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Cornuvesica: A little known mycophilic genus with a unique biology and unexpected new species

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ABSTRACT

Little is known about the biology of the monotypic genus *Cornuvesica* (*Microascales*), apart from that isolates are notoriously difficult to culture on artificial media. A recent collection of material resembling this genus from freshly made wounds on *Gmelina arborea* in Indonesia, provided an opportunity to reconsider all available material of *Cornuvesica falcata*, type species of the genus. In addition to morphological comparisons, multigene phylogenetic analyses were made using sequences of the SSU, ITS, LSU and TEF-1α genes. Our results showed that the holotype of *Cor. falcata* from pine in Canada differed from all other material previously considered to represent this species and also from the new Indonesian collections. The collections considered represented three additional species that we describe here as new. Three New Zealand isolates and an isolate from UK were respectively described as *Cor. acuminata* and *Cor. crypta*, while the Indonesian isolates were described as *Cor. magnispora*. Phylogenies based on the SSU and LSU data sets showed that *Cornuvesica* spp. do not belong in the *Ceratocystidaceae* as previously suggested, but represent a distinct lineage in the *Microascales* that has yet to be named. Results showed that culture filtrates from other fungi or ferric chloride markedly stimulated the growth of *Cor. magnispora*.

Introduction

The unusual and poorly known genus *Cornuvesica* includes a single ophiostomatoid fungus (Wingfield *et al.* 1993; Seifert *et al.* 2013) that has morphological features such as short-beaked, minute ascomata with 2-celled falcate ascospores, and two distinct thielaviopsis-like asexual states. These characteristics, unusual for the ophiostomatoid fungi, have resulted in *Cornuvesica* being poorly suited for inclusion in treatments of other ophiostomatoid genera broadly placed in the *Ceratocystidaceae* (*Microascales*) and the *Ophiostomatales* (Upadhyay 1981; Hausner *et al.* 1993; Wingfield 1993).

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The type species, *Cornuvesica falcata*, was initially placed in *Ceratocystis* (Wright & Cain 1961), due to its similarity to *Ceratocystis minuta* (now *Ceratocystiopsis minuta*). It was later transferred to *Ceratocystiopsis* (*Cop.*) together with other ophiostomatoid species with falcate ascospores (Upadhyay 1981). Hausner *et al.* (1993) reduced *Ceratocystiopsis* to synonymy with *Ophiostoma* based on phylogenetic analyses of partial rDNA sequences. They, however, excluded some species, including *Cop. falcata*, due to its distant phylogenetic relatedness to *Ophiostoma* and unique morphological features. Viljoen *et al.* (2000) settled the generic placement of *Cop. falcata* by introducing a new, monotypic genus, *Cornuvesica*, to accommodate this species. Hausner & Reid (2004) showed, based on SSU sequences, that the genus represents a monophyletic lineage in the *Microascales*, sister to *Ceratocystis*. *Cornuvesica* was assigned to the *Ceratocystidaceae* together with *Ceratocystis*, *Thielaviopsis*, and *Ambrosiella* (Réblová *et al.* 2011; De Beer *et al.* 2013), genera that have most recently been revised by De Beer *et al.* (2014).

The biology of *Cornuvesica falcata* remains an enigma. The species was first isolated and described from decaying pine wood in Canada (Wright & Cain 1961). The fungus has also been reported from Britain where it was isolated from the stumps of fallen birch, beech, and oak trees (Rayner & Hudson 1977), and New Zealand, where it was isolated from the galleries of bark beetles infesting larch and pine (Hutchison & Reid 1988). Although *Cornuvesica* is closely related to *Ceratocystis*, *Graphium*, and *Knoxdaviesia* (De Beer *et al.* 2013), genera known for their association with arthropod vectors, a strong association between *Cornuvesica* and arthropods has not been confirmed. The only suggestion that such an association might exist, is that the fungus has been found in bark beetle galleries (Hutchison & Reid 1988), but for the present such evidence remains circumstantial.

Cornuvesica falcata grows poorly in artificial media. Wright & Cain (1961) failed to grow the fungus in culture. Rayner & Hudson (1977) and Hutchison & Reid (1988) obtained the fungus in culture, but growth was extremely slow (less than 1–1.6 mm per day). They, however, noticed that the presence of other fungi on culture plates stimulated growth. Rayner & Hudson (1977) reported an improvement of mycelial growth and ascomatal production when *Cor. falcata* grew on plates contaminated with other fungi such as *Acremonium butyri* or *Trichoderma* spp. The source of this stimulation remained unknown (Rayner & Hudson 1977). Hutchison & Reid (1988) noted that *Cor. falcata* in bark beetle galleries always occurred together with *Gliocladium roseum*. They prepared the media with culture extracts of *G. roseum* on which *Cor. falcata* grew and observed greater numbers of ascomata and larger conidia. Kawchuk *et al.* (1993) later investigated the stimulatory agent produced by *G. roseum*. It was a small hydrophilic compound with a weight of 1 kDa and tolerant in autoclaving, but its function was not considered (Kawchuk *et al.* 1993). Most recently, Hausner *et al.* (2003) argued that the stimulating agent might not be 'solely of fungal origin' after they observed greater numbers of ascomata and increased mycelial growth of the New Zealand isolates on three different artificial media.

Not directly related to *Cornuvesica* but relevant to the present study, Thanh *et al.* (2002), when describing a new yeast species, made the discovery that *Debaryomyces mycophilus* required the presence of other commonly occurring fungi for its survival in culture. These fungi included a *Fusicoccum* sp. and a *Graphium* sp., the culture filtrates of which improved the growth of the yeast. The growth promoting factors were identified as siderophores (Thanh *et al.* 2002). These authors further discovered that supplementing 300 μM ferric chloride ($FeCl_3$) to the culture media produced the same effect as the presence of the *Fusicoccum* and *Graphium* species, confirming the role of these two fungi as donors of siderophores (Thanh *et al.* 2002).

The present study emerged from the recent discovery of a fungus resembling *Cornuvesica falcata* on wounds freshly made on the stems of *Gmelina aborea* trees in Kalimantan, Indonesia. The aim of the investigation was to identify this species, but also to reconsider the taxonomy of *Cor. falcata* based on morphology and DNA sequence comparisons by including all available material of this fungus from international collections. Since the Indonesian fungi grew very poorly in culture, we also explored techniques to enhance growth in culture, and at the same time considered the possible reasons for such behaviour in an attempt to better understand the biology of the genus.

Materials and methods

Isolates

A block of bark (approx. 10 cm^2) was removed from the trunks of *Gmelina arborea* trees growing in Northern Kalimantan, Indonesia and close to the town of Berau, and a small wedge of wood was removed to expose the xylem vessels. Six weeks after wounding, pieces of wood were removed from the wounds, placed in plastic bags and transported to the laboratory for study. Small ascomata were observed on the wood samples placed under a dissection microscope and fine tendrils of ascospores were found emerging from them. These ascospore tendrils were lifted from the ascomata using a sterile needle and transferred to malt extract agar (MEA: 2 % Biolab malt extract, 2 % Difco agar) supplemented with streptomycin (400 mg L^{-1}). This resulted in cultures that grew extremely slowly.

All the isolates generated in this study were deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa, and the ex-type cultures in the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands. Dried cultures were deposited with the National Collection of Fungi in South Africa (PREM). For comparative purposes, the only four existing cultures of *Cor. falcata* were obtained from international culture collections (Table 1). In addition, herbarium specimens for all collections of this fungus were sourced for detailed examination.

Growth in culture and culture characteristics

Isolates were grown on three different media that have previously been shown to enhance the growth of *Cornuvesica falcata* in culture. These included a medium amended with a mycelial

Table 1 – Isolates and herbarium specimens previously treated as *Cornuvesica falcata* that were included in the present study.

Voucher specimen and cultures	Host plant	Substrate	Origin of locality	References
[a]TRTC 33037 (holotype)	*Pinus strobus*	Sapwood	Canada, Ontario, York Co., NW of Nobleton	Wright & Cain 1961
[b]Herb. IMI 200338	*Betula pendula*	Sections cut from stumps	UK, England, Suffolk, King's Forest, coll. March 1975, by ADM Rayner	Rayner & Hudson 1977
[c]ATCC 36538	*Betula* sp.	Unspecified	UK, England (deposited by HJ Hudson)	Viljoen *et al.* 2000
–	*B. pendula*, *Fagus sylvatica*, *Quercus robur*	Sections cut from stumps	UK, England, Norfolk, near Thetford	Rayner & Hudson 1977
[d]UAMH 9701 (=UM 487)	*Larix* sp.	Beetle galleries in the inner bark or outer sapwood	New Zealand, Taupo, Kaingaroa State forest, Waiotapu, compartment 5 (1982/05/08)	Hutchison & Reid 1988, Hausner *et al.* 2003
–	*Pinus radiata*	Beetle galleries in the inner bark or outer sapwood	New Zealand, Auckland, Woodhill State Forest, compartment 37 (1982/05/14); Coromandel, Whangapoua State Forest, off road 41 (1982/05/19)	Hutchison & Reid 1988
[d]UAMH 9702 (=UM 792 = WIN(M) 792); UAMH 9703 (=UM 793)	*Pinus radiata*	Unspecified	New Zealand, Taupo, Kaingaroa State Forest, compartment 1212	Hausner *et al.* 1993, 2003, Hausner & Reid 2004, Réblová *et al.* 2011

a Royal Ontario Museum Mycology Herbarium (TRTC).
b CABI Bioscience UK Centre Herbarium (Herb. IMI).
c American Type Culture Collection (ATCC).
d The University of Alberta Microfungus Collection and Herbarium (UAMH).

extract of *Gliocladium roseum* (Kawchuk *et al.* 1993), that was obtained from the CMW collection, a composite medium (CM) described by Hausner *et al.* (1993), which included 17 g cornmeal agar, 10 g malt extract, 1 g yeast extract, and 10 g agar in 1 L water and a medium used by Viljoen *et al.* (2000), which included 10 g malt extract, 10 g yeast extract, 5 g tryptone, 2 g yeast carbon base, and 20 g agar in 1 L water. In addition, an MEA medium amended with 300 μM $FeCl_3$ (Thanh *et al.* 2002) was used, in an attempt to stimulate the growth of cultures.

