC03B	KX713619	KX713619	n/a	KX713723	n/a	Hymenoptera (*Camponotus castaneus/americanus*)	USA (South Carolina)
	SC30	KX713629	KX713622	KX713699	KX713727	n/a	Hymenoptera (*Camponotus castaneus/americanus*)	USA (South Carolina)
	SC27	KX713630	KX713621	n/a	KX713726	n/a	Hymenoptera (*Camponotus castaneus/americanus*)	USA (South Carolina)
	SC09B	KX713631	KX713620	KX713698	KX713724	n/a	Hymenoptera (*Camponotus castaneus/americanus*)	USA (South Carolina)
	SC03A	n/a	n/a	KX713697	KX713722	n/a	Hymenoptera (*Camponotus castaneus/americanus*)	USA (South Carolina)
	SC36	n/a	KX713623	n/a	KX713728	n/a	Hymenoptera (*Camponotus castaneus/americanus*)	USA (South Carolina)
	SC100	n/a	KX713624	KX713696	KX713725	n/a	Hymenoptera (*Camponotus castaneus/americanus*)	USA (South Carolina)
	SCX	n/a	KX713625	n/a	KX713729	n/a	Hymenoptera (*Camponotus castaneus/americanus*)	USA (South Carolina)
O. kniphofioides	Ophkni975	KC610790	KF658679	KC610739	KF658667	KC610717	Hymenoptera	Colombia
O. konnoana	EFCC 7295	EF468958	n/a	n/a	EF468862	EF468915	Coleoptera	Korea
	EFCC 7315	EF468959	n/a	EF468753	EF468861	EF468916	Coleoptera	Korea
O. lloydii	OSC 151913	KJ878924	KJ878891	KJ878970	KJ879004	KJ878948	Hymenoptera	Ecuador
O. longissima	TNS F18448	KJ878925	KJ878892	KJ878971	KJ879005	n/a	Hemiptera	Japan
	HMAS_199600	KJ878926	n/a	KJ878972	KJ879006	KJ878949	Hemiptera	China
	EFCC 6814	n/a	EF468817	EF468757	EF468865	n/a	Hemiptera	Korea
O. melolonthae	OSC 110993	DQ522548	DQ518762	DQ522331	DQ522376	n/a	Coleoptera	n/a
O. myrmecophila	HMAS_199620	KJ878927	KJ878893	KJ878973	KJ879007	n/a	Hymenoptera	China
	CEM1710	KJ878928	KJ878894	KJ878974	KJ879008	n/a	Hymenoptera	China
	TNS 27120	KJ878929	KJ878895	KJ878975	KJ879009	n/a	Hymenoptera	Japan

Table 2. (Continued).

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	KJ878896	KJ878976	KJ879010	n/a	Coleoptera	Japan
O. nigrella	EFCC 9247	EF468963	EF468818	EF468758	EF468866	EF468920		Korea
O. nutans	OSC 110994	DQ522549	DQ518763	DQ522333	DQ522378	n/a	Hemiptera	n/a
O. odonatae	TNS F18563	n/a	KJ878877	n/a	KJ878992	n/a	Odonata	Japan
	TNS 27117	n/a	KJ878878	n/a	n/a	n/a	Odonata	Japan
O. oecophyllae	OECO1	KX713635	n/a	n/a	n/a	n/a	Hymenoptera (*Oecophylla smaragdina*)	Australia
O. ootakii	J14	KX713651	n/a	KX713682	KX713709	n/a	Hymenoptera (*Polyrhachis moesta*)	Japan
	J13	KX713652	KX713600	KX713681	KX713708	n/a	Hymenoptera (*Polyrhachis moesta*)	Japan
O. ponerinarum	HUA 186140	KC610789	KC610767	KC610740	KF658668	n/a	Hymenoptera (*Paraponera clavata*)	Brazil, Colombia, Ecuador
O. pulvinata	TNS-F 30044	GU904208		GU904209	GU904210	n/a	Hymenoptera (*Camponotus obscuripes*)	Japan
O. purpureostromata	TNS F18430	KJ878931	KJ878897	KJ878977	KJ879011	n/a	Coleoptera	Japan
O. ravenelii	OSC 110995	DQ522550	DQ518764	DQ522334	DQ522379	DQ522430	Coleoptera	n/a
	OSC 151914	KJ878932	n/a	KJ878978	KJ879012	KJ878950	Coleoptera	USA
O. rhizoidea	NHJ 12529	EF468969	EF468824	EF468765	EF468872	EF468922	Coleoptera	n/a
	NHJ 12522	EF468970	EF468825	EF468764	EF468873	EF468923	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	KJ878898	KJ878979	KJ879013	n/a	Hemiptera	Japan
O. sp.	Gh41	KX713656	n/a	KX713668	KX713706	n/a	Hymenoptera (*Polyrhachis* sp.)	Ghana (Atewa)
	TNS F18495	KJ878934	KJ878899	KJ878980	KJ879014	n/a	Hemiptera	USA
	OSC 151904	KJ878935	n/a	KJ878981	KJ879015	KJ878951	Hemiptera	USA
	OSC 151905	KJ878936	KJ878900	KJ878982	KJ879016	KJ878952	Hymenoptera	Guyana
O. sphecocephala	OSC 110998	DQ522551	DQ518765	DQ522336	DQ522381	DQ522432	Hymenoptera	n/a
O. stylophora	OSC 111000	DQ522552	DQ518766	DQ522337	DQ522382	DQ522433	Coleoptera	n/a
	OSC 110999	EF468982	EF468837	EF468777	EF468882	EF468931	Coleoptera	n/a
O. tricentri	CEM 160	AB027330	AB027376	n/a	n/a	n/a	Hemiptera	n/a
O. unilateralis	OSC 128574	DQ522554	DQ518768	DQ522339	DQ522385	DQ522436	Hymenoptera	Thailand
	SERI2	KX713627	n/a	KX713676	KX713731	n/a	Hymenoptera (*Camponotus sericeiventris*)	n/a
	SERI1	KX713628	KX713626	KX713675	KX713730	n/a	Hymenoptera (*Camponotus sericeiventris*)	Brazil (Atlantic Rainforest)
O. variabilis	ARSEF 5365	DQ522555	DQ518769	DQ522340	DQ522386	DQ522437	Diptera	n/a
	OSC 111003	EF468985	EF468839	EF468779	EF468885	EF468933	n/a	USA
O. yakusimensis	HMAS_199604	KJ878938	KJ878902	n/a	KJ879018	KJ878953	Hemiptera	China

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. *Ophiocordyceps*. The *O. unilateralis* clade was resolved as a monophyletic group of 23 species with bs = 100 % and *O. oecophyllae* sp. nov. as its sister taxon with bs = 77 %. We refer to the *O. unilateralis* core clade as the clade formed by the following species: *O. kimflemingiae* sp. nov., *O. camponoti-hippocrepidis* sp nov., *O. camponoti-renggeri* sp. nov., *O. albacongiuae* sp. nov., *O. camponoti-nidulantis* sp. nov., *O. camponoti-atricipis*, *O. camponoti-floridani* sp. nov., *O. camponoti-balzani*, *O. camponoti-rufipedis*, *O. camponoti-femorati* sp. nov., *O. camponoti-chartificis* sp. nov., *O. camponoti-bispinosi*, *O. pulvinata*, *O. blakebarnesii* sp. nov., *O. rami*, *O. naomipierceae* sp. nov., *O. ootakii* sp. nov., *O. halabalaensis*, *O. camponoti-saundersi*, *O. satoi* nom. et. stat. nov., *O. polyrhachis-furcata* and *O. camponoti-leonardi*. There 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-melanotici*, *O. camponoti-indiani* 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

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

Etymology: Named after the ant host genus, *Daceton*.

Specimens examined: **Brazil**, Reserva Adolpho Ducke, Manaus, Amazonas, on *Daceton armigerum* (Latreille) (Myrmicinae, Daceti), 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 Hirsutella-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 (Yanoviak 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).

Ophiocordyceps 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**, Licuala State Forest, Wongaling Beach, Queensland, on *Oecophylla smaragdina* (Formicinae: Oecophyllini), 8 Jun. 2015, S. Abell, T.S. Marney, R.G. Shivas, **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 synnemata), ampulliform, 3–10 × 3–4.5 µm, with an apical sterigma-like appendage up to 10 µm, at maturity phialidic, 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. Conidiogenous 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.

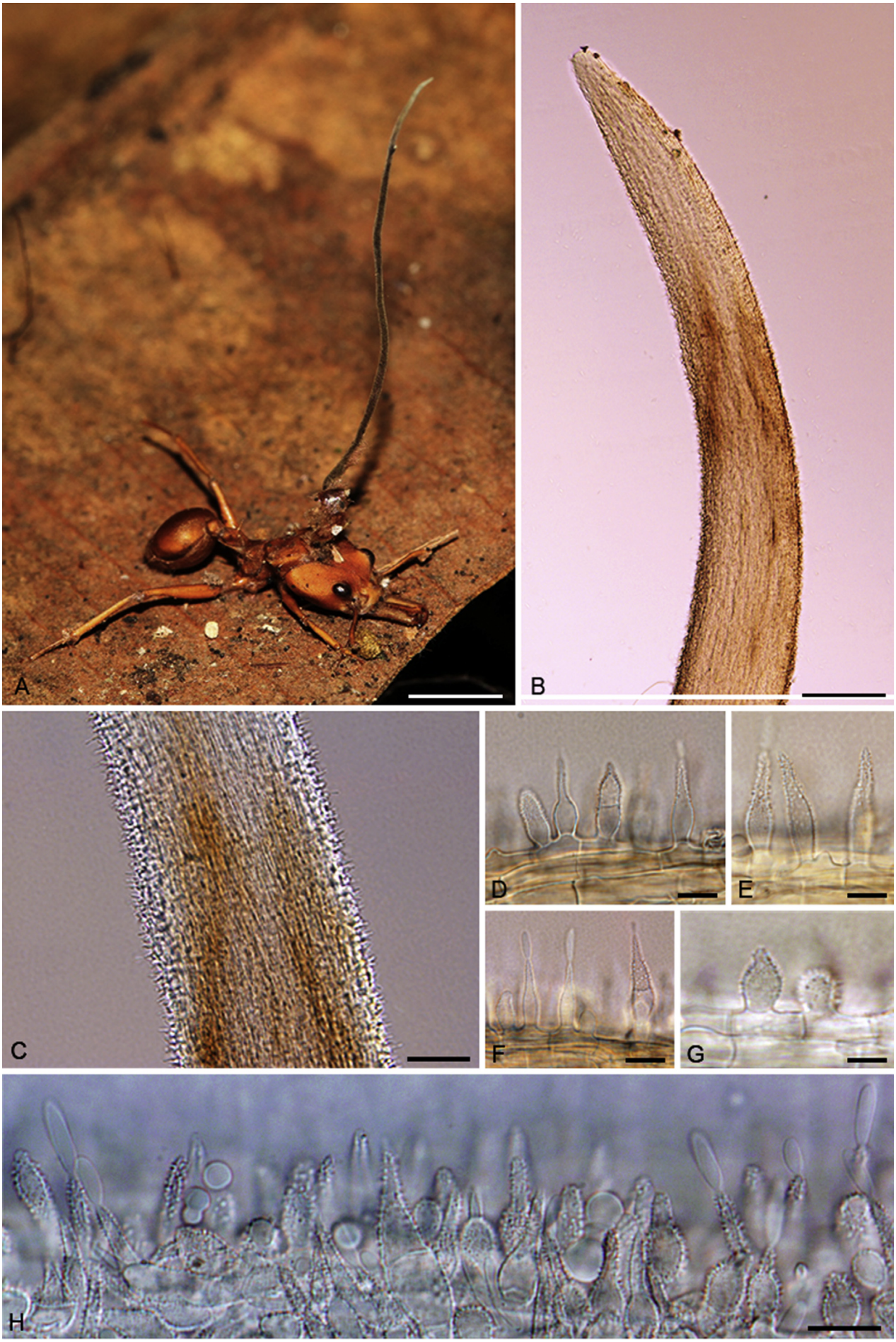

Fig. 1. *Ophiocordyceps daceti*. **A.** Infected *Daceton armigerum* on the leaf litter. **B.** Cross-section of the synnema. **C.** Close-up of synnema showing the *Hirsutella* hymenium. **D–F.** Verrucose phialides. **G.** Phialides at early developmental stage. **H.** Close-up of the hymenium of verrucose *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. Asci 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 capilliconidiophore; 25–30 µm, bearing a terminal capilliconidium, 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* (Formicinae: 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. Asci 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. rufipes*, 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-chartificis 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. Asci 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 capilliconidiophore; variable in length (65–)75–90(–95) µm; bearing a single terminal capilliconidium, 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* (Formicinae: 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. Asci 8-

