Finding the missing link: Resolving the Coryneliomycetidae within Eurotiomycetes

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Coryneliales
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systematics
Tripospora

Abstract
Species belonging to the Coryneliaceae and parasitizing Podocarpaceae hosts were collected from different locations in South Africa and studied morphologically by light microscopy and molecularly by obtaining partial nrDNA (ITS-1/5.8S/ITS-2, 18S and 28S) gene sequences. The position of the Coryneliaceae within the Eurotiomycetidae was not confirmed and a new subclass, Coryneliomycetidae, was introduced. While Eurotiomycetidae usually form cleistothecia/gymnothecia with evanescent, unitunicate asci, and Chaeothomycetidae mostly perithecia with bitunicate/fissitunicate to evanescent asci, Coryneliomycetidae form pseudothecial mazaedial ascomata, initially with double-walled asci with the outer layer deliquescing, resulting in passive ascospor release. The Coryneliomycetidae thus occupies a unique position in the Eurotiomycetes. Furthermore, epitypes were designated for Corynelia iberata, the type species of Corynelia (type genus of the family, order and subclass), Lagenulopsis bispora, the type species of Lagenulopsis, and Tripospora tripolis the type species of Tripospora, with Lagenulopsis and Triplospora confirmed as belonging to the Coryneliomycetidae. Corynelia iberata resolved into three clades, one on Afrocarpus (= Podocarpus) falcatus and A. gracillim, and two clades occurring on P. latifolius, herein described as C. africana and C. fructigena. Morphologically these three species are not readily distinguishable, although they differ in spore dimensions, ascomata shape, ornamentation and DNA phylogeny. It is likely that several more species from other parts of the world are currently erroneously placed in C. iberata.

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INTRODUCTION

Recent molecular phylogenetic evidence places the Coryneliales (sole family Coryneliaceae) as basal within the Eurytomomyctidae (Geiser et al. 2006). This order has a unique set of morphological characters including ascocarpoid ascomata, spermgonia, initially bitunicate asci with an outer wall layer that breaks away early in their development so that they appear unitunicate at maturity, and ascospores that are liberated passively if you get permission from the copyright holder. Nothing in this license impairs or restricts the author’s moral rights.

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### Table 1  Details of specimens/strains included in the molecular analyses.