To study culture characteristics, growth rates and the effect of an iron supplement, two culture media were used. The New Zealand and British isolates were plated on CM and the Indonesian isolates on MEA. To assess the effect of an iron supplement, the medium was amended with 300 μM $FeCl_3$ and a control was without an iron supplement. The cultures were prepared by placing a 5 mm diam agar plug bearing actively growing mycelium from a 4-week-old culture at the centre of a 90 mm Petri dish. The cultures were grown in the dark for 21 d at temperatures ranging from 15 °C to 35 °C at 5 °C intervals with five replicates for each test isolate at each temperature. Two diameter measurements, perpendicular to each other, were made for each culture and the average of the diameters was computed.

Three Indonesian isolates (CMW 37280, 37282, 37284) were tested for their sensitivity to the antibiotic cycloheximide at 0.05 % (500 mg L^{-1}). The cultures were prepared by placing a 5 mm diam agar plug containing actively growing mycelium at the centre of a 90 mm Petri dish. The cultures, including five replicate plates for each isolate, were placed in the dark at temperatures ranging from 15 °C to 35 °C at 5 °C intervals for 21 d. The sensitivity to cycloheximide was described as percentage inhibition of colony areas (πr^2) on media containing cycloheximide *versus* the controls (Hausner *et al.* 1993).

Effect of extracellular metabolites on the growth of the Indonesian isolate

Four fungal species were chosen for the extraction of extracellular metabolites: *Fusicoccum* sp. (CMW 325) and *Graphium* sp. (CMW 729) that had previously been shown to produce an extracellular metabolite facilitating iron uptake in a yeast (Thanh *et al.* 2002), *Ceratocystis* sp. (CMW 36613) and a hypocrealean fungus (CMW 37146) that was found growing together with the Indonesian *Cornuvesica* sp. on the wood from which this fungus was isolated.

Four 500 mL flasks containing 250 mL yeast-malt broth (YM broth: 5 g malt extract, 0.5 g yeast extract, 250 mL water) were inoculated with mycelium of each of the four test fungi and placed in a shaker at 150 rpm near UV light at 21 °C for 5 d. Culture filtrates were collected from the flasks using a vacuum filter. The 50 ml filtrates were then added to 450 ml yeast-malt extract agar (YMA: 2 % malt extract, 0.2 % yeast extract, 2 % agar). The culture filtrate medium was autoclaved at 121 °C for 20 min and 30 mL was then dispensed into 90 mm Petri dishes.

Six different sets of YMA were prepared: one without culture filtrate used as a control, one with 300 μM $FeCl_3$ and four representing each of the different culture filtrates. Five mm diam plugs containing mycelium of the Indonesian

Cornuvesica sp. (CMW 37282) were placed at the centres of 90 mm Petri dishes. Five replicate plates for each of the six test media (control, iron, and four culture filtrates) were incubated at 25 °C and 30 °C in the dark for 7 d. These two temperatures were shown to support the best growth of the isolate in the growth study. The growth was assessed by measuring the average of the colony diameters, which included two diameter measurements perpendicular to each other for each plate.

Microscopy and morphology

A study of the morphology of isolates was undertaken using a Zeiss Axioskop2 Plus compound microscope or a Zeiss Discovery V12 dissection microscope. Images were captured by an AxioCam ICc 3 camera and drawings were prepared using a drawing tube. All the microscopic features were studied on glass slides with specimens mounted in 85 % lactic acid other than for those of the herbarium specimens that were examined in 10 % KOH. Measurements of characteristic morphological features were made using the Axiovision 4.8 software. Twenty-five to fifty measurements were made for each structure depending on their availability. Sizes of structures are presented with a 95 % confidence level.

DNA preparation, PCR, and sequencing

Fungal isolates were grown in YM broth supplemented with 300 μM $FeCl_3$. After one to two weeks of incubation at 25 °C on a rotary shaker set at 150 rpm, fungal mycelium was harvested by centrifugation. DNA samples were prepared from the fresh fungal mycelium using PrepMan Ultra reagent (Applied Biosystems, Foster City, California) following the method used by Duong *et al.* (2012). DNA samples were kept at −20 °C for further analyses.

Four gene regions were amplified, sequenced, and used in phylogenetic analyses. These included the large subunit (LSU), the small subunit (SSU) and the internal transcribed spacers 1 and 2 (ITS1-ITS2) of the ribosomal DNA, and partial sequence of the translation elongation factor-1 alpha (TEF-1α) gene. The primers for PCR amplification and sequencing were LR0R and LR5 (Vilgalys & Hester 1990) for LSU, NS1, and NS4 (White *et al.* 1990) for SSU, ITS1, and ITS4 (White *et al.* 1990) for ITS1-ITS2 and EF2F (5′-GGTCAYYTGATCTACCAGTG-3′; designed in this study) and EF2R (Jacobs *et al.* 2004) for TEF-1α. The protocols for PCR and sequencing were the same as those described by Duong *et al.* (2012).

Phylogenetic analyses

To investigate the taxonomic relationship between *Cornuvesica* and other families in the *Microascales*, SSU, and LSU datasets were compiled from SSU and LSU sequences of *Cornuvesica* and other representative species residing in different families and orders in the *Hypocreomycetidae*. Representative species in the *Xylariales* and *Lulworthiales* were used as outgroups in the analyses of SSU and LSU datasets. The ITS and EF-1α datasets were compiled from ITS and EF-1α sequences of all *Cornuvesica* isolates included in this study (Table 2). *Knoxdaviesia proteae*, *Ceratocystis fimbriata*, and *Huntiella* (*Ceratocystis*) *moniliformis* were used as outgroup taxa in the analyses of ITS dataset. Due to the unavailability of EF-1α sequences for *K. proteae*, only *C. fimbriata*, and *H. moniliformis* were used as outgroup taxa in the analyses of the EF-1α dataset.

Each dataset was analysed separately. Alignments were done using an online version of MAFFT 7 (Katoh & Standley 2013). In the case of the ITS and EF-1α datasets, optimal substitution models were determined using jModelTest 2 (Darriba *et al.* 2012). Maximum parsimony (MP) analyses were performed in MEGA 6 (Tamura *et al.* 2013) where tree-bisection-reconnection branch-swapping algorithm was used; bootstrap replications were set at 1000 and gaps and missing data were included. Maximum likelihood (ML) analyses of ITS and EF-1α datasets were performed in MEGA 6 (Tamura *et al.* 2013) where a subtree-pruning-regrafting heuristic search level 5 was used; the bootstrap replication was set at 1000 and gaps and missing data were included. ML analyses of SSU and LSU datasets were performed using raxmlGUI

Table 2 – Isolates sequenced in this study including GenBank accession numbers.

Species	Isolate no.	Geographical origin	Host	GenBank accession numbers			
				LSU	SSU	ITS	EF
Cor. crypta	[ET]ATCC 36538 = [a]CMW 37652	Britain	*Betula* sp.	KP888984	KP888956	KP888975	KP888968
Cor. acuminata	UAMH 9701 = CMW 37653	New Zealand	*Larix* sp.			KP888974	KP888969
Cor. acuminata	[T]UAMH 9702 = CMW 37654	New Zealand	*Pinus radiata*	KP888987	KP888959	KP888972	KP888970
Cor. acuminata	UAMH 9703 = CMW 37655	New Zealand	*P. radiata*			KP888973	KP888971
Cor. magnispora	[b]CBS 134698 = CMW 37280	Indonesia	*Gmelina arborea*	KP888985	KP888957	KP888976	KP888964
Cor. magnispora	CMW 37281	Indonesia	*G. arborea*			KP888977	KP888960
Cor. magnispora	[T]CBS 134697 = CMW 37282	Indonesia	*G. arborea*	KP888986	KP888958	KP888978	KP888962
Cor. magnispora	CMW 37283	Indonesia	*G. arborea*			KP888981	KP888963
Cor. magnispora	CMW 37284	Indonesia	*G. arborea*			KP888982	KP888967
Cor. magnispora	CMW 37285	Indonesia	*G. arborea*			KP888980	KP888961
Cor. magnispora	CMW 37286	Indonesia	*G. arborea*			KP888983	KP888966
Cor. magnispora	CBS 134699 = CMW 37287	Indonesia	*G. arborea*			KP888979	KP888965

ET: ex-epitype, T: ex-holotype.
a Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (CMW).
b Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands (CBS).