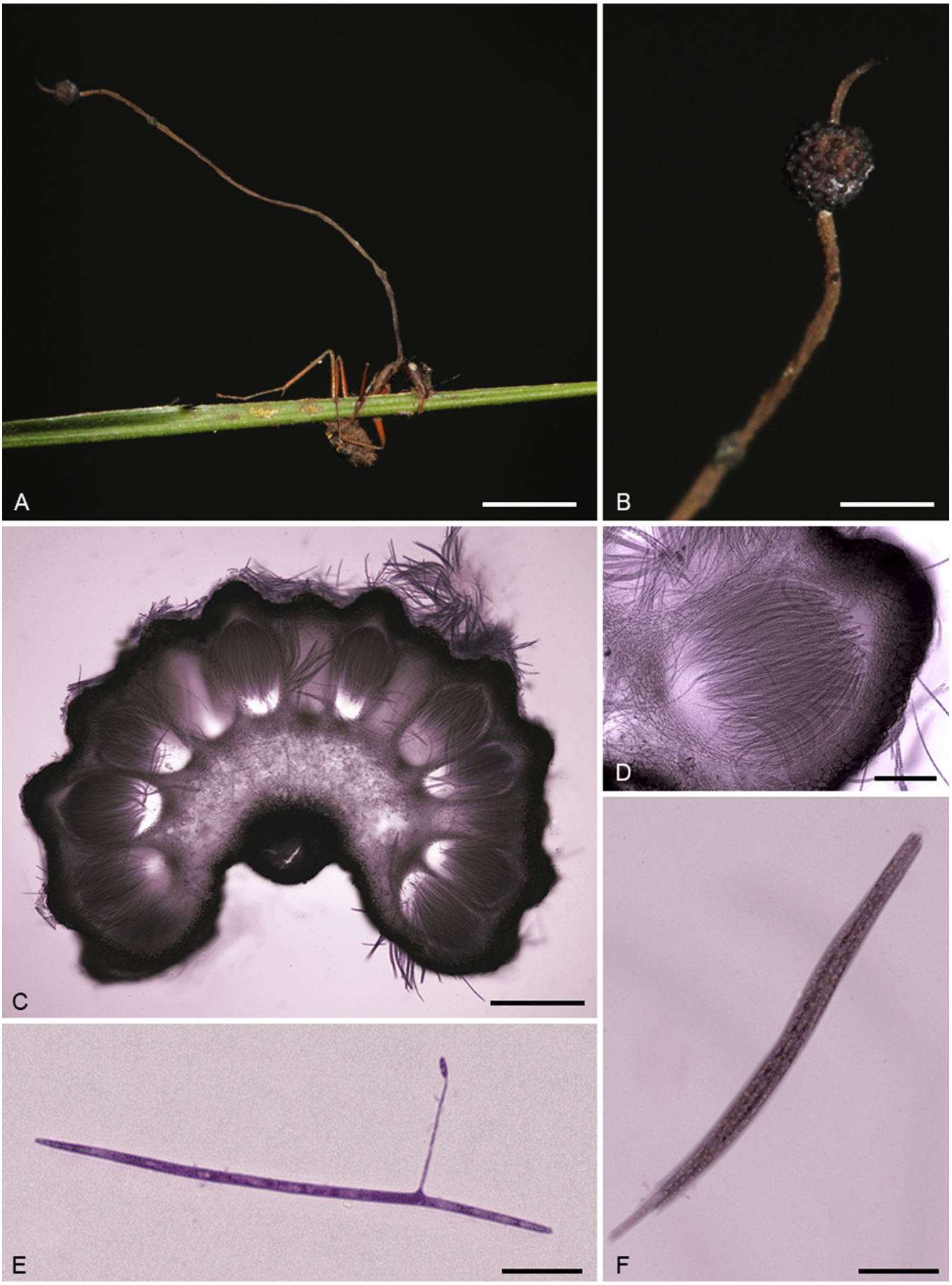

Fig. 3. *Ophiocordyceps camponoti–sexguttati*. **A.** *Camponotus sexguttatus* biting into vegetation with the long stroma arising from its dorsal pronotum. **B.** Close-up of the ascoma. **C.** Section through ascoma showing the perithecial arrangement. **D.** Close-up of perithecium. **E.** Long ascospores 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.

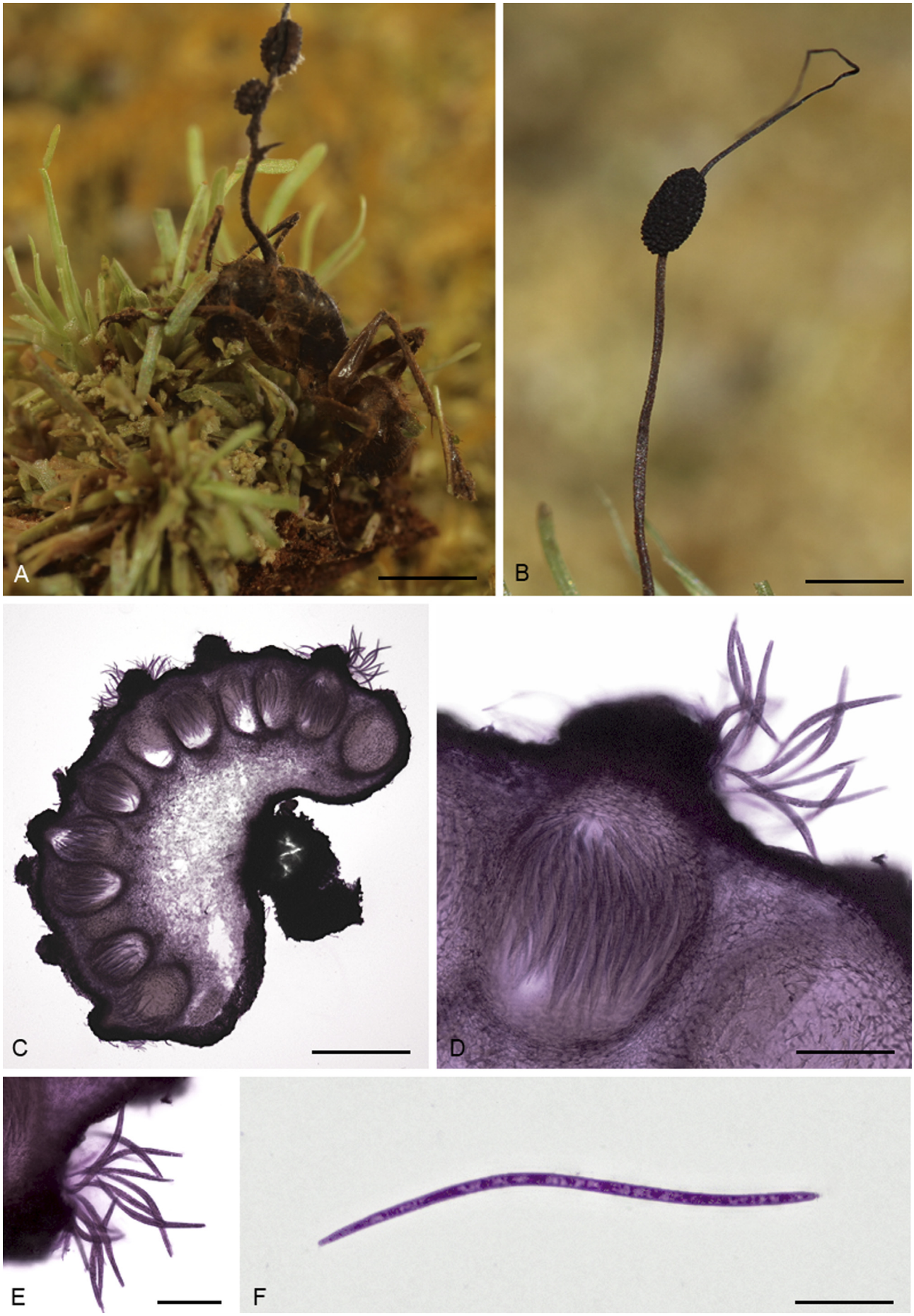

Fig. 4. *Ophiocordyceps camponoti–renggeri*. **A.** *Camponotus renggeri*, dead and attached to bryophytes on the base of trees. **B.** Close-up of the fertile part (ascoma). **C.** Section through ascoma showing the perithecial arrangement. **D.** Close-up of perithecium. **E.** Asci. **F.** Ascospores. Scale bars: A = 5 mm, B = 1 mm, C = 250 µm, D = 50 µm, E = 70 µm, F = 20 µm.

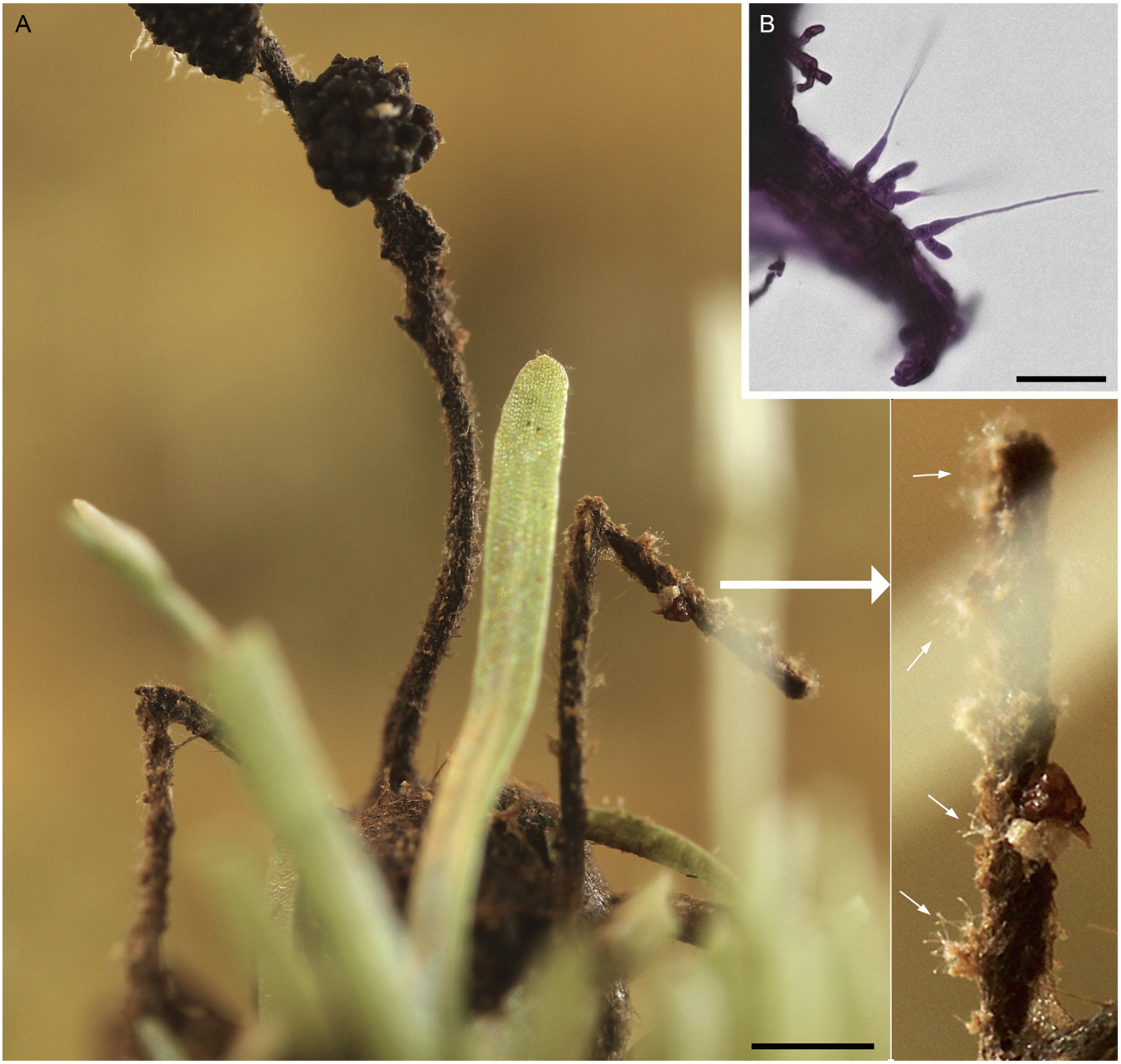

Fig. 5. *Ophiocordyceps camponoti–renggeri* (asexual morph). **A.** Ant biting into the moss carpet with the antennae raised, showing detail of the sporodochia. **B.** Phialides (hirsutella B-type). Scale bars: A = 1 mm, B = 30 µm.