| Species                  | Specimen number\(^1\) | Substrate                  | Collector  | Collection date | Location                  | GenBank accession numbers\(^2\) |
|--------------------------|------------------------|----------------------------|------------|-----------------|---------------------------|---------------------------------|
| **Caliciopsis orientalis** CBS 138.64 (ex-type culture) | Tsuga canadensis | A. Funk | 26 Nov. 1960 | Canada: Ontario | KP81690 DQ470987 DQ471039 |
| **Caliciopsis pinea** CBS 138.64 | Pinus strobus | – | – | Canada: Ontario | KP81691 DQ678097 DQ678043 |
| **Corynelia africana** PREM 57242 (holotype) = ARW 247 | Podocarpus latifolius | A.R. Wood | 20 Nov. 2000 | South Africa: Western Cape | KP81693 KP81714 KP81719 |
| PREM 59200 = ARW 656 | Podocarpus latifolius, leaves | A.R. Wood | 17 Oct. 2004 | South Africa: Western Cape | KP81694 – – |
| PREM 61194 = ARW 671 | Podocarpus latifolius, leaves | A.R. Wood | 14 July 2006 | South Africa: Western Cape | KP81695 – – |
| PREM 61196 = ARW 673 | Podocarpus latifolius, leaves | A.R. Wood | 14 July 2006 | South Africa: Western Cape | KP81696 – – |
| PREM 61198 = ARW 675 | Podocarpus latifolius, leaves | A.R. Wood | 14 July 2006 | South Africa: Western Cape | KP81697 – – |
| PREM 61201 = ARW 678 | Podocarpus latifolius, leaves | A.R. Wood | 14 July 2006 | South Africa: Western Cape | KP81698 KP81715 – |
| PREM 61204 = ARW 688 | Podocarpus latifolius, leaves | A.R. Wood | 18 July 2006 | South Africa: Eastern Cape | KP81699 – – |
| PREM 61205 = ARW 682 | Podocarpus latifolius, leaves | A.R. Wood | 15 July 2006 | South Africa: Western Cape | KP81700 – – |
| PREM 61206 = ARW 683 | Podocarpus latifolius, leaves | A.R. Wood | 15 July 2006 | South Africa: Western Cape | KP81701 – – |
| ARW 681 | Podocarpus latifolius | A.R. Wood | 15 July 2006 | South Africa: Western Cape | KP81692 – – |
| UD 259 | Podocarpus cf. latifolius, leaves | U. Damm | 1 July 2007 | South Africa: Western Cape | KP81702 – – |
| **Corynelia fructigena** PREM 57240 (holotype) = ARW 250 | Podocarpus latifolius, fruits | A.R. Wood | 20 Nov. 2000 | South Africa: Western Cape | KP81704 KP81716 KP81720 |
| PREM 59201 = ARW 657 | Podocarpus latifolius, fruits | A.R. Wood | 17 Oct. 2004 | South Africa: Western Cape | KP81705 – – |
| ARW 684 | Podocarpus latifolius, leaf | A.R. Wood | 15 July 2006 | South Africa: Western Cape | KP81703 – – |
| **Corynelia uberata** PREM 61203 = ARW 680 | Afrocarpus falcatus, leaves | A.R. Wood | 15 July 2006 | South Africa: Western Cape | KP81706 – – |
| PREM 61207 (epitype) = ARW 686 | Afrocarpus falcatus, leaves | A.R. Wood | 15 July 2006 | South Africa: Western Cape | KP81707 – – |
| **Lagenulopsis bispora** PREM 57232 (epitype) = ARW 249 | Podocarpus latifolius | A.R. Wood | 20 Nov. 2000 | South Africa: Western Cape | KP81709 KP81717 KP81721 |
| PREM 59202 = ARW 655 | Podocarpus latifolius, leaves | A.R. Wood | 17 Oct. 2004 | South Africa: Western Cape | KP81710 – – |
| PREM 61197 = ARW 674 | Podocarpus latifolius, leaves | A.R. Wood | 14 July 2006 | South Africa: Western Cape | KP81711 – – |
| ARW 685 | Podocarpus latifolius, leaves | A.R. Wood | 15 July 2006 | South Africa: Western Cape | KP81708 – – |
| **Tripospora tripos** PREM 61200 = ARW 677 | Afrocarpus falcatus, leaves | A.R. Wood | 14 July 2006 | South Africa: Western Cape | KP81712 KP81718 – |
| PREM 61202 (epitype) = ARW 679 | Afrocarpus falcatus | A.R. Wood | 14 July 2006 | South Africa: Western Cape | KP81713 – – |

\(^1\) ARW: personal number of Alan Wood; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; PREM: South African National Collection of Fungi (NCF), Mycology Unit, Biosystematics Division, Plant Protection Research Institute, Agricultural Research Council, Rooecdraai, Pretoria, South Africa; UD: personal number of Ulrike Damm.

\(^2\) ITS: internal transcribed spacers and intervening 5.8S nrDNA; LSU: partial 28S nrDNA; SSU: partial 18S nrDNA.
The yellowwoods (Podocarpus) are iconic trees of the Afro-montane forests of South Africa, being long sought after for their valuable timber. Currently four species are recognised from South Africa (Afrocarpus falcatus (= Podocarpus falcatus), Podocarpus elongatus, P. henkelii, and P. latifolius) (Barker et al. 2004). Application of these names by early workers was highly confusing, in particular specimens of A. falcatus were frequently referred to as P. elongatus or A. gracilior, whereas P. latifolius was frequently referred to as P. elongatus or P. thunbergii. Podocarpus elongatus as currently understood has a limited distribution in the most south-westerly mountains of the Western Cape Province of South Africa.