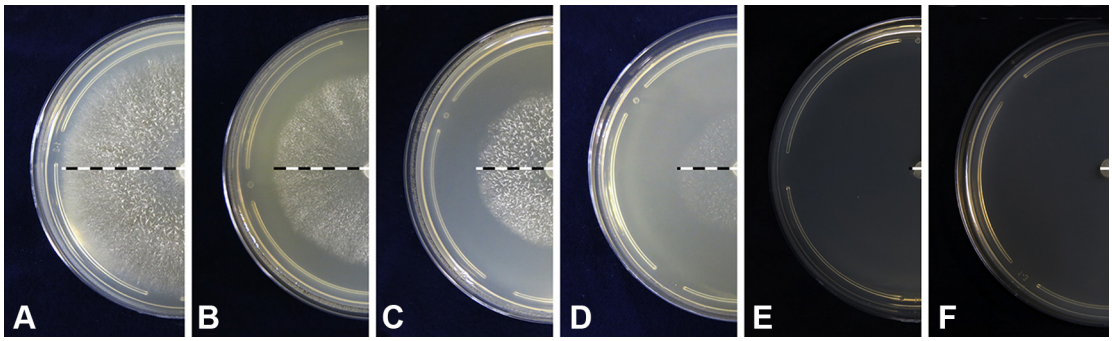

Fig 1 – Growth of *Cornuvesica magnispora* on the YMA media amended with culture filtrates, ferric chloride or nothing in the dark for 7 d at 30 °C. From A to D each plate represents different culture filtrate of the following fungi. (A). *Fusicoccum* sp. (CMW 325). (B). *Ceratocystis* sp. (CMW 36613). (C). *Graphium* sp. (CMW 729). (D). A hypocrealean fungus (CMW 37146). (E). Supplemented with 300 μM $FeCl_3$. (F). With no supplement. Scale bars: a single unit white or black bar = 3.5 mm.

(Silvestro & Michalak 2012) with the GTR+G+I model selected. Ten parallel runs, each with four threads and 1000 bootstrap replicates, were conducted. Bayesian inference (BI) analyses were performed using MrBayes 3.2 (Ronquist *et al.* 2012) applying the GRT+G+I model. Ten parallel runs, each with 2.000.000 generations, were conducted. Trees were sampled at every 100th generation. Trees sampled in the burn-in phase (25 % of the trees sampled) were discarded and posterior probabilities were calculated from the remaining trees.

Results

Isolates

Twelve *Cornuvesica* isolates were considered in this study. These included a British isolate purchased from the American type culture collection (ATCC, U.S.A.), three New Zealand isolates purchased from the University of Alberta Microfungus Collection and Herbarium (UAMH, Canada) and eight isolates collected from the wounded *Gmelina arborea* trees in Northern Kalimantan (Table 2). In addition, herbarium specimens of the holotype of *Cor. falcata* were obtained from the Royal Ontario Museum Mycology Herbarium (TRTC, Canada) and a British specimen of *Cor. falcata* described by Rayner & Hudson (1977) was obtained from CABI Bioscience UK Centre herbarium (Herb. IMI, U.K.) (Table 1).

Growth in culture and cultural characteristics

Of the four culture media screened for the growth of *Cor. falcata*, CM showed the best result for the British and New Zealand isolates and MEA provided the best substrate for growth of the Indonesian isolates. The growth study was thus performed on these two media. Neither the British nor the New Zealand isolates grew at temperatures above 30 °C and they displayed extremely slow growth at other temperatures (7–20 mm diam in 21 d). The Indonesian isolates grew slowly at all temperatures (7–27 mm diam in 21 d). The iron supplement showed a positive effect on the Indonesian isolates but almost no effect on the British and New Zealand isolates. The sensitivity of the Indonesian isolates to cycloheximide was assessed between 20 °C and 30 °C where vigorous growth occurred. All three isolates showed sensitivity. The details of growth characteristics for each isolate are provided in the taxonomy section.

Effect of extracellular metabolites on the growth of an Indonesian isolate

Culture filtrates of all four test fungi produced some level of stimulation to the growth of the Indonesian *Cornuvesica* sp. (Fig 1). This growth stimulation was greater at 30 °C than at 25 °C. The *Fusicoccum* sp. (CMW 325) culture extract resulted in colonies of *Cornuvesica* sp. growing 5–6 times (63 mm diam at 25 °C, 75 mm at 30 °C) larger than the control (12 mm). The *Ceratocystis* sp. (CMW 36613) extract resulted in 4–5 times larger (51 mm at 25 °C, 62 mm at 30 °C) colonies, the *Graphium* sp. (CMW 729) in 3–4 times larger (40 mm at 25 °C, 50 mm at 30 °C) colonies and the hypocrealean fungus (CMW 37146) in 3 times larger (38 mm at 25 °C, 40 mm at 30 °C) colonies. All the cultures grown on the medium supplemented with culture filtrates produced sexual states in 14 d. Amongst these, the medium including the *Fusicoccum* sp. (CMW 325) extract gave rise to sexual structures first.

Phylogenetic analyses

The SSU dataset consisted of 65 taxa and had 1014 characters including alignment gaps, of which 654 were constant and 261 were parsimony-informative. The LSU dataset consisted of 73 taxa and had 745 characters including alignment gaps, of which 336 were constant and 362 were parsimony-informative. The SSU sequence (GB: AY271797) generated from a New Zealand isolate of *Cornuvesica* (UAMH 9702) in Hausner & Reid (2004) did not match with SSU sequence generated from the same isolate in this study and that sequence was consequently not included in the analyses. The ITS dataset consisted of 640 characters, of which 330 characters were constant and 148 characters were parsimony-informative. The EF-1α dataset had 921 characters, of which 683 characters were constant and 71 characters were parsimony-informative.

As determined by jModelTest 2, the GTR+I substitution model was used in the analyses of ITS and EF-1α. GTR+G were used in the analyses of SSU and LSU datasets.

Trees resulting from analyses of SSU and LSU differed in topologies. However, all the major lineages representing different families and orders could clearly be identified. In all the analyses of SSU and LSU datasets, isolates of *Cornuvesica* consistently formed a strongly supported monophyletic clade and were relatively closely related to species in the *Ceratocystidaceae* and *Gondwanamycetaceae*. In the analyses of SSU dataset, *Cornuvesica* species formed a monophyletic clade with members of the *Ceratocystidaceae* and *Gondwanamycetaceae* and resided in the *Microascales*. This relationship was, however, not clear in the LSU dataset. Phylogenetic trees resulting from ML analyses of the SSU and LSU datasets are presented in Fig 2 and Fig 3 respectively.

Phylogenetic analyses of ITS and EF-1α datasets resulted in trees with the same topology in all (MP, ML, and BI) of the analyses (Fig 4). Isolates from Indonesia always grouped together forming a well-supported lineage. The three isolates from New Zealand previously known as *Cop. falcata* also formed a distinct, well-supported lineage, while the single isolate from Britain was distinct from the other two lineages in all trees. Although the data sets were small, the three lineages encompassing the *Cornuvesica* isolates considered in this study consistently grouped together with strong statistical support, distinguishing them from the *Knoxdaviesia* and *Ceratocystis* outgroup taxa.

Taxonomy

Phylogenetic analyses and morphological comparisons revealed a well-supported monophyletic lineage in the *Microascales* that included three distinct taxa, described here as new species, that are morphologically distinct from the type species, *Cornuvesica falcata*. Morphology and phylogenetic analyses confirmed that isolates previously treated as *Cor. falcata* from Britain (Rayner & Hudson 1977) and New Zealand (Hutchison & Reid 1988) as well as those collected in the present study from Indonesia, were all distinct from the holotype of *Cor. falcata* originally collected in Canada. Because the morphological descriptions of *Cor. falcata* subsequent to the original species description represented various different species as shown in the present study, a new description for *Cor. falcata* based only on the holotype specimen is provided. This was then compared and contrasted with the three new species recognised in this study.

Cornuvesica falcata (E.F. Wright & Cain) C.D. Viljoen, M.J. Wingf. & K. Jacobs, Mycol. Res. 104: 366. 2000. emend. Marinc., T.A. Duong, Z.W. De Beer, M.J. Wingf. Figs 5A–C, 7E, F.

Synonyms: *Ceratocystis falcata* E.F. Wright & Cain, Can. J. Bot. 39: 1226. 1961

Ceratocystiopsis falcata (E.F. Wright & Cain) H.P. Upadhyay, Monogr. *Ceratocystis*, and *Ceratocystiopsis*, p. 125. 1981.

On a mounted microscope slide enclosed in the type specimen, *Ascomata* brown, beak darker than base, 60–90 μm diam. *Ascospores* hyaline, falcate, gradually attenuating towards both ends, with one end more attenuated, straight or slightly curved, 1-septate, septum indistinct, supra-median, (22–)25–27(–30) × 1–2 μm (avg. 26.1 × 1.3 μm), thin-walled, smooth. No asexual structures observed.

Specimen examined: **Canada**: *Ontario*, York Co., NW of Nobleton, on the sapwood of *Pinus strobus*, 1 July 1957, *R.F. Cain*, TRTC 33037, holotype.

Notes — The holotype specimen included wood pieces and two microscope slides. Unfortunately no evidence of *Cor. falcata* could be found on the wood pieces but the microscope slides were fairly well-preserved. On the one slide, ascomata on a wooden substrate and ascospores were clearly visible (Figs 5A–C, 7E, F). Wright & Cain (1961) reported that they did not observe any asexual state on the specimen and also failed to grow the fungus in artificial media. The only possibility to confirm the phylogenetic position of this species would be to collect fresh material from the same host and origin, which could then be designated as an epitype.