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: Hirsutella A-type associated with the apical part of stroma. Hirsutella 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 m^2.

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.

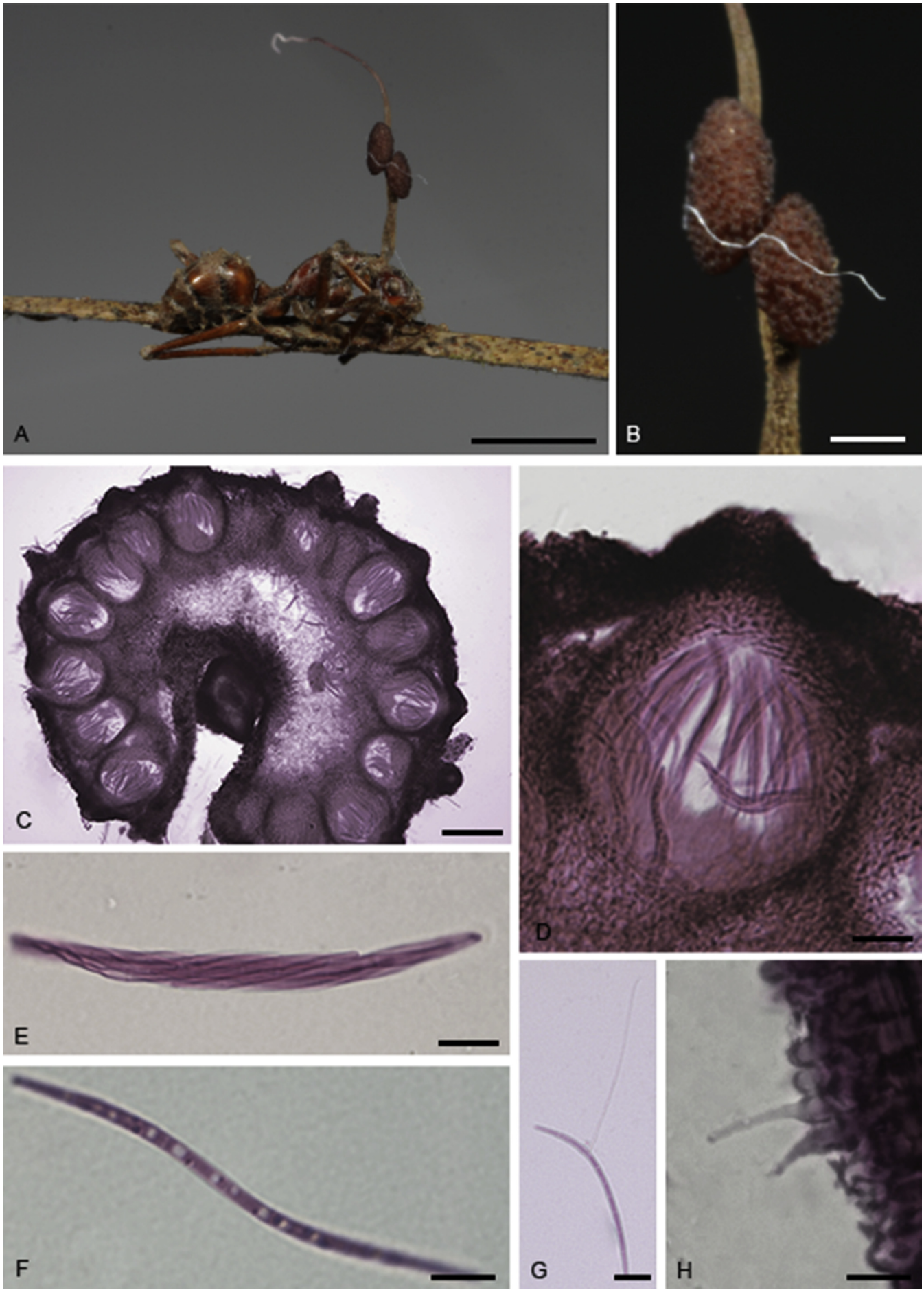

Fig. 6. *Ophiocordyceps camponoti–chartificis*. **A.** *Camponotus chartifex* biting onto a palm leaf. **B.** Close-up of the ascoma. **C.** Cross section of the ascoma showing the perithecial arrangement. **D.** Close-up of the perithecium. **E.** Ascus with ascospores arranged within. **F.** Non-germinated 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.

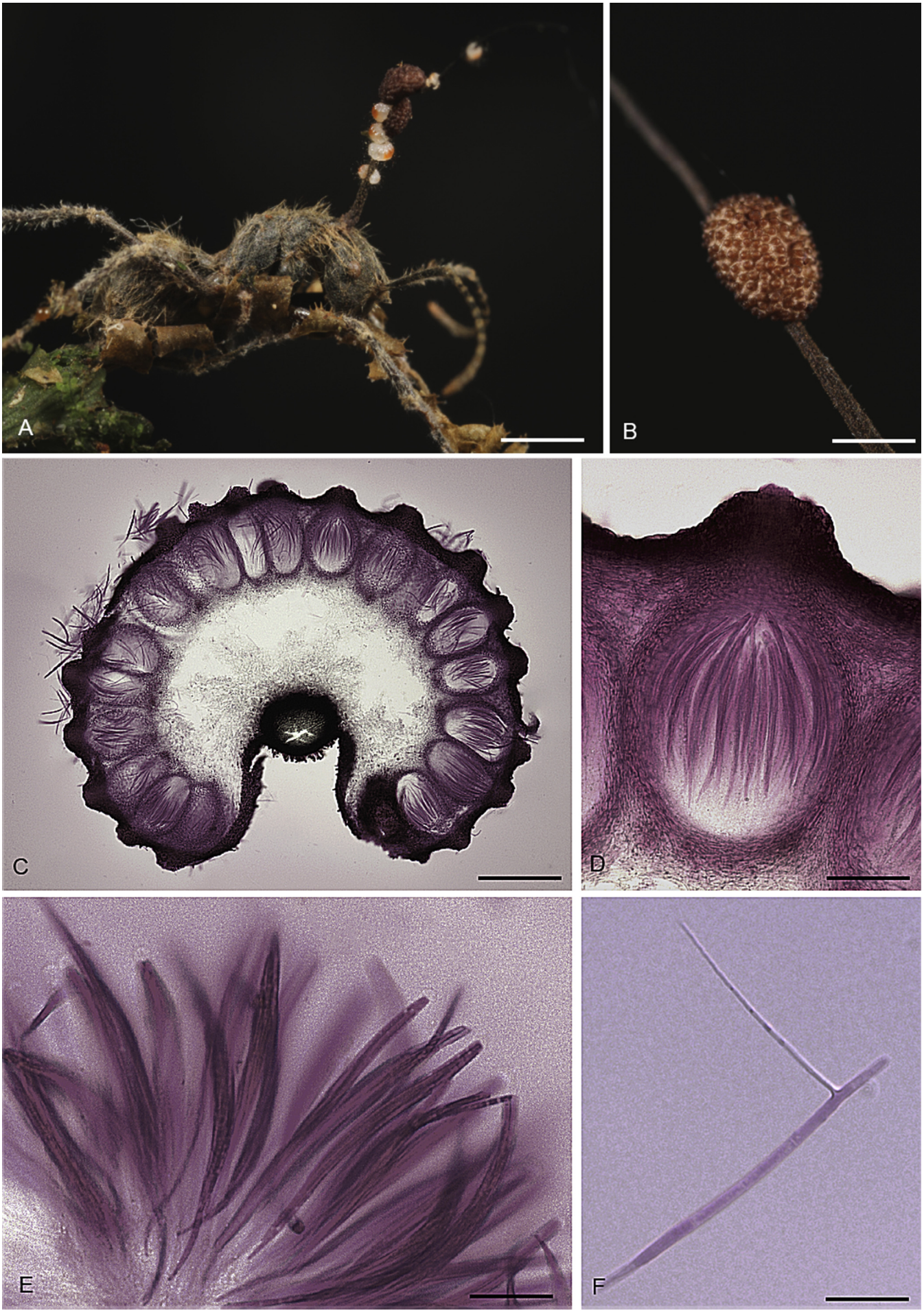

Fig. 7. *Ophiocordyceps camponoti–nidulantis* (sexual morph). **A.** *Camponotus nidulans* infected and biting into a leaf (with fly larvae on the stroma). **B.** Close-up of the ascoma. **C.** Section through ascoma showing the perithecial arrangement. **D.** Close-up of perithecium. **E.** Asci. **F.** Ascospore with capilliconidium. Scale bars: A = 3 mm, B = 1 mm, C = 200 µm, D = 75 µm, E=F = 20 µm.