Corynelia uberata and Tripospora tripods on Podocarpus spp. and Coryneliospora fructicia on fruit of Rapanea melanophloes (Myrsinaceae) are the only members of the Coryneliaceae recorded from South Africa (Doidge 1950, Crous et al. 2000). Corynelia uberata was one of the first South African plant parasitic fungi to be collected and described by European explorers, being collected in 1772 by Carl Peter Thunberg during his travels in this country (Doidge 1950). Thunberg made his collection in ‘sylvis Houtniquas Cap.’ (forests of the Outeniqua mountains) on A. falcatus (Juel 1918). This species has been recorded historically as being ‘extremely common throughout southern Africa on leaves, twigs and fruit of Yellow-woods’ (Doidge 1950: 58), Phillips (1927) noted that in the forests around the town of Knysna (on the southern slopes of the Outeniqua mountains) in some years P. latifolius ‘bear scarcely a single podocarpium that is not diseased by Corynelia’. It has also been observed as very common on leaves and fruit, at times up to 100 % of fruit, of A. gracilior in Ethiopia (Assefa et al. 2014, 2015).

Lagenulopsis bispora was first described as a form of Corynelia clavata, namely as C. clavata f. macrospora. Fitzpatrick (1920) described it as the new species C. bispora, and later established the genus Lagenulopsis to accommodate it (Fitzpatrick 1942a). The species is easy to identify as it is the only one in the family with the character combination of two ascosporas per mature ascus and ostiolute ascomata. Originally described from material collected near Ruwenzori (now in Uganda), L. bispora has also been recorded from Fiji, Jamaica, Malawi, and Mexico (Fitzpatrick 1942a, Benny et al. 1985c, Johnston & Minter 1989). Benny et al. (1985c) listed a specimen collected in South Africa in 1926, which was found amongst specimens of C. uberata received from K (K(M) 187631, pers. comm. Begonía Aguirre-Hudson). Recently a number of specimens of L. bispora have been collected on P. latifolius at several sites in the Western and Eastern Cape Provinces, South Africa. It is usually rare, and easily overlooked. The aim of the present paper is to morphologically re-evaluate the Coryneliaceae based on fresh material of Corynelia, Lagenulopsis, and Tripospora, and to study the phylogenetic placement of the family/order Coryneliales/Coryneliaceae and the three genera based on newly generated DNA sequence data.

**MATERIALS AND METHODS**

**Specimens and isolates**

Ascomata on P. latifolius and A. falcatus were collected at several sites in the Western and Eastern Cape Provinces of South Africa (Table 1). Type specimens (holo- and paratypes) of the species studied as well as additional specimens are located at the fungaria of the Royal Botanic Garden, Kew, UK (K(M)), the Swedish Museum of Natural History, Stockholm, Sweden (S), the Museum of Evolution, Uppsala University, Uppsala, Sweden (UPS), the University of Florida Herbarium, Gainesville, Florida, USA (FLAS) and of the Plant Pathology Herbarium, Cornell University, Ithaca, New York, USA (CUP). Specimens, or high resolution photos of specimens, were obtained from these herbaria. The newly collected specimens including holotype specimens of new species were deposited in the National Collection of Fungi, Pretoria, South Africa (PREM). Isolates of Caliciopsis spp. from the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, the Netherlands were included as well. All descriptions are based on holo- or eotypes. Features of additional specimens are included, if they deviate from the type specimens. Host plant nomenclature follows currently accepted names according to The Plant List (http://www.theplantlist.org) and the African Plant Database (http://www.ville-ge.ch/musinfo/bd/cjb/africa/index.php?langue=en).