Cornuvesica crypta Marinc., T.A. Duong, Z.W. De Beer, M.J. Wingf., **sp. nov.** — MycoBank MB811610; Figs 5D–G, 6A, D, E, 7A–D.

Etymology — name refers to the fact this species was obscured in having been treated as *Cor. falcata*.

On wood, *Ascomata* scattered or clustered in groups, superficial, embedded in loose mycelial mat on which asexual states are formed, sparsely covered with brown conidiophores, with conical opening distinctly recognisable, dark brown to blackish, 70–100 μm diam. *Peridium* firm, pseudoparenchymatous, *textura epidermoidea* to *angularis*. *Ostiolar hyphae* convergent, compactly adhered to each other, pale brown to hyaline. *Asci* evanescent. *Ascospores* hyaline, falcate, gradually attenuating towards both ends, with one end more attenuated and frequently curved like a hook, straight or slightly curved, 1-septate, septum indistinct, supra-median, (21–)25–27(–31) × 1–2 μm (avg. 26 × 1.4 μm), thin-walled, smooth. *Asexual states* thielaviopsis-like with two distinct ranges of conidial dimensions. *Micro-* and *Macro-conidiophores* micronematous, semi-macronematous, mostly unbranched, cylindrical, septated. *Micro-conidiogenous cells* phialidic, collarette indistinct, hyaline, discrete, mostly intercalary, cylindrical gradually tapering to the apex, straight or curved, (21–)28–32(–47) μm long, (3–)3.5–4(–5) μm wide near the base, 2–3 μm wide near the apex. *Macro-conidiogenous cells* phialidic, collarette indistinct, pale brown to brown, discrete or integrated, intercalary or terminal, cylindrical gradually tapering to the apex, straight or curved. *Micro-conidia* hyaline, oblong with round ends, (4–)5–5.5(–7) × 2(–2.5) μm (avg. 5.4 × 2.1 μm), smooth, in chains, endogenous. *Macro-conidia* hyaline, oblong with truncate ends, (5–)6–7(–9) × 3–3.5(–4) μm (avg. 6.5 × 3.3 μm), smooth, in chains, endogenous.

Culture characteristics — Colonies showing irregular growth, white near the centre, becoming transparent towards the edge, flat, mycelium mostly submerged, not densely compact, showing no effect on medium, becoming fertile in 7 d. There was no growth at 30 °C and 35 °C and at other temperatures showed extremely slow growth, reaching 7 mm diam at 15 °C in 21 d, 20 mm at 20 °C and 19 mm at 25 °C. The addition of 300 μM $FeCl_3$ to the medium showed no effect on the growth.

Specimens examined: **United Kingdom**: *England*, Suffolk, King's Forest, on a slice from cut stump of *Betula pendula* after 21 d incubation in a damp chamber (N.G. Refs. TL796721), Mar. 1975, *A.D.M. Rayner*, Herb. IMI 200338, holotype; England, *Betula* sp., deposited by H.J. Hudson, a dried culture of ATCC

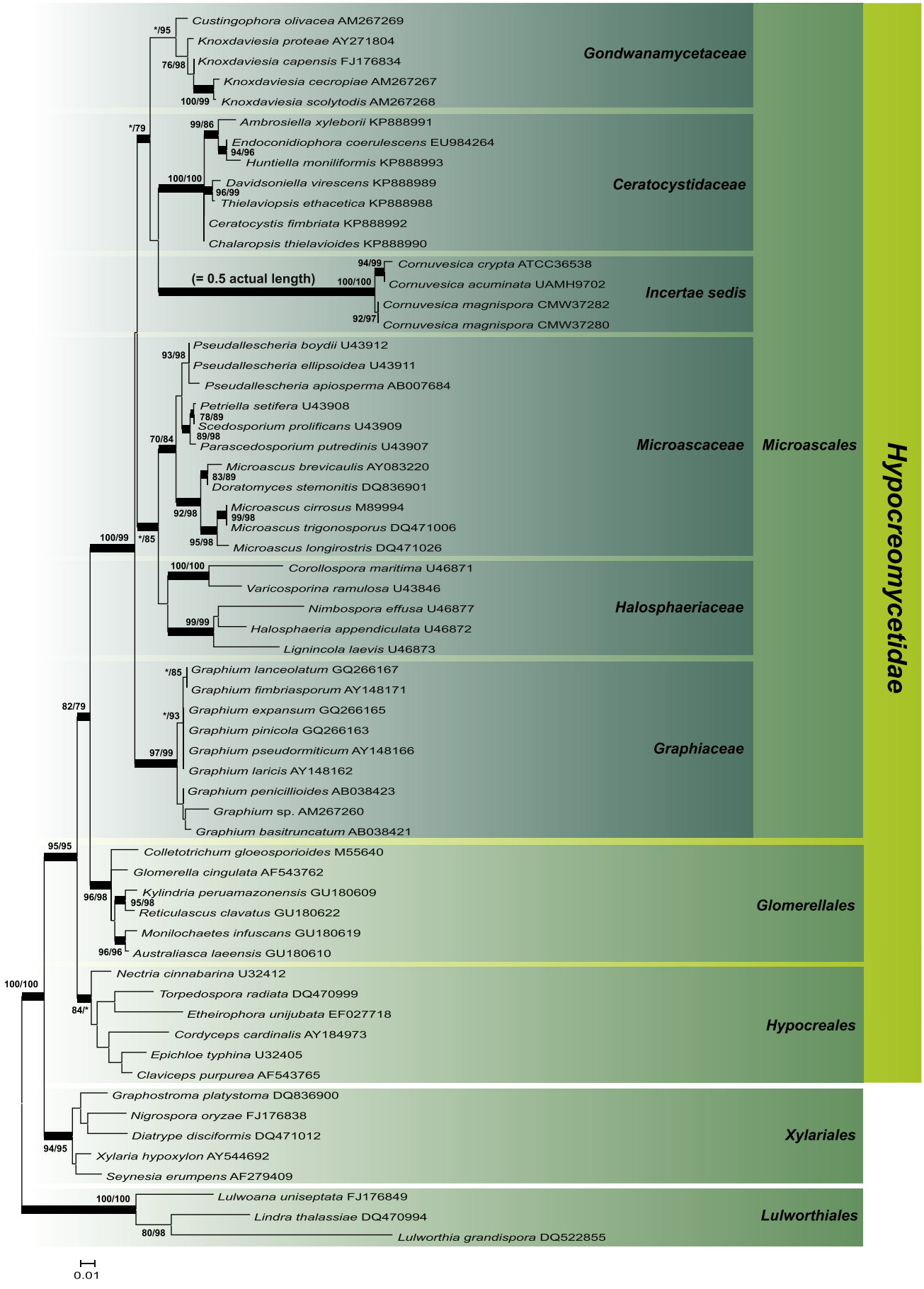

Fig 2 – Phylogenetic tree derived from ML analyses of SSU dataset. Bootstrap supports (≥75 %) for ML/MP are presented at nodes. Thick branches represent BI posterior probabilities ≥95 %. To aid with tree presentation, the branch length for the *Cornuvesica* clade has been shortened by half as indicated in the tree.