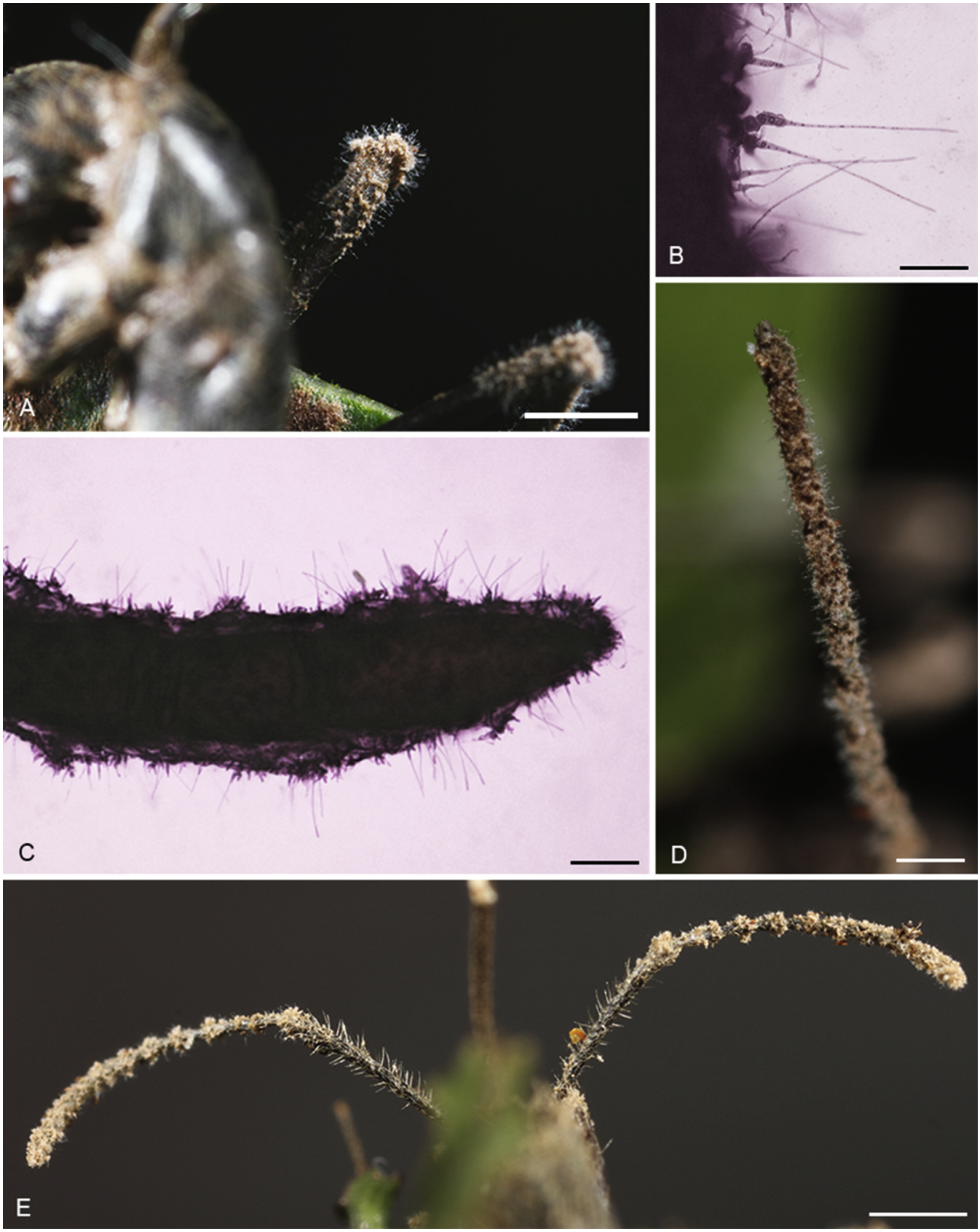

Fig. 8. *Ophiocordyceps camponoti–nidulantis* (asexual morph). **A.** Leg joints with hirsutella-like phialides. **B.** Close-up of leg phialides. **C.** Close-up of antenna covered with *Hirsutella* phialides. **D.** Antenna covered with phialides. **E.** Typical antennal display exhibited by the ant after being killed by *O. camponoti-nidulantis*, exposing the *Hirsutella* hymenium. Scale bars: A = 1 mm, B = 40 µm, C = 100 µm, D = 0.5 mm, E = 0.5 mm.

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,

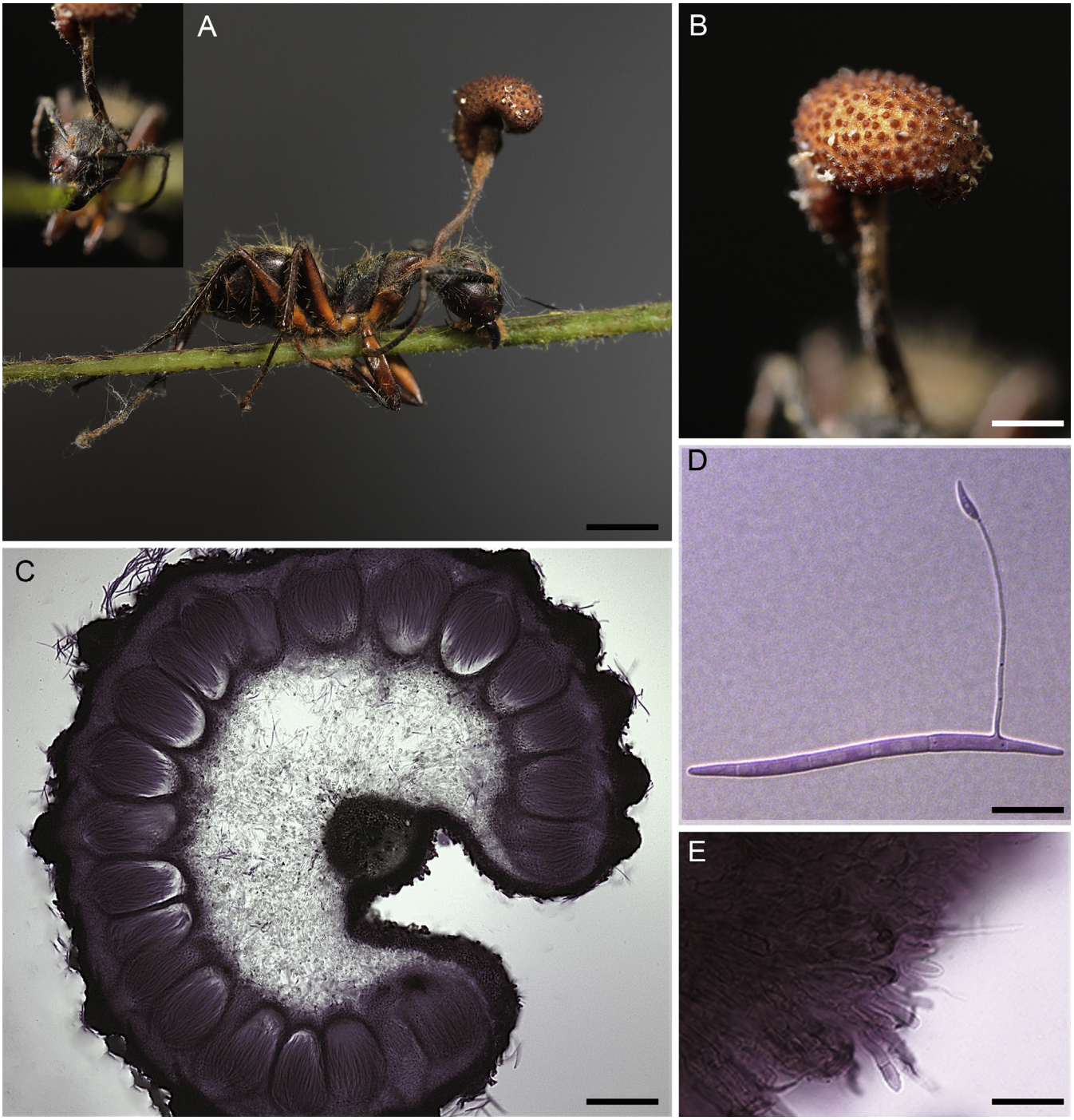

Fig. 9. *Ophiocordyceps camponoti–femorati*. **A.** *Camponotus femoratus* biting a palm leaf. **B.** Close-up of the ascoma. **C.** Cross section showing the perithecial arrangement. **D.** Ascospore with capilliconidia **E.** Hirsutella A-type phialide on the stroma. Scale bars: A = 1 mm, B = 0.3 mm, C = 200 µm, D = 20 µm, E = 5 µm.

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 *C. 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

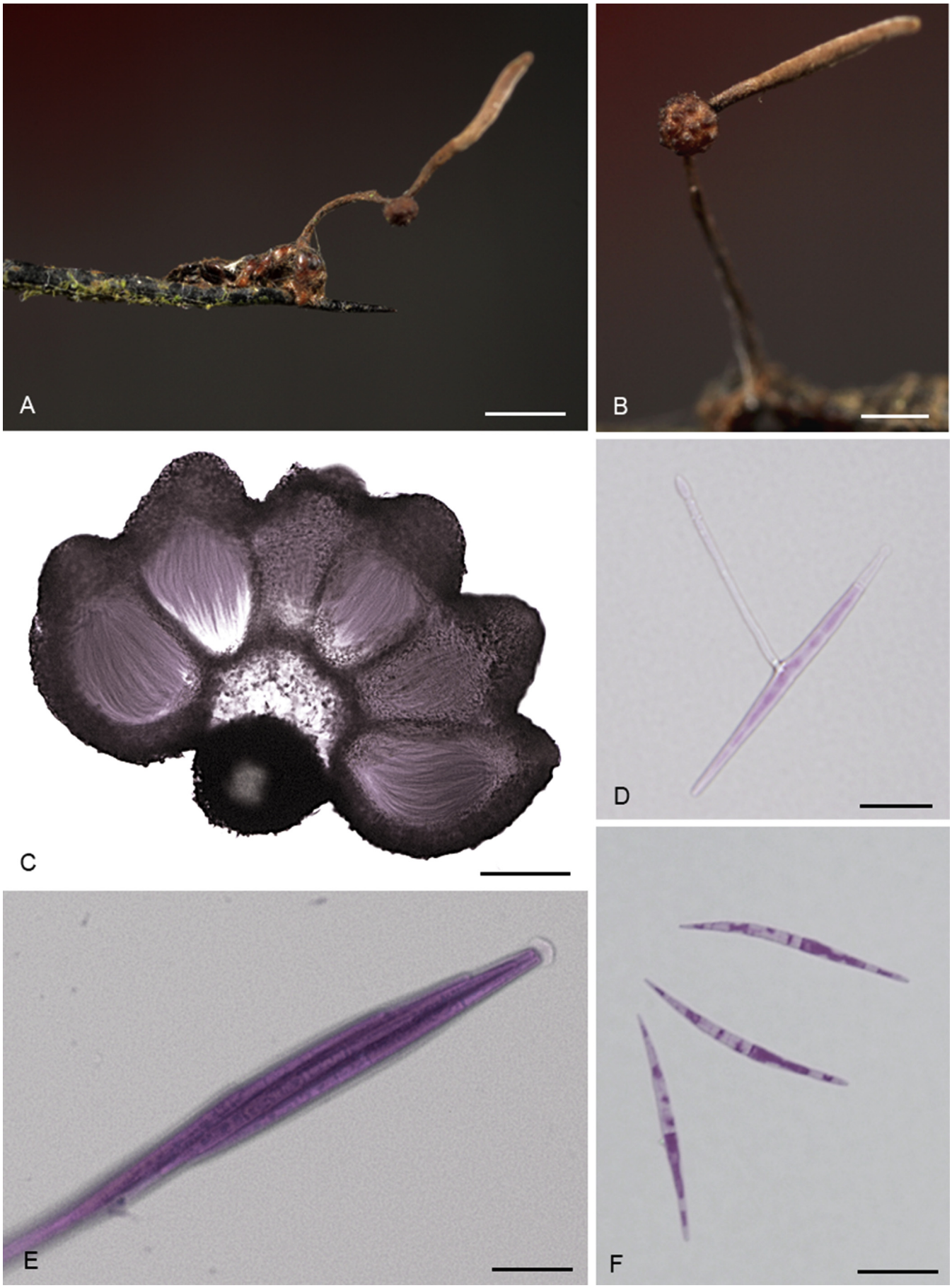

Fig. 10. *Ophiocordyceps camponoti-hippocrepidis*. **A.** Minute *Camponotus hippocrepis* (ca. 1.5 mm) biting onto a palm spine. **B.** Close-up of the ascoma. **C.** Cross-section of the ascoma showing the perithecial arrangement. **D.** Ascospore with capilliconidiophore with verrucose apical portion. **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-floridani 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, biguttulate, 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 kimflemingiae 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.