**DNA extraction, amplification and analysis**

Genomic DNA was isolated from fungal ascomata scraped from the surface of leaves or fruits and grinded with a mortar and pestle in liquid nitrogen, following the protocol of Lee & Taylor (1990). The 5.8S ribosomal gene with the two flanking internal transcribed spacers (ITS-1 and ITS-2), a partial sequence of the 28S rDNA gene (LSU) and of the 18S rDNA gene (SSU) were amplified and sequenced using the primer pairs ITS-1F (Gardes & Bruns 1993) + ITS-4 (White et al. 1990), NL1 + NL4 (O’Donnell 1993), and NS1 + NS8 (White et al. 1990), respectively, as well as NS4, NS5, NS2, and NS3 as internal sequence primers for SSU (White et al. 1990). The novel sequences were added to sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov) using the megablast function, including the SSU sequence AF242262 which is derived from a specimen that had also been collected from South Africa (Winka 2000), and ITS and SSU sequences of Corynelia uberata from Afrocarpus falcatus from a recent study in Ethiopia (Assefa et al. 2014). Three alignments were made: SSU and LSU separately for higher order placement and ITS for species identification and placement. The alignments were assembled and manually adjusted using Sequence Alignment Editor v. 2.0a11 (Rambaut 2002). Phylogenetic analyses were performed using PAUP v. 4.0b10 (Swoford 2003). Alignment gaps were treated as ‘fifth base’ and all characters were unordered and of equal weight. Maximum parsimony analyses were performed using the heuristic search option with 100 random sequence additions and tree-bisection-reconstruction (TBR) as the branch-swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the trees obtained was evaluated by 1000 bootstrap replications with 100 random sequence additions (Hillis & Bull 1993). Other measures including tree length, consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were also calculated. Gaps in the SSU alignment longer than 10 bp were coded as single indels. Sequences derived in this study were lodged at GenBank (Table 1), and the alignments and derived trees in TreeBASE.

**Morphology**

Observations were made with a Zeiss V20 Discovery stereo-microscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and an AxioCam MRC5 camera and ZEN software. Measurements and photographs were made from structures mounted in clear lactic acid, derived from 30 observations (×1 000 magnification) unless otherwise stated, with the extremes given in parentheses. The terminology of Minter (2006a) was used for descriptions.

**RESULTS**

**Phylogenetic analysis**

The SSU sequence alignment contained 39 sequences (including the outgroup) and 1 257 characters (968 characters constant, 103 variable and parsimony-uninformative, 186 cha-
characters parsimony-informative) and the heuristic search resulted in two equally most parsimonious trees (TL = 621 steps, CI = 0.604, RI = 0.861, RC = 0.520, HI = 0.396), of which one is shown in Fig. 1. The two trees differed only with regard to the position of Monascus purpureus (GenBank DQ782881) in the Aspergillaceae. Within the Coryneliales clade, the sequences of C. ubera group together (72 % bootstrap), with C. africana and C. fructigena (99 % bootstrap support) as sister lineages (99 % bootstrap support). Lagunulopsis bispora clustered basal to the Corynelia spp. (99 % bootstrap support). Calicopsis pinea represents the most basal species in the Coryneliales (100 % bootstrap support). The Coryneliales/Coryneliomycetidae formed a strongly supported sister clade (100 % bootstrap support) to Chaetothyriomycetidae and Eurotiomycetidae, representing a separate subclass; not clustering (bootstrap support < 50 %) with either of them, but with closer affinities to Chaetothyriomycetidae than to Eurotiomycetidae.

Twelve equally most parsimonious trees (TL = 904 steps, CI = 0.446, RI = 0.686, RC = 0.306, HI = 0.554) (Fig. 2) were generated from the LSU alignment consisting of 35 sequences (including the outgroup) and 536 characters, of which 291 were constant, 47 variable and parsimony-uninformative, and 198 parsimony-informative. The trees differed mainly with regard to the position of genera in the Chaetothyriomycetidae and the Aspergillaceae. Species of Corynelia clustered together with high support (100 % bootstrap support) and had as more basal sister species Tripospora tripos and Lagunulopsis bispora. Similar to the SSU phylogeny, Calicopsis represented the most basal lineages in the Coryneliales/Coryneliomycetidae (100 % bootstrap support). The Coryneliomycetidae clustered with the

![Fig. 1](image-url) The first of two equally most parsimonious trees obtained from the SSU sequence alignment. Branches present in the strict consensus tree are thickened and novel sequences generated in this study are shown in bold face. The subclass, order and family (from right to left) of the included species are shown to the right of the tree using blocks of different colours. Bootstrap support values > 50 % based on 1 000 replicates are shown at the nodes. The scale bar indicates 10 changes and the tree was rooted to Dothidea sambuci (GenBank AY544722).
**Eurotiomycetidae** with low bootstrap support (75 %) forming a sister clade to the *Mycocaliciaceae*.