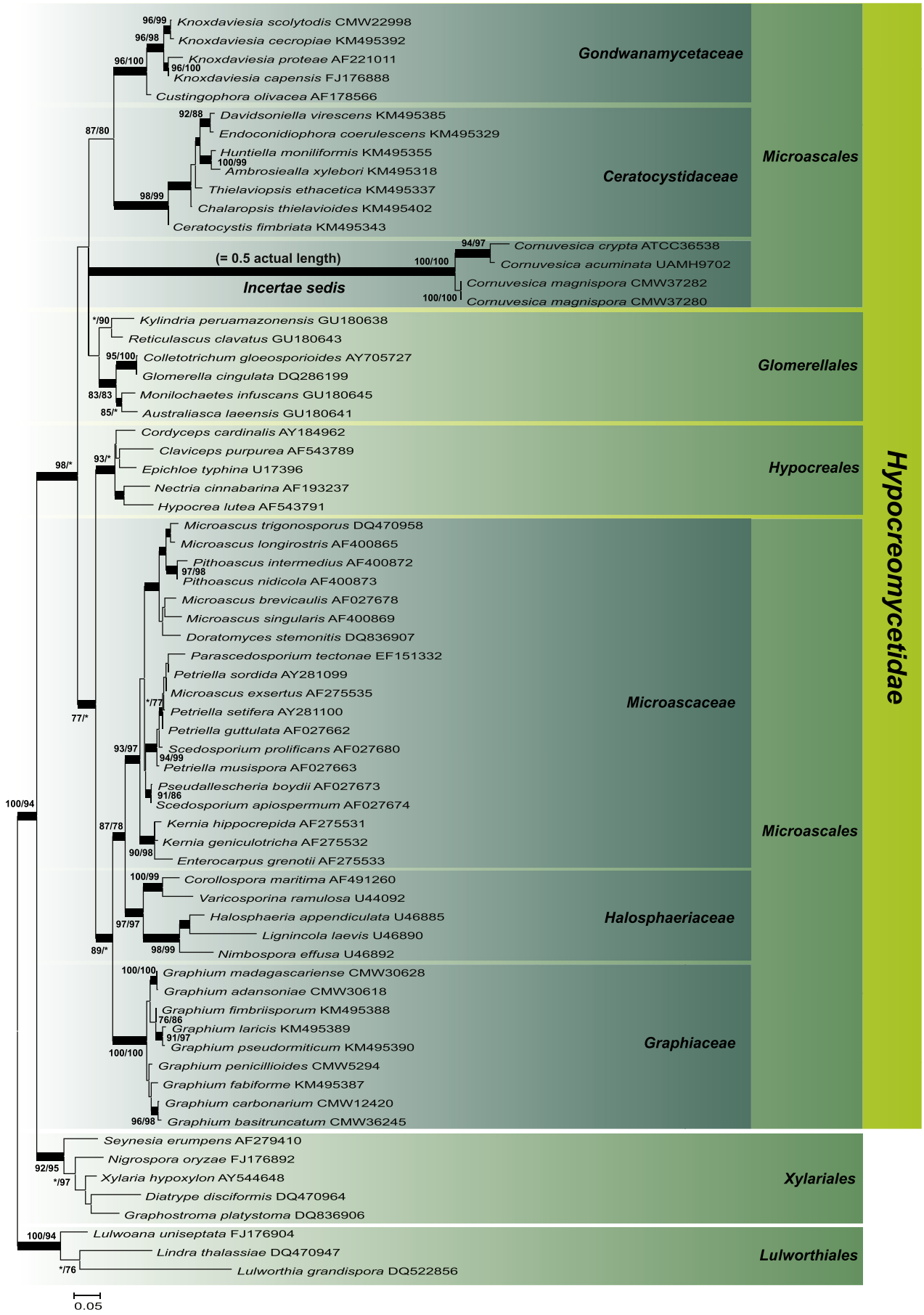

Fig 3 – Phylogenetic tree derived from ML analyse of LSU dataset. Bootstrap supports (≥75 %) for ML/MP are presented at nodes. Thick branches represent BI posterior probabilities ≥95 %. To aid with tree presentation, the branch length for the *Cornuvesica* clade has been shortened by half as indicated in the tree.

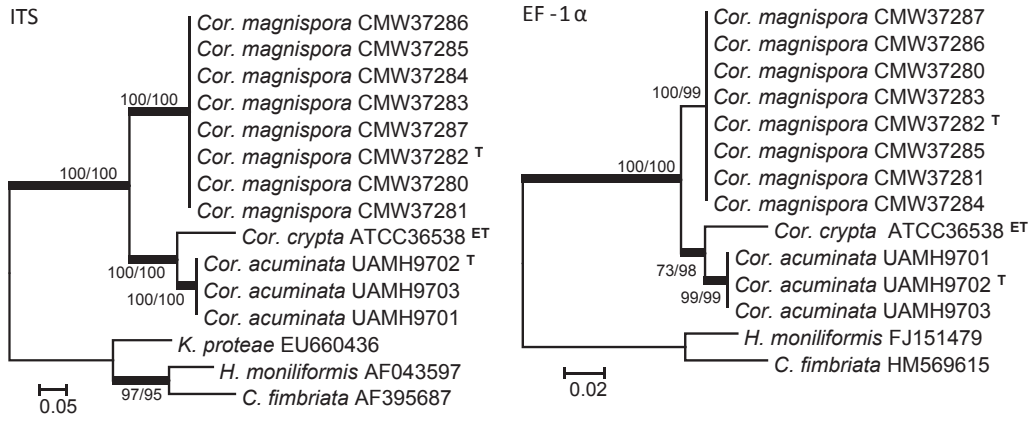

Fig 4 — Phylogenetic trees derived from ML analyses of ITS and EF-1α datasets. Bootstrap supports (≥75 %) for ML/MP are presented at nodes. Thick branches represent BI posterior probabilities ≥95 %. ET: ex-epitype, T: ex-holotype.

36538, PREM 60965, ***epitypus hic designatus***, ex-epitype ATCC 36538 = CMW 37652.

Notes — *Cornuvesica crypta* was initially reported as *Ceratocystis falcata* by Rayner & Hudson (1977). We have designated the herbarium specimen they deposited in Herb. IMI as holotype. A culture of the same fungus was deposited by H. J. Hudson to ATCC, with the only information linked to it that it originated from a *Betula* sp. in England. The possibility exists that this isolate was obtained from the same material as the Herb. IMI specimen. However, in the absence of evidence confirming a link between the specimen and isolate, we consider the two as distinct.

Rayner & Hudson (1977) were able to obtain the asexual form of the fungus from single ascospore cultures on artificial media and described it as a *Chalara* (*Thielaviopsis*) state. The conidia of these isolates were 'oblong with truncate ends, 5–8 × 1.5–2.0 μm' (Rayner & Hudson 1977). We observed two thielaviopsis-like asexual states on the original herbarium specimen, with distinct ranges of conidial dimensions, with micro-conidia (4–7 × 2–2.5 μm) more abundant (Fig 7A, B). Rayner & Hudson (1977) might have overlooked the presence of macro-conidia (4.5–9 × 2.5–4 μm) on the specimen due to their scarcity compared to micro-conidia. In the present study, the living culture produced conidia measuring 5–11.5 × 2–3 μm (avg. 8 × 2.2 μm), resembling the conidia of smaller size, but longer than those found on the specimens and described in original report (Rayner & Hudson 1977). The same isolate was used by Viljoen *et al.* (2000) to supplement the description of asexual state of the type species, *Cor. falcata*, when they established the genus *Cornuvesica*.

Cornuvesica crypta and *Cor. falcata* are very similar in their sexual states. Discovery of the asexual state of *Cor. falcata* in the future could provide valuable morphological characteristics to distinguish these two species from each other. We treat *Cor. crypta* as a species different to *Cor. falcata* based on the locality from which it was collected and the substrate on which it was found. It is also morphologically distinct from the other species of *Cornuvesica* described in this study.

Cornuvesica acuminata Marinc., T.A. Duong, Z.W. De Beer, M.J. Wingf., **sp. nov.** — MycoBank 811611; Figs 5H–K, 6B, F, 7G–L.

Etymology — the name refers to the fact that the ascospores taper abruptly towards their ends.

On iron-enriched MEA, *Ascomata* superficial, scattered or some in groups, often embedded in well-developed mycelium on which asexual states are formed, covered with aerial hyphae, with a conical opening, dark brown to blackish, 135–160 μm diam. *Peridium* firm, pseudoparenchymatous, *textura epidermoidea* to *angularis*. *Ostiolar hyphae* convergent, compactly adhered to each other, pale brown to hyaline, 5–14 μm long. *Asci* evanescent. *Ascospores* hyaline, falcate, attenuated at the same degree at both ends, sharply attenuating at ¼ from each end, straight or slightly curved, 1-septate, septum indistinct, supra-median, (21–)27–29(–35.5) × (1–)1.5(–2.5) μm (avg. 27.8 × 1.6 μm), thin-walled, smooth, emerging in a tendril. *Asexual states* thielaviopsis-like with two distinct ranges of conidial dimensions. *Micro-conidiophores* abundant, micronematous, semi-macronematous, occasionally branched, hyaline, smooth, cylindrical, septated. *Macro-conidiophores* scarce, macronematous, formed on hyphae originated from perithecium, straight, unbranched or branched, pale brown to brown, smooth, septated. *Micro-conidiogenous cells* phialidic, collarette indistinct, hyaline, discrete or integrated, intercalary, rarely terminal, cylindrical gradually tapering to the apex, straight or curved, (20–)26–29(–35) μm long, (2–)3(–4) μm wide near the base, (1–)2 μm wide near the apex. *Macro-conidiogenous cells* phialidic, collarette indistinct, pale brown to brown, discrete or integrated, intercalary or terminal, cylindrical gradually tapering to the apex, straight or curved, (24–)29–32(–37) μm long, (3–)5–6.5(–9) μm wide at the base tapering into (2–)3–4(–5.5) μm at the apex. *Micro-conidia* hyaline, oblong with truncate to slightly round ends or infrequently one end enlarged, (4–)5–6(–9) × (1.5–)2(–2.5) μm (avg. 5.5 × 1.9 μm), aseptate, smooth, in chains, endogenous. *Macro-conidia* hyaline, doliform to rectangular, (5–)7.5–9(–14.5) × (2–)3(–3.5) μm (avg. 8.1 × 2.8 μm), aseptate, smooth, in chains, endogenous.