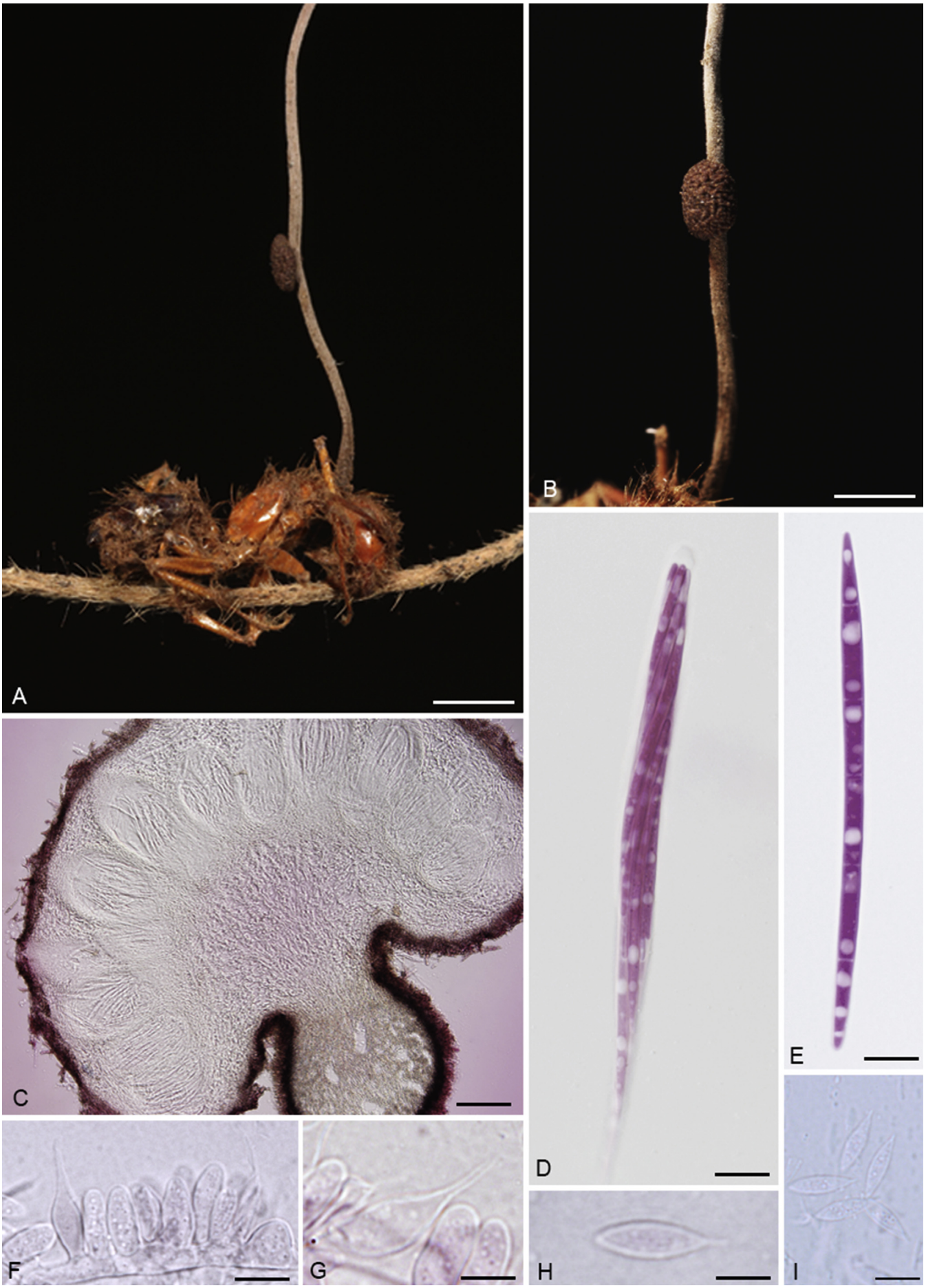

Fig. 12. *Ophiocordyceps camponoti–floridani*. **A.** *Camponotus floridanus* infected, biting into a plant. **B.** Close-up of the disc-shape ascoma attached to the stroma. **C.** Cross-section of the ascoma. **D.** Ascus. **E**. Ascospore. **F–G.** *Hirsutella* phialides. **H–I.** Limoniform conidia. Scale bars: A = 2 mm, B = 1 mm, C = 100 µm, D–F = 10 µm, G–I = 5 µm.

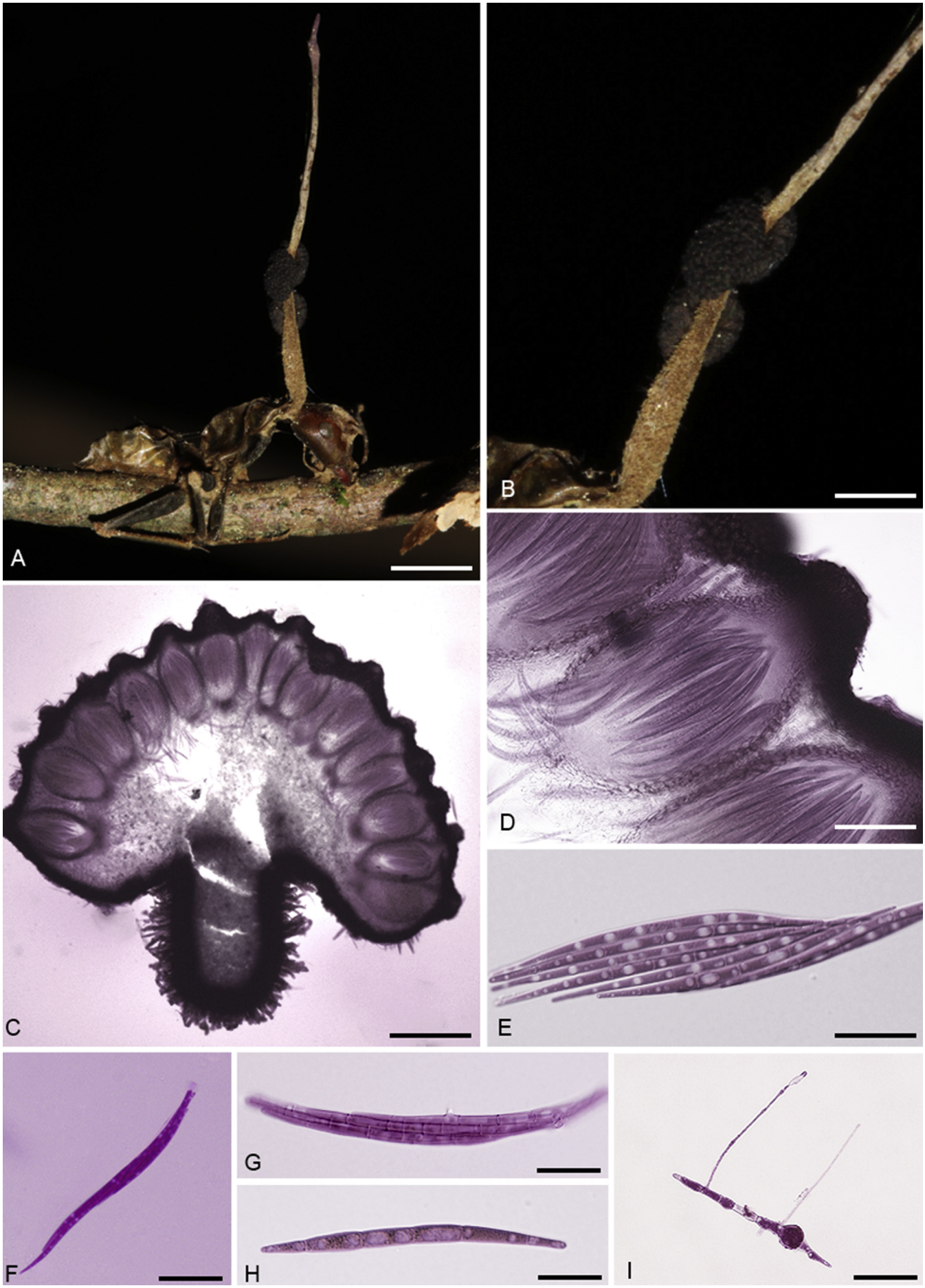

Fig. 13. *Ophiocordyceps kimflemingiae*. **A.** *Camponotus castaneus* biting a twig. **B.** Close-up of the stroma showing two ascomatal plates attached on it. **C.** Ascoma section and Perithecia arranged on its surface. **D.** Perithecium. **E.** Cluster of ascospores. **F–G.** Ascus. **H.** Ascospore. **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 blakebarnesii 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. *chromaiodes* (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: Hirsutella 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. *chromaiodes*, 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. kimflemingiae*).

Ophiocordyceps naomipierceae 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 stromata, 120−150 × 35−50 µm, phialides very abundant along the whole stromata; 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, vermiform, cylindrical, 150−180 × 7 µm. Ascospore hyaline, straight to gently curved, vermiform, 75−105 × 5−6 µm, 4−6-septate; tapering at apex.

Asexual morph: Paraisaria-like phialides produced profusely along the whole stromata; 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 understorey shrub in coastal forest; very common, associated with epizootics.

Ophiocordyceps ootakii 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**, Matsuoyama (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, vermiform, straight to gently curved, 85−100 × 3 µm, 5-septate, tapering at both ends.

Asexual morph: Hirsutella 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, Kimitu-gun, Tanjinyama-mura, Myôken-zan, 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.** Ascus. **F–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.

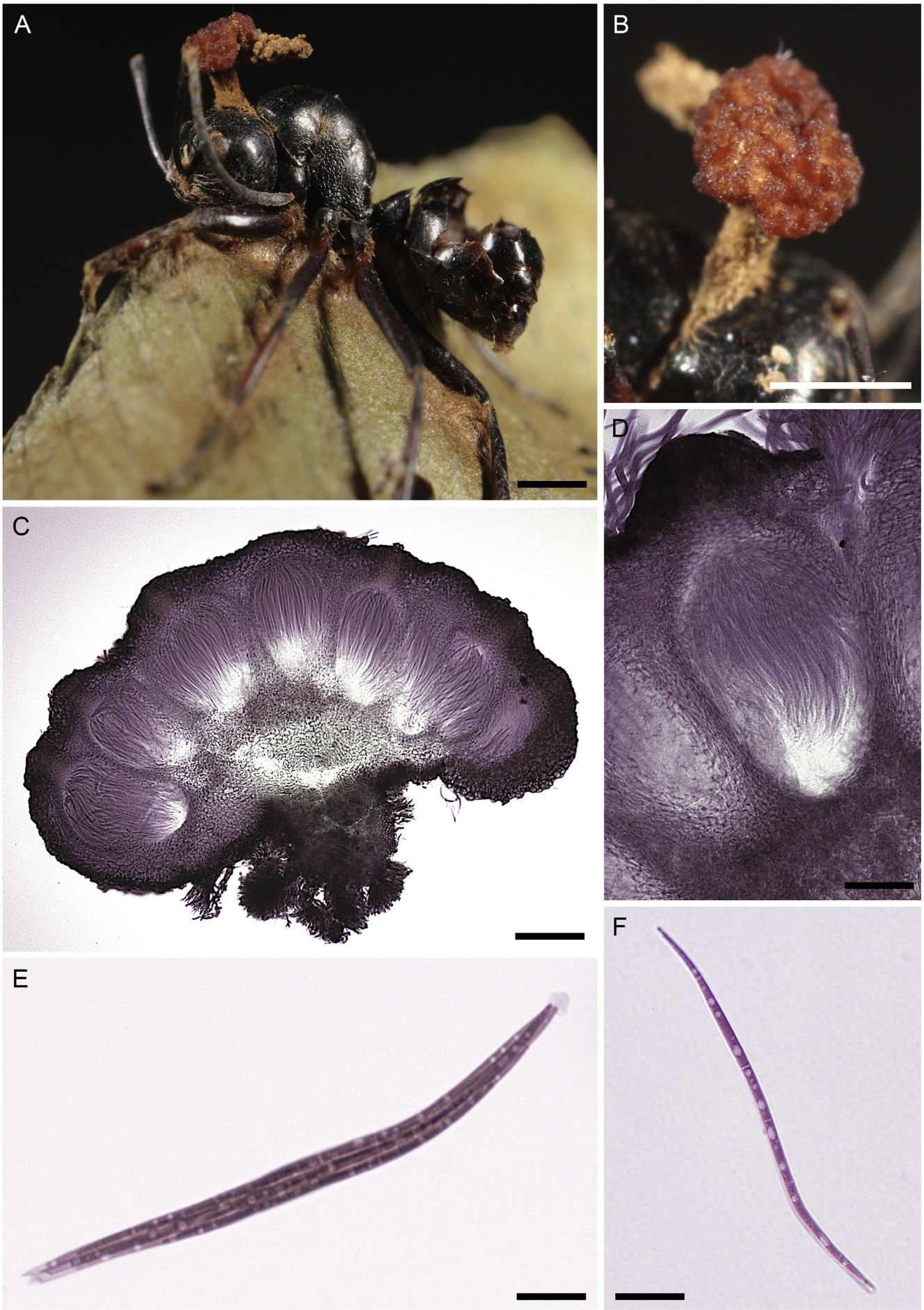

Fig. 15. *Ophiocordyceps naomipierceae* (sexual morph). **A.** *Polyrhachis* sp. biting the edge of a leaf. **B.** Close-up of the orange ascoma. **C.** Cross-section of the ascoma. **D.** Perithecium. **E.** Ascus. **F.** Ascospore. Scale bars: A = 0.5 mm, B = 1 mm, C = 100 µm, D = 50 µm, E–F = 20 µm.