The ITS sequence alignment contained 42 sequences (including the outgroup) and 496 characters (239 constant, 56 variable and parsimony-uninformative, and 200 parsimony-informative characters) and the heuristic search resulted in three equally most parsimonious trees (TL = 549 steps, CI = 0.761, RI = 0.921, RC = 0.701, H1 = 0.239), of which the first is shown in Fig. 3. The three trees differed mainly in the order of strains belonging to the same species. The *Corynelia* strains studied here clustered in three distinct clades, one of which is identified as *C. uberata* (100 % bootstrap support) and two clades for which the names *C. fructigena* (88 % bootstrap support) and *C. africana* (96 % bootstrap support) are proposed below. These latter two clades clustered with available sequences of *C. portoricensis* and *C. tropica*, all of which formed a sister clade to *C. uberata* s.str. The *C. africana* clade contained one sequence from GenBank (accession JX968551) which was deposited in the database as *C. uberata* from *P. latifolius* in South Africa. The *C. uberata* clade recognised in the present study contains several sequences from GenBank: JX968546, JF811342, JF811343, JF811344, and JN998125 from *A. gracilior* (as *P. falcatus*) trees in Ethiopia, JF895471 from *P. latifolius* in South Africa, and JX968544 and JX968545 from *Podocarpus* sp. in Kenya. The remaining isolates included in this study represented *Lagenulopsis bispora* (100 % bootstrap support) and *Tripospora triplos* (100 % bootstrap support).

**Morphology**

The ascomatal stromata and fertile extensions observed were as described previously (Fitzpatrick 1920, 1942a, Benny et al. 1985b, c, Minter 2006a, b). There were however small differences noticed in ascomatal shape and ascospore size between specimens initially identified as *C.uberata*. These differences

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**Fig. 2** The first of 12 equally most parsimonious trees obtained from the LSU sequence alignment. Branches present in the strict consensus tree are thickened and novel sequences generated in this study are shown in bold face. The subclass, order and family (from right to left) of the included species are shown to the right of the tree using blocks of different colours. Bootstrap support values > 50 % based on 1 000 replicates are shown at the nodes. The scale bar indicates 10 changes and the tree was rooted to *Dothidea sambuci* (GenBank NG_027611).
Fig. 3 The first of three equally most parsimonious trees obtained from the ITS sequence alignment. Branches present in the strict consensus tree are thickened and novel sequences generated in this study are shown in bold face. The species names are shown to the right of the tree and specimen or GenBank accession numbers are shown at the leaves. Bootstrap support values > 50% based on 1 000 replicates are shown at the nodes. The scale bar indicates 10 changes and the tree was rooted to Eupenicillium parvum (GenBank DQ536524).

were consistent with the host species or organ on which the fungi were collected (Fig. 4) and corresponded to consistent differences in the molecular analysis using the rDNA ITS region (Fig. 3). Therefore, two new species are described below.

Fresh specimens of C. uberata and the two species here described were examined and found to have asci with uniformly thick walls when immature, which broke down with maturity, as described by Johnston & Minter (1989) and interpreted by them as bitunicate. The asci with developing spores were frequently observed to also have a thick wall structure surrounding them (Fig 5), which in progressively more developed asci was seen as breaking or thinner and eventually was not visible on asci ready to liberate the ascospores, as illustrated by Minter (2006a). Fully mature asci had only a thin wall. Young asci of Lagenulopsis bispora and Tripos tripospora were observed to also have a uniformly thick wall, presumably bitunicate, whereas mature asci were all thin-walled (Fig. 10).

Taxonomy

Corynemeliomycetidae A.R. Wood, Damm, J.Z. Groenew., Cheew. & Crous, subclass. nov. — MycoBank MB814491

Type order. Coryneliales Seaver & Chardon (1926).