Culture characteristics — Colonies showing irregular growth, white near the centre, becoming transparent towards the edge, flat, mycelium mostly submerged, not densely compact, showing no effect on medium, becoming fertile in 7 d. No

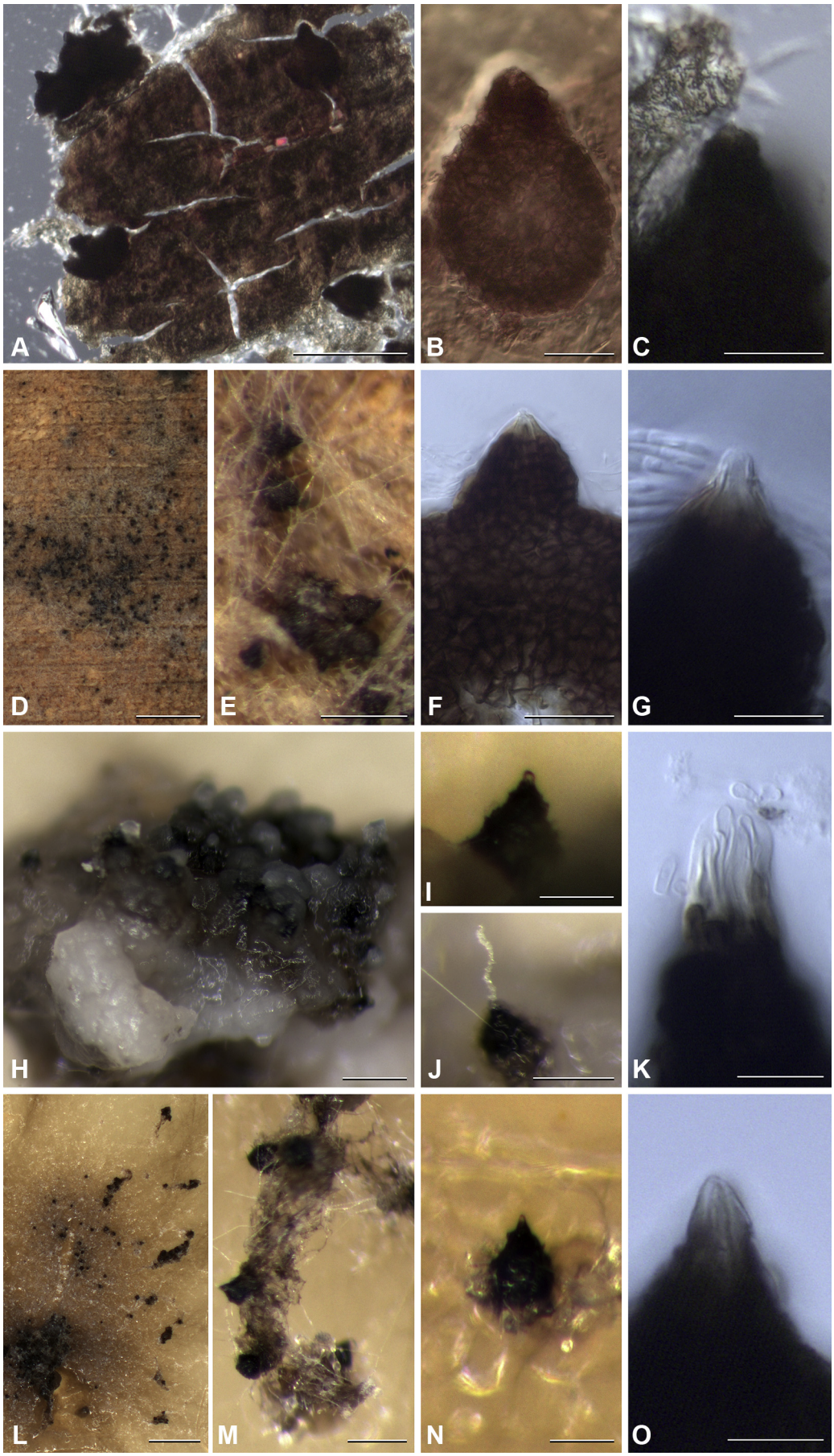

Fig 5 – Photographic images of ascomata. (A–C). *Cornuvesica falcata* (TRTC 33037). (D–G). *Cor. crypta* (Herb. IMI 200338). (H–K). *Cor. acuminata* (UAMH 9702). (L–O). *Cor. magnispora* (CBS 134697 = CMW 37282). A, D, E. Ascomata on the host substrate. H, L, M. Ascomata on the artificial media. B, F, I, N. A single ascoma. J. A single ascoma with a cirrhus. C, G, K, O. Convergent ostiolar hyphae. Scale bars: D, L = 2 mm, H = 500 μm, A, E, J, M = 250 μm, I, N = 100 μm, B, C, F = 25 μm, G, K, O = 10 μm.

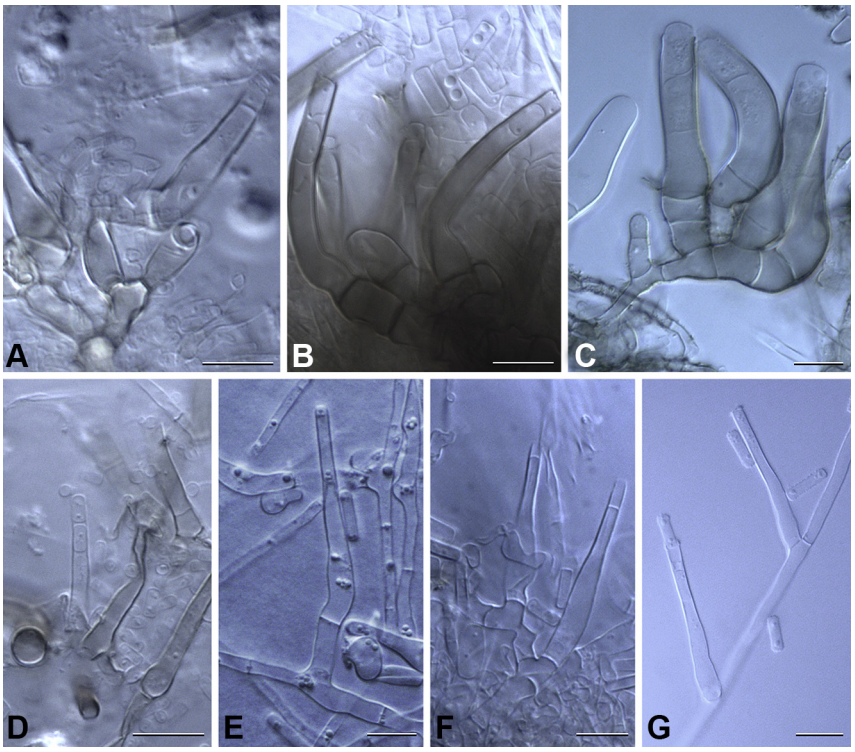

Fig 6 – Photographic images of conidiophores. (A, D). *Cornuvesica crypta* (Herb.IMI 200338). (E). *Cor. crypta* (ATCC 36538). (B, F). *Cor. acuminata* (UAMH 9702). (C, G). *Cor. magnispora* (CBS 134697 = CMW 37282). (A–C). Conidiophores of macro-conidia. (D–G). Conidiophores of micro-conidia. Scale bars: A–G = 10 μm.

growth occurred at 30 °C and 35 °C and extremely slow growth at other temperatures, reaching 8 mm diam at 15 °C in 21 d, 7 mm at 20 °C and 8 mm at 25 °C. The addition of $FeCl_3$ to the medium showed no effect on the growth.

Specimens examined: **New Zealand**: *North Island*, Taupo, Kaingaroa State forest, compartment 5, isolated from beetle galleries in the inner bark or outer sapwood of *Pinus radiata*, May 1982, *J. Reid*, PREM 60969, holotype, a dried culture of UAMH 9702, ex-holotype UAMH 9702 = CMW 37654; compartment 1212, *Pinus radiata*, PREM 60970, a dried culture of UAMH 9703, culture UAMH 9703 = CMW 37655; compartment 1212, *Larix* sp., PREM 60971, a dried culture of UAMH 9701, culture, UAMH 9701 = CMW 37653.

Notes — *Cornuvesica acuminata* was identified as *Cop. falcata* by Hutchison & Reid (1988) from the North Island of New Zealand, with the first report of *Chalara* spp. (*Thielaviopsis*) with two distinct ranges of conidial dimension. In the past, the ex-holotype (UAMH9702) has been included in various phylogenetic analyses erroneously as *Cor. falcata* (Hausner *et al.* 1993; Hausner & Reid 2004; Réblová *et al.* 2011).

Cornuvesica acuminata can be distinguished from the other species in the genus by its abruptly tapering ascospores and distinct conidial dimensions. Hausner *et al.* (1993) reported that *Cor. acuminata* (as *Cop. falcata*) was insensitive to 0.01 % cycloheximide, displaying a colony growth area reduction of about 30–50 %.

Cornuvesica magnispora Marinc., T.A. Duong, Z.W. De Beer, M.J. Wingf., **sp. nov.** — MycoBank 811612; Figs 5L–O, 6C, G, 7M–R, 8A–I.

Etymology — name refers to the larger conidia as compared with other species in the genus.