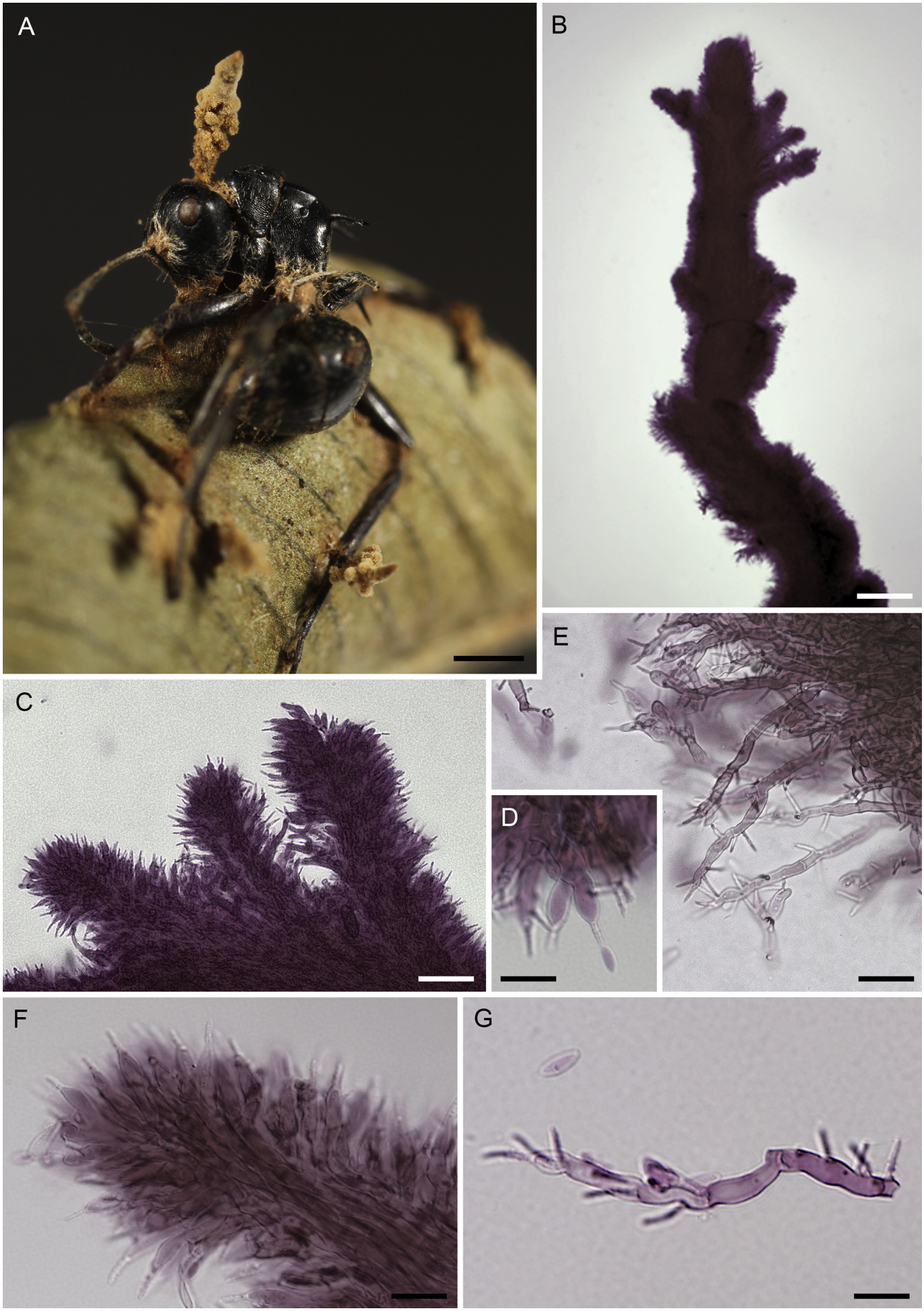

Fig. 16. *Ophiocordyceps naomipierceae* (asexual morph). **A.** *Polyrhachis* sp. with stromata arising from leg joints and dorsal pronotum. B. Synnema. **C.** Close-up of synnema. **D.** Close-up phialides **E.** Phialides. **F.** Close-up synnema showing apical phialides. **G.** Individual long phialide with multiple verrucose necks. Scale bars: A = 0.5 mm, B = 100 µm, C = 20 µm, D = 15 µm, E–G = 10 µm.

Fig. 17. *Ophiocordyceps ootakii*. **A.** *Polyrhachis moesta* biting on a leaf edge. **B.** Close-up showing two ascomatal plates attached to the stroma. **C.** Cross-section of ascoma. **D.** Ascus. **E.** Ascospore. **F.** Hirsutella A-type phialides on stroma. Scale bars: A–B = 1 mm, C = 250 µm, D–E = 20 µm, F = 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. Asci 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 macromorphological 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. naomipierceae* (Australia) are similar to *O. satoi* regarding the production of multiple stalks (Luangsa-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 septation, 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.

Ophiocordyceps 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. kniphofioides* 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 vermiform, multi-septate ascospores that do not germinate into secondary structures (e.g. capilliconidiophores) 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 capilliconidia after landing on surrounding substrata (Araújo & Hughes 2017).

Although the topological relationship between *O. unilateralis* core clade and *O. kniphofioides* 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. kniphofioides* sub-clade, rather than a member of *O. kniphofioides* sub-clade as presented by Sanjuán *et al.* (2015). This novel result allows us to consider the monophyly of *O. unilateralis* core clade + *O. oecophyllae* + *O. kniphofioides* sub-clade, forming a strictly ant-pathogenic clade within *Ophiocordyceps*. However, this is still a working hypothesis and further more inclusive studies are needed. Thus, we currently refer to the *O. kniphofioides* sub-clade as the clade formed by the species: *O. monacidis* comb. nov. et stat. nov., *O. kniphofioides 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. kniphofioides* clades produce ascospores that do not disarticulate into part-spores. No species within the *O. kniphofioides* sub-clade has ascospores that germinate in vitro, to produce either capilliconidiophores or hyphae. Conversely, production of capilliconidiophore 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. naomipierceae*, *O. camponoti-floridani*, *O. blakebarnesii*, *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 capilliconidiophores.

Ophiocordyceps camponoti-indiani produces ascospores measuring 75 × 5 µm exhibiting up to three capilliconidiophores 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 indianus* 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 between capilliconidia 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

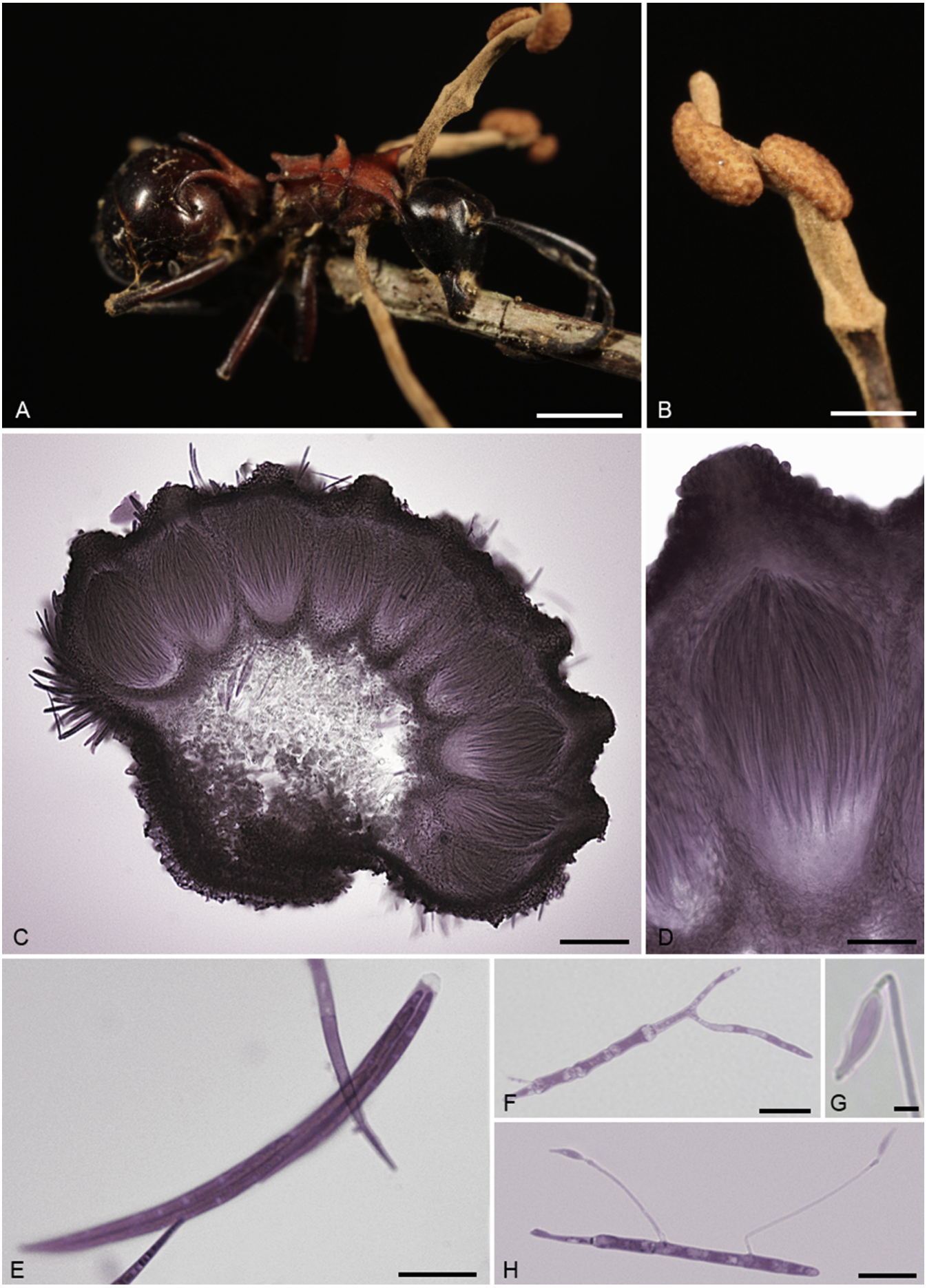

Fig. 18. *Ophiocordyceps satoi*. **A.** *Polyrhachis lamellidens*. with three stromata arising from its body. **B.** Close-up stroma with two ascomatal cushions. **C.** Cross-section of the ascoma, showing the perithecial arrangement. **D.** Close-up perithecium. **E.** Ascus. **F.** Ascospore germinating on agar plate after 3–5 d. **G.** Capilliconidium. **H.** Ascospore with two capilliconidiophores 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.