On iron-enriched MEA, *Ascomata* superficial, scattered or some in groups, often embedded in well-developed mycelium on which asexual states are also formed, covered with aerial hyphae, with a conical opening, 80–135 μm diam. *Peridium* firm, pseudoparenchymatous, *textura epidermoidea* to *angularis*. *Ostiolar hyphae* convergent, compactly adhered to each other, pale brown to hyaline, similar to the peridial cells but less pigmented, 5–10 μm high. *Asci* evanescent. *Ascospores* hyaline, falcate, gradually attenuated at the same degree at both ends, straight or curved, (23.5–)30.5–32(–40) × (1–)1.5(–2) μm (avg. 31.4 × 1.6 μm), 1-septate, septum indistinct, median, thin-walled, smooth. *Asexual states* thielaviopsis-like with two distinct ranges of conidial dimensions observed. *Micro-conidiophores* abundant, straight, unbranched or branched, hyaline or rarely pale brown, smooth, 0–2-septated, 5.5–19.5 × 3–4.5 μm (avg. 11.9 × 3.8 μm). *Macro-condiophores* less common than those of smaller size, formed on hyphae originated from perithecium, straight, unbranched or branched, pale brown to brown, smooth, 6.5–71.5 × 4.5–10 μm (avg. 32.3 × 7.6 μm), 0–7-septated. *Micro-conidiogenous cells* phialidic, collarette indistinct, hyaline or occasionally pale brown, discrete or integrated, intercalary or terminal, cylindrical gradually tapering to the apex, 14.5–35.5 μm long, 2–4.5 μm wide at the base, 2–3 μm at the apex. *Macro-conidiogenous cells* phialidic, collarette indistinct, pale brown, discrete or integrated, intercalary or terminal, cylindrical gradually tapering to the apex, 23.5–54 μm long, 8–13 μm wide at the base, 5.5–9 μm at the

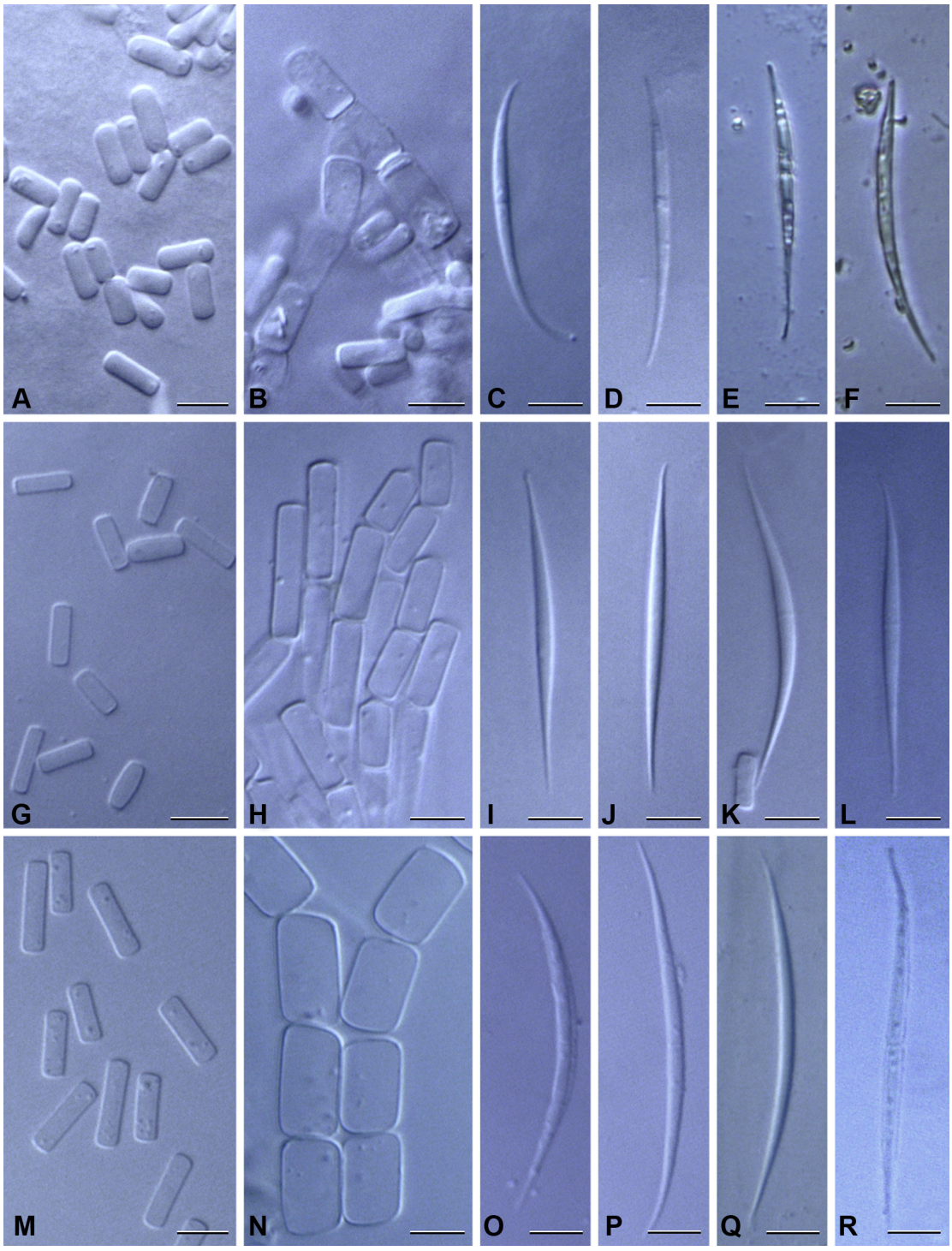

Fig 7 – Photographic images of spores. (A–D). *Cornuvesica crypta* (Herb.IMI 200338). (E, F). *Cor. falcata* (TRTC 33037). (G–L). *Cor. acuminata*. (M–R). *Cor. magnispora*. A, G, M. Micro-conidia. B, H, N. Macro-conidia. C–F, I–L, O–R. Ascospores. Scale bars: A–R = 5 μm.

apex. *Micro-conidia* hyaline, oblong with truncate ends, (4–)7–8(–10.5) × (1.5–)2(–2.5) μm (avg. 7.4 × 2.0 μm), aseptate, smooth, in chains, endogenous. *Macro-conidia* hyaline, doliiform, (7–)10–10.5(–18) × (5–)6(–9.5) μm (avg. 10.1 × 5.9 μm), aseptate, smooth, in chains, endogenous.

Culture characteristics— Colonies on iron-enriched MEA showing circular shape, transparent to umber, with radially striated olivaceous veins, flat, mycelium submerged with aerial hyphae woolly, evenly dispersed, density sparse, showing no effect on medium, becoming fertile in 7 d. The Indonesian

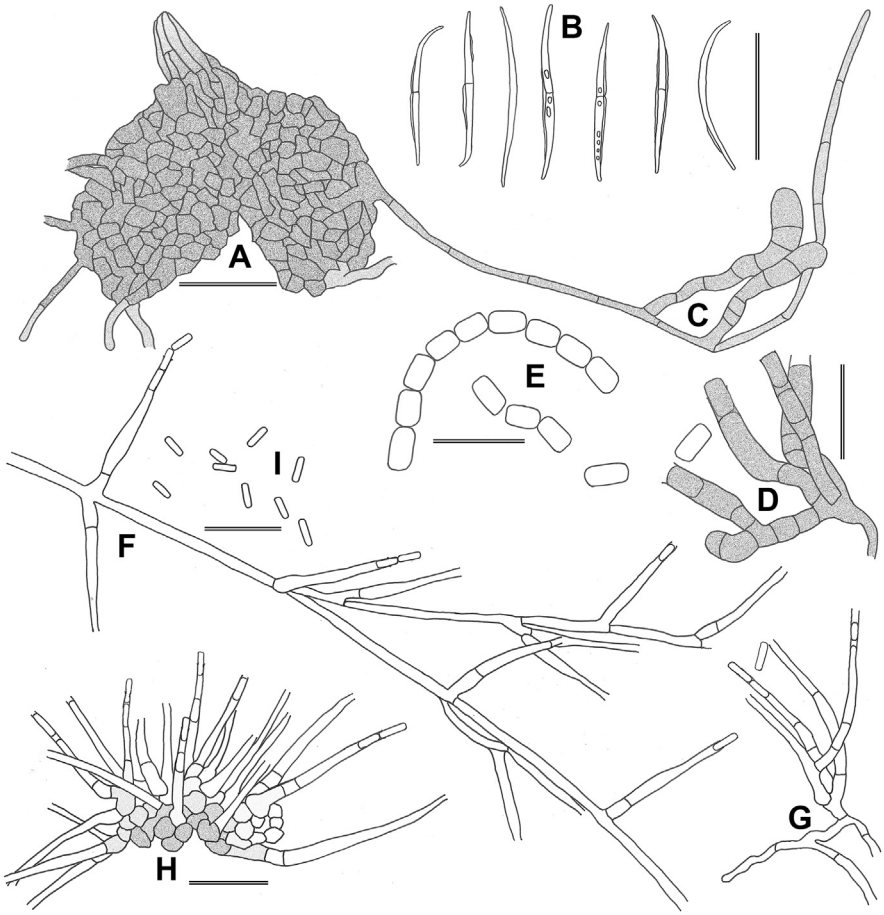

Fig 8 – Drawings of *Cornuvesica magnispora*. (A). A single ascoma with an extended hypha bearing young macro-conidiophores (C). (B). Ascospores, (C). Young macro conidiophores. (D). Mature macro-conidiophores. (E). Macro-conidia. (F, G). Micro-conidiophores from a fresh isolate. (H). Microconidiophores from an isolated sub-cultured a few time in the laboratory. (I). Micro-conidia. Scale bars: A–I = 25 μm.

isolate (CMW 37282) grew at all temperatures but extremely slowly on MEA, reaching 14 mm at 15 °C in 21 d, 24 mm at 20 °C and 25 °C, 43 mm at 30 °C, 7 mm at 35 °C. The addition of $FeCl_3$ to the medium showed a positive effect on the growth with the isolates reaching 32 mm at 20 °C (1.3 times larger than the control), 66 mm at 25 °C (2.7 times larger), 80 mm at 30 °C (1.9 times larger), 15 mm at 35 °C (2 times larger) but no effect at 15 °C. The other two isolates (CMW 37280, CMW 37287) showed a similar growth pattern and effect.