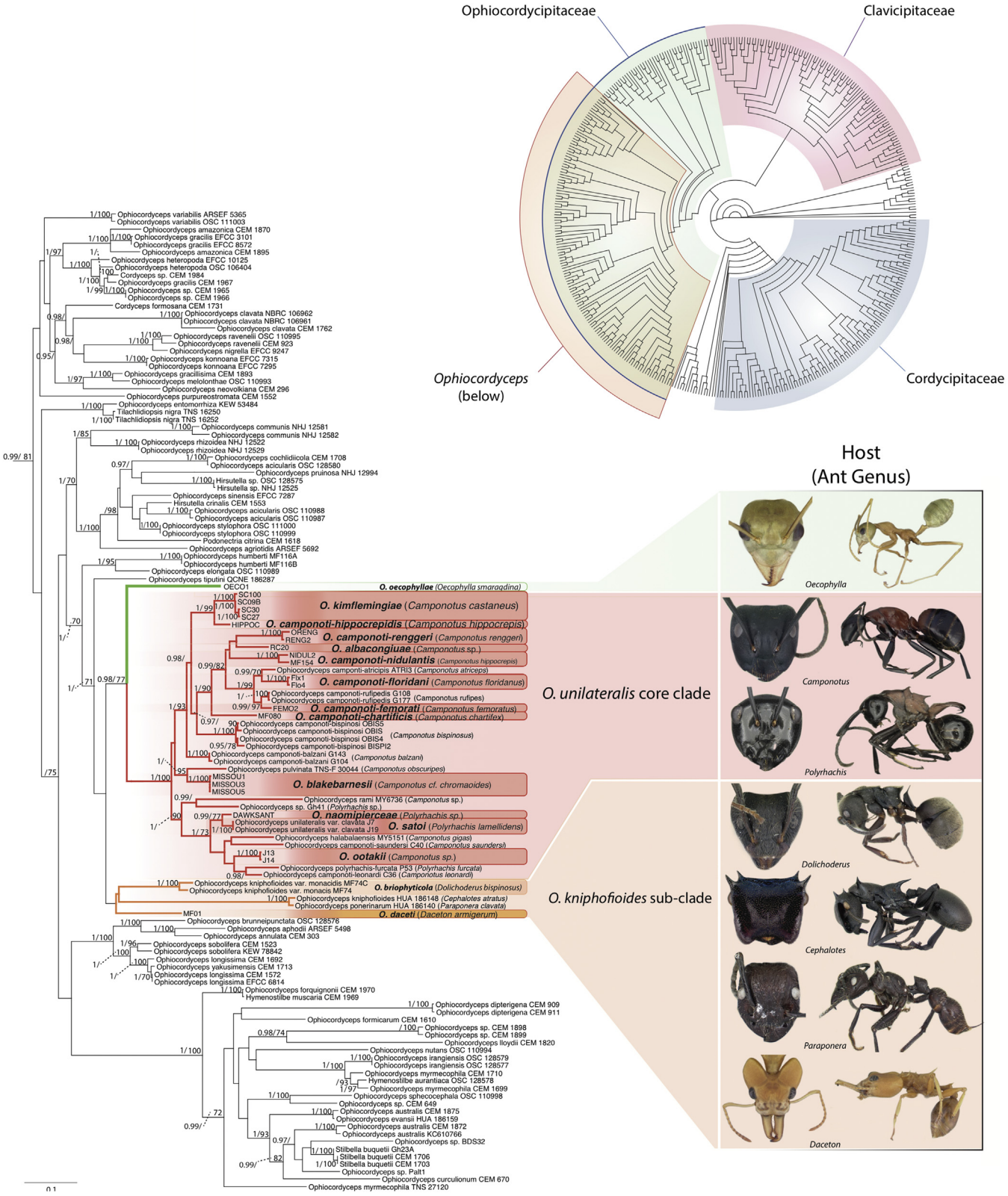

Fig. 19. Maximum Likelihood tree of *Ophiocordyceps* obtained with a combined dataset of SSU, LSU, *tef*, *RPB1* and *RPB2* based on Bayesian/RAxML analysis with only >0.95/70 shown. Species proposed in this study are highlighted. Ant figures correspond to the ant genera infected by each clade within hirsutelloid *Ophiocordyceps*. 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 *Ophiocordyceps* highlighted. (ant images from www.AntWeb.org and the photographers: *Oecophylla*, *Camponotus*, *Dolichoderus*, *Cephalotes*, *Paraponera* and *Daceton*: April Nobile, *Polyrhachis*: Will Ericson.

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

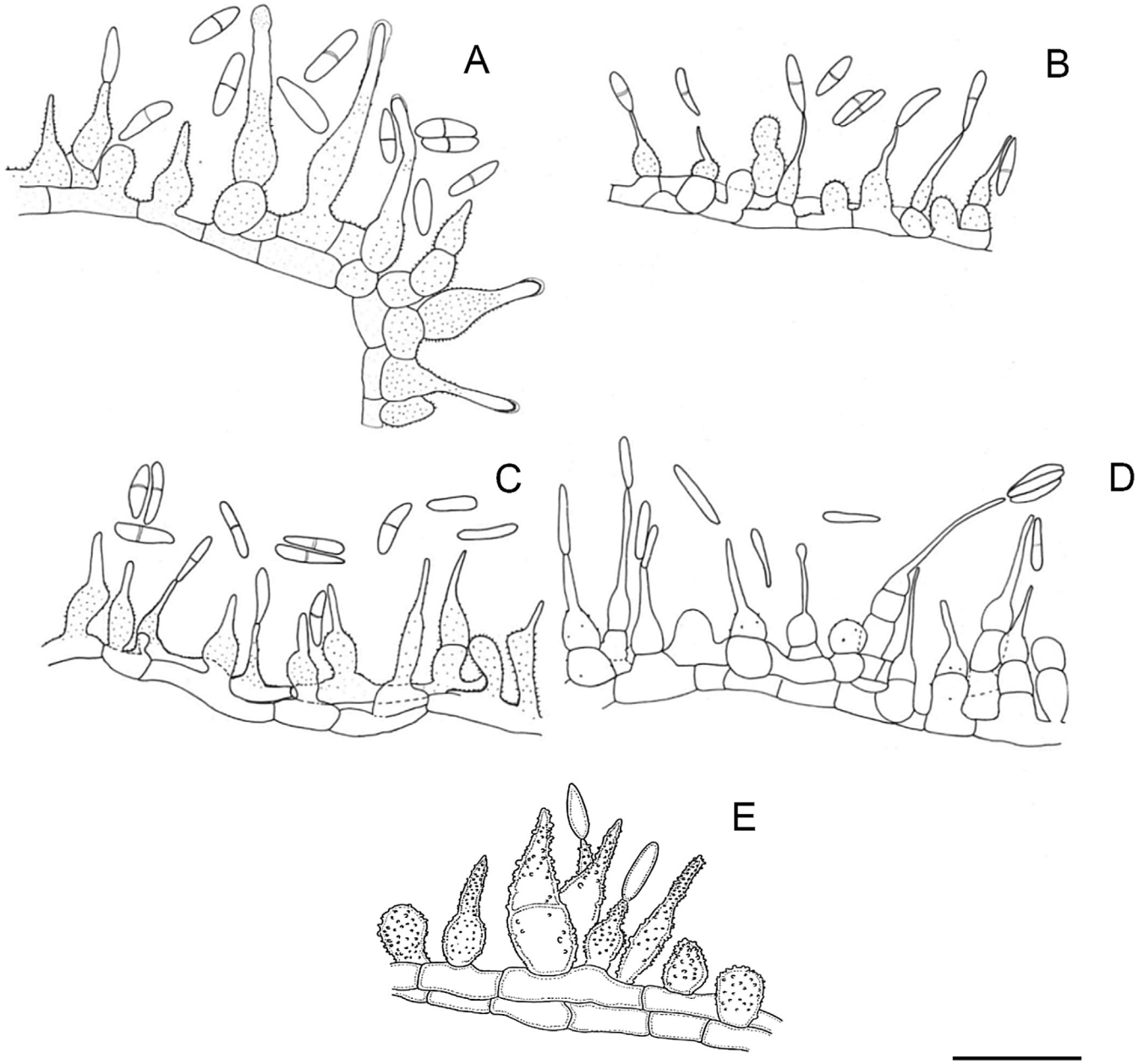

Fig. 20. Comparison of the phialide morphology for the species within the *O. kniphofioides* clade (A-phialides and conidia). **A.** *O. kniphofioides* sensu stricto. **B.** *O. monacidis*. **C.** *O. ponerinarum* **D.** *O. kniphofioides* var. *gnamptogenyos*. **E.** *O. daceti* sp. nov. (A–D. Evans & Samson 1984; E. This study). Scale bar = 10 µm.

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. camponoti-renggeri*, *O. camponoti-nidulantis*, *O. camponoti-balzani* and *O. camponoti-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

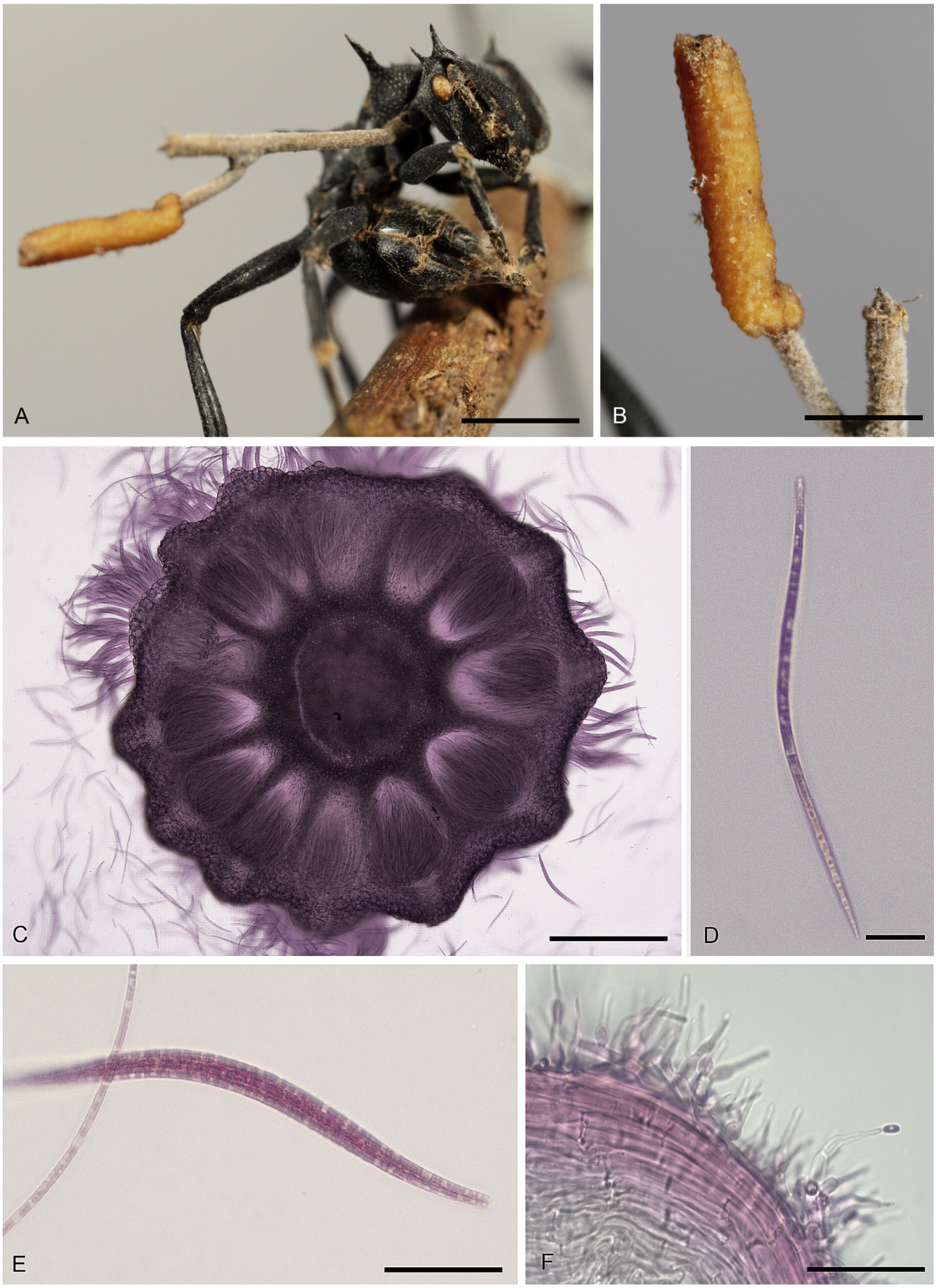

Fig. 21. *Ophiocordyceps kniphofioides sensu stricto*. **A.** *Cephalotes atratus* with the stroma arising laterally from pronotum. **B.** Close-up of the ascoma. **C.** Section of the ascoma showing the immersed perithecia. **D.** Ascospore. **E.** Ascus. **F.** Hirsutella-like phialides, present along 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 kniphofioides 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.

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).

New combinations

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

Ophiocordyceps kniphofioides (H.C. Evans & Samson) G.H. Sung *et al.*, Stud. Mycol. 57: 44. 2007. Fig. 23.
Basionym: *Cordyceps kniphofioides* H.C. Evans & Samson, Trans. Brit. Mycol. Soc. 79: 434. 1982, on *Cephalotes atratus*.

Ophiocordyceps dolichoderi (H.C. Evans & Samson) Araújo, H.C. Evans & D.P. Hughes, **comb. et stat. nov.** MycoBank MB822352.
Basionym: *Cordyceps kniphofioides* var. *dolichoderi* H.C. Evans & Samson, Trans. Brit. Mycol. Soc. 79: 437. 1982, on *Dolichoderus attelaboides*.
Synonym: *Ophiocordyceps kniphofioides* var. *dolichoderi* (H.C. Evans & Samson) G.H. Sung *et al.*, Stud. Mycol. 57: 44. 2007.

Ophiocordyceps ponerinarum (H.C. Evans & Samson) T. Sanjuan & R.M. Kepler, Fungal Biol. 119: 911. 2015.
Basionym: *Cordyceps kniphofioides* var. *ponerinarum* H.C. Evans & Samson, Trans. Brit. Mycol. Soc. 79. 441. 1982, on *Paraponera clavata*.
Synonym: *Ophiocordyceps kniphofioides* var. *ponerinarum* (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 kniphofioides* var. *monacidis* H.C. Evans & Samson, Trans. Brit. Mycol. Soc. 79: 439. 1982.
Synonym: *Ophiocordyceps kniphofioides* 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. 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.

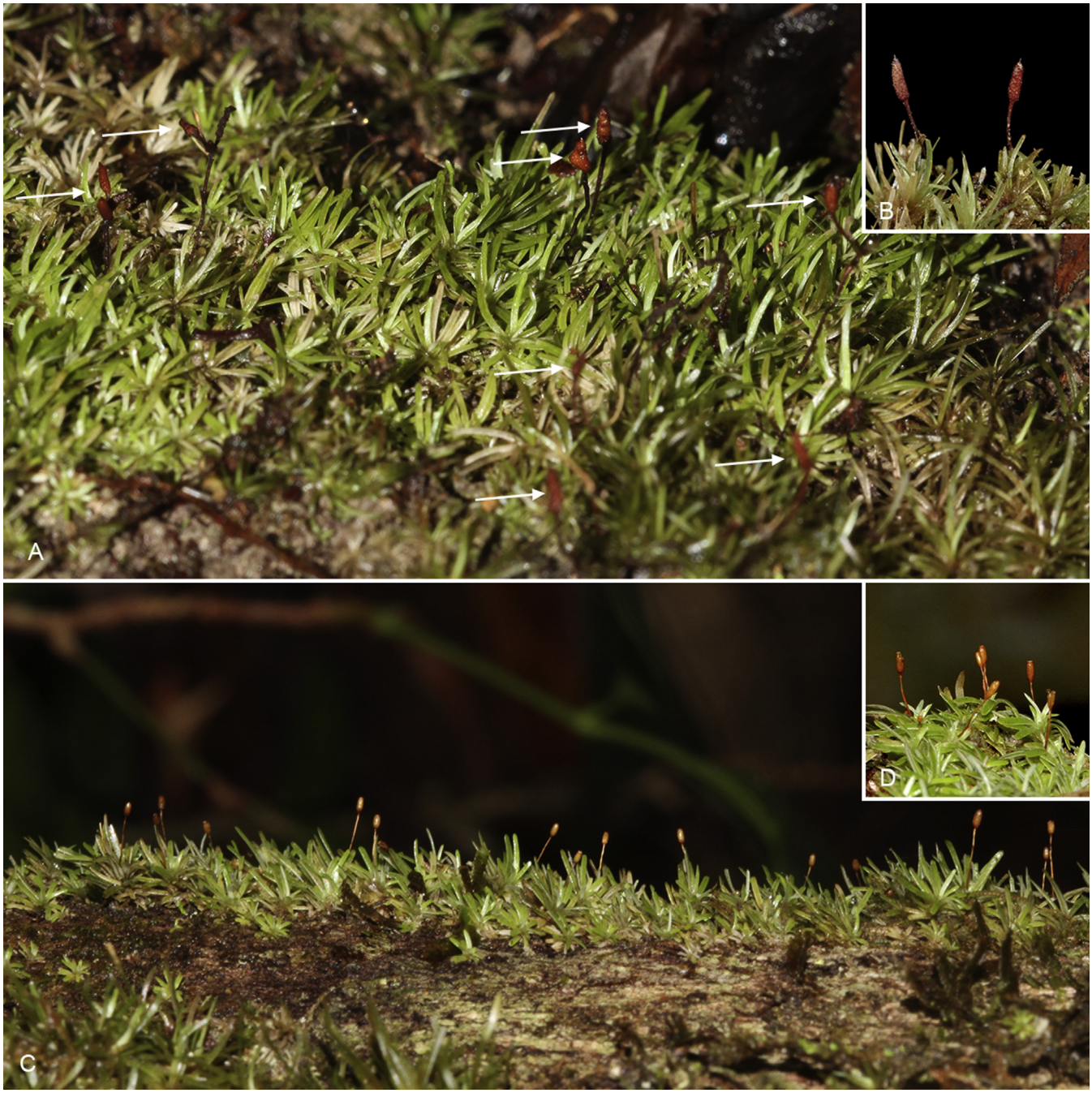

Fig. 25. A–B. *Ophiocordyceps monacidis* stromata among the moss *Octoblepharum albidum*. The ants die hidden underneath the moss carpet just exposing the fungal structures (white arrows). **C–D.** *O. albidum* on the tree surface with its orange sporophytes.

Behaviour manipulation

All myrmecophilous hirsutelloid species (*O. unilateralis* core clade + *O. oecophyllae* + *O. kniphofioides* 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. kniphofioides* clade display a less sophisticated type of manipulation of the host compared to those in the *O. unilateralis* clade. *O. kniphofioides 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-floridani*, sister species in the phylogeny, bite onto

Fig. 26. *Daceton armigerum* infected by *Ophiocordyceps daceti*. **A.** Infected ant attached on the leaf petiole, in its original upside–down position. **B.** Close-up showing the attachment exclusively by the host legs with no apparent fungal attachment structures. **C.** Close-up showing the early stages of *O. daceti* emerging from the host's dorsal pronotum.

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.

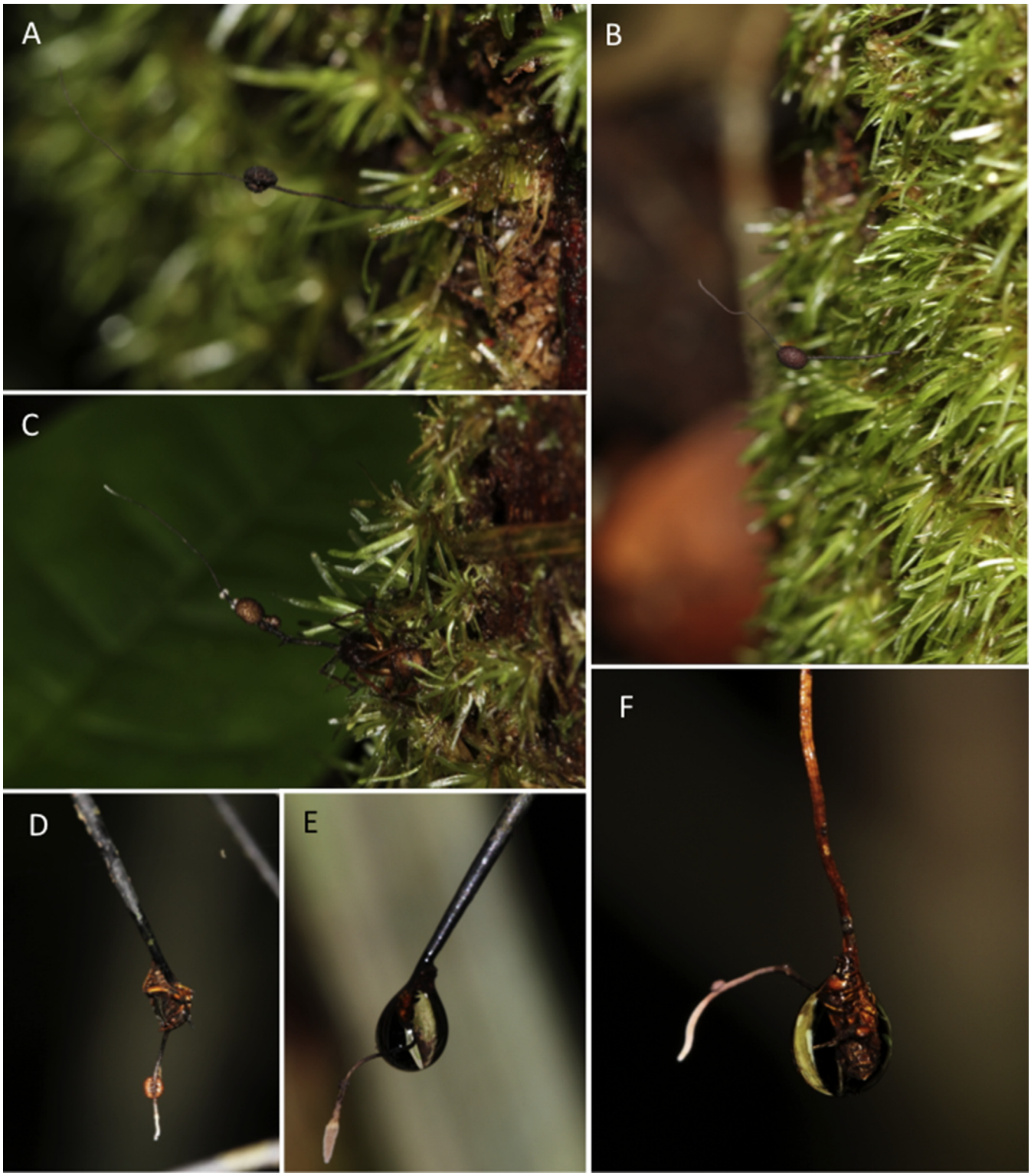

Fig. 27. Different behavioural manipulation within the *O. unilateralis* complex. **A–C.** Dead *O. camponoti-renggeri* as they are typically found, among moss at the base of trees. **D–E.** Smaller ants (e.g. *O. camponoti–bispinosi*, *O. camponoti–hippocrepidis* and *O. camponoti–femorati*) die often at the very tip of palm spines and epiphytes where water droplets form, providing continuous water resource.

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