Specimens examined: **Indonesia**: *Kalimantan*, isolated from freshly made wounds of *Gmelina arborea*, Dec. 2010, *M.J. Wingfield*, PREM 60967, holotype, a dried culture of CBS 134679, culture ex-holotype CBS 134697 = CMW 37282; PREM 60966, a dried culture of CBS 134698, culture CBS 134698 = CMW 37280; PREM 60968, a dried culture of CBS 134699, culture CBS 134699 = CMW 37287, other cultures CMW 37281, CMW 37283–37286.

Notes — *Cornuvesica magnispora* can be distinguished from the other species by its conidial dimensions and its larger ascospores. Adding iron supplement to the cultures that had been stored for less than one year enhanced growth at all temperatures. In contrast, there was no effect on the cultures that had been maintained in a collection at 5 °C for more than two years. *Cornuvesica magnispora* isolates showed sensitivity to 0.05 % cycloheximide. Addition of this antibiotic resulted in the colony growth areas being decreased by 72–80 % at 20 °C, by 58–62 % at 25 °C, and by 71–93 % at 30 °C.

Discussion

Ceratocystiopsis spp. owe their notoriety to the fact that many species occur together with ophiostomatoid fungi (Wingfield *et al.* 1993), in some cases economically important, and living in association with bark beetles that infest conifers. In this regard, the fungus known as *Ceratocystiopsis falcata* has frequently been encountered on woody substrates and for many years was assumed to be related to other species of *Ceratocystiopsis*. Viljoen *et al.* (2000) showed this not to be true and established *Cornuvesica* to accommodate what they (and others) believed was a single species. The overall results of this study have shown that previous collections of *Cor. falcata* represent different species and that these are closely

related to fungi in the *Ceratocystidaceae* and the *Gondwanamycetaceae* (*Microascales*). In this regard, they are unrelated to species of *Ceratocystiopsis* in which *Cor. falcata* was accommodated for many years. The three new *Cornuvesica* species described in this study shared the common morphological features with the type species, *Cor. falcata*. These include minute ascomata of less than 160 μm diam, indistinct ostiolar hyphae, 2-celled falcate ascospores with attenuated ends and two distinct thielaviopsis-like asexual states. Variations in the sizes of either ascospores or conidia and the ascospore morphology provide relatively obvious morphological features by which these species can easily be identified.

Results of this study have clearly shown that *Cornuvesica* is not monotypic, as was assumed for many years (Rayner & Hudson 1977; Hutchison & Reid 1988; Viljoen *et al.* 2000; De Beer *et al.* 2013). This is perhaps not surprising given the fact that fungi, very similar to the type species, *Cor. falcata*, have been found in many different parts of the world, and on very different but generally woody substrates. Prior to the ready availability of DNA sequence comparisons, it would not have been possible to recognise the cryptic species that have emerged from this study. We thus also expect that additional species of *Cornuvesica* will be found in the future.

Prior to the availability of DNA sequence data for phylogenetic inference, the taxonomic position of *Cornuvesica* in relation to other ophiostomatoid fungi was confused. Early studies based on a partial SSU and LSU rDNA sequences showed that *Cornuvesica* (as *Cop. falcata*) was more closely related to *Ceratocystis* than to *Ophiostoma* (Hausner *et al.* 1993). It was also suggested that *Cornuvesica* shared a common ancestry with other Microascalean taxa (Hausner *et al.* 1993; Hausner *et al.* 2000) and that it represented a monophyletic lineage with *Knoxdaviesia* (= *Gondwanamyces*) and *Ceratocystis* s. str., exclusive of *Sphaeronaemella* (Hausner & Reid 2004). Réblovà *et al.* (2011) segregated the latter genera into two families, *Gondwanamycetaceae* and *Ceratocystidaceae*, in the *Microascales*. The former family includes *Knoxdaviesia* and *Custingophora*, and the latter *Cornuvesica*, *Ceratocystis*, and *Ambrosiella* (Réblovà *et al.* 2011; De Beer *et al.* 2013). The phylogenetic analyses of SSU and LSU datasets in the present study showed that *Cornuvesica* spp., including *Cor. acuminata*, *Cor. crypta* and *Cor. magnispora*, form a discrete clade that represents a unique lineage, separated from the *Ceratocystidaceae* in the *Microascales*. This is also strongly supported by the distinct morphology and the unusual biology of *Cornuvesica* spp. The appropriate placement of this genus at the family level should be explored further in a multigene phylogenetic study, including protein-coding genes, that incorporate species representative of all genera currently included in the *Microascales*.

The ascomata of *Cornuvesica* spp. do not have well-defined ostiolar hyphae. In the first description of *Cor. falcata* (as *Ceratocystis falcata*), the presence of ostiolar hyphae was not mentioned but the small ascomata were described as terminating in 'slightly projecting, rounded hyaline cells' (Wright & Cain 1961). In the preserved slide representing the holotype, it was possible to recognise these hyaline cells that are easily differentiated from the dark cells of the ascomatal neck (Fig 5B, C). Rayner & Hudson (1977) reported that the ostiolar hyphae of *Cor. crypta* (as *Ceratocystis falcata*) were absent. We were, however, able to observe the convergent hyaline cells at the apices of the ascomata in *Cor. crypta*, which are more prominent than those on the type specimen (Fig 5F, G). Amongst the three newly recognised species, ostiolar hyphae were most obvious in *Cor. acuminata* (Fig 5K).

Macro-conidia were closely associated with the presence of a sexual state in species of *Cornuvesica*. In *Cor. magnispora*, we observed that the hyphae on which conidiophores giving rise macro-conidia were formed, originated from the bases of the ascomata (Fig 8A). Most conidiophores producing macro-conidia were also found close to the bases of the ascomata in *Cor. acuminata*. Hutchison & Reid (1988) made a similar observation for this fungus, which he treated as *Cop. falcata* (now *Cor. acuminata*) stating that, 'the larger phialospores formed only in immediate association with the perithecia'. In the present study, macro-conidia were absent in species such as *Cor. crypta* in which no ascomata were found.

The survival of *Cornuvesica* species appears to be dependent on the presence of other fungi growing in their vicinity. Evidence for this fact has emerged from previous studies (Rayner & Hudson 1977; Hutchison & Reid 1988) and it was clear in the present investigation. There is also some evidence to suggest that different fungi provide a growth stimulatory effect in different species of *Cornuvesica*. For example Rayner & Hudson (1977) showed that *Acremonium butryi* and *Trichoderma* spp., as contaminants in culture plates, stimulate the growth and ascomatal production in *Ceratocystis falcata* (now *Cor. crypta*). Likewise, Hutchison & Reid (1988) showed that the presence of *Gliocladium roseum* in the galleries of a bark beetle stimulated the growth and sprulation of *Ceratocystis falcata* (now *Cor. acuminata*). Results of the present study added credence to this view where a *Ceratocystis* sp. in the *C. fimbriata* complex (De Beer *et al.* 2014) and an undescribed hypocrealean fungus appeared to contribute to the growth of *Cor. magnispora*. There was no evidence of mycoparasitism and we speculate that *Cornuvesica* spp. lack the ability to produce certain extracellular metabolites and have acquired the capacity to derive these from other fungi that grow in association with them.

Relatively simple experiments testing the effect of filtered culture extracts in this study showed conclusively that filtrates from four test fungi increased the growth of *Cor. magnispora* dramatically. Here, colony diameters were up to 7 times larger than those of the controls, which lacked the various filtrates. The filtrate of the *Fusicoccum* sp. tested provided the greatest stimulatory effect and this was the same *Fusicoccum* sp. that Thanh *et al.* (2002) used to enhance the growth of a new *Debaryomyces* sp. Thanh *et al.* (2002) purified the culture extract of this fungus as well as that of a *Gliocladium* sp. and showed that the extracellular metabolite promoted the growth of the yeast was a siderophore. Siderophores are low molecular weight compounds produced by microorganisms that have a high level of affinity for ferric iron (Winkelman 2007). It seems reasonable to assume that the stimulatory effect on the growth of *Cor. magnispora* is similar to that described by Thanh *et al.* (2002) but additional studies would be needed to confirm this.

Adding an iron supplement to the culture medium showed a similar stimulatory effect on *Cor. magnispora* to that found when culture filtrates of other fungi were added to the growth media. This suggests that *Cor. magnispora* is similar to the

dependency on iron that Thanh *et al.* (2002) showed for *Debaryomyces mycophilus*. However, a confusing aspect of the present study was that this effect was clear for cultures that had been stored for less than one year but it did not occur in cultures of this fungus that had been in storage for more than two years. Similarly, the effect was not seen for *Cor. acuminata* or *Cor. crypta* that were sourced from culture collections and had been stored for long periods of time. Those isolates could simply have lost their vigour due to long-term storage.

During routine isolations from woody substrates, apparently saprobic fungi are commonly encountered that grow very poorly, if at all, on artificial culture media. This could be due to many different factors but the fact that it could be due to a reliance of metabolites produced by other microbes has received little attention. An apparently commensal symbiosis, where growth appears only in the presence of other fungi as has been observed in *Cor. magnispora* in this study, suggests that many apparently saprobic fungi are not collected because commonly used culture media cannot support their growth. Thus, enrichment of isolation media with fungal culture filtrates could offer interesting opportunities to enhance isolation success. Such symbioses might also, at least partially explain the descrepency in numbers of fungi isolated from various substrates and those known to be present as shown in contemporary metagenomic studies (Fierer *et al.* 2007; Kemler *et al.* 2013; Cuadros-Orellana *et al.* 2013).

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