Mostly plant pathogenic and usually biotrophic, with internal mycelium that is early erumpent producing coriaceous to car-
Fig. 4  a–f. Corynelia uberata on Afrocarpus falcatus. a. In habit; b, c. stromata with Neodevriesia coryneliae; d–f. stromata on leaves and stems showing dumbbell-shaped ascomata. — g–k. Corynelia fructigena on Podocarpus latifolius fruit. g. In habit, infected fruit persistent on trees; h. often only the podocarpium is infected, but the seed can also be infected; i, j. flask-shaped ascomata; k. occasional infected fruit several years old occur. — l–q. Lagenulopsis bispora on Podocarpus latifolius leaves. l. Adaxial surface showing chlorotic area associated with stromata; m. abaxial surface of same leaf showing stromata; n–q. smooth narrowly flask-shaped ascomata. — r–w. Corynelia africana on Podocarpus latifolius leaves. r. In habit; s, t. flask-shaped immature ascomata; u. mature ascomata with pronounced curvature; v. mature ascomata with shaggy appearance at dehiscence; w. fully opened ascomata showing wide clefts at tips.
bonaceous stromata. *Stromata* reduced or cushion-like, developing to variously shaped loculate fertile extensions that form ascomata or spermogonia. *Spermogonia* sessile, with minute ostiole, spermatia unicellular, elongate, hyaline. *Ascomata* clustered or individual, pseudothecial, with a mazaedial chamber above the ascus-bearing base, black, thick- and smooth or rough-walled, opening by an ostiole or dehiscence grooves. *Interascal tissue* absent. *Asci* clavate, spathulate to capitate, mostly 8-spored or 2–3-spored, initially thick double-walled, the outer layer breaking and sloughing away with elongation, becoming long-stalked and thin-walled, without apical release mechanism, evanescent. *Ascospores* aseptate, dark, variable, smooth-walled to prominently ornamented, aggregating in a mass above the ascus layer. Sexual propagules do not germinate in culture.

*Coryneliales* Seaver & Chardón, Sci. Surv. Porto Rico & Virgin Islands 8: 40. 1926

*Type family.* Coryneliaceae Sacc. (1886).

Characters as for subclass.
**Coryneliaceae** Sacc., in Berl. & Voglino, Syll. Fung., Addit. I–IV (Abellini): 193. 1866

*Type genus. Corynelia Ach. (1823).*

**Characters as for subclass.**

**Notes** — The phylogenetic position of the Coryneliales, being variable and not closely associated with any of the three existing subclasses within the Eurotiomycetes (Fig. 1, 2) necessitated the establishment of the new subclass, **Coryneliomycetidae**. This new subclass is also supported by unique morphological features. While **Eurotiomycetidae** form cleistothecia/gymnothecia with usually evanescent, unitunicate asci and **Chaetothyriomycetidae** mostly perithecia with bitunicate/fissitunicate to evanescent asci (Geiser et al. 2006, Hibbett et al. 2007), **Coryneliomycetidae** form pseudothecial mazaedial ascomata with initially double-walled asci with the outer layer deliquescing, without an apical release mechanism resulting in passive ascospore release (Johnston & Minter 1989), and produce spermogonia (Geiser et al. 2006). The subclass **Coryneliomycetidae** consists of a single order with a single family only.

**Corynelia Ach., in Fr., Syst. Mycol. (Lundae) 2: 534. 1823**

*Type species. Corynelia uberata Fr. (1818).*

Phytopathogenic on **Podocarpaceae.** **Stromata** subcircular or elongated, coalescing, black, often crowded, bearing fertile extensions as ascomata or spermogonia. **Spermogonia** may or may not be present, if present globose, ovoid or irregularly compressed, sessile, loculate, with minute ostiole, spermatia unicellular, elongate, hyaline. **Ascomata** formed inside stromatic tissue then extending beyond the stromata as variously shaped cylindrical extensions, black, shiny, roughened, rather uniform, straight or slightly curved, rounded at base and tip, or constricted in the middle giving the ascomata a dumbbell shape, apex with a dehiscence zone splitting either along a single transverse groove or along several radiating grooves resulting in 2–several apical lobes, transverse groove may be indistinguishable; interior a locule, the lower portion containing the asci and the distal portion free ascospores. **Paraphyses** not observed. **Asci** arising from basal cushion, maturing sequentially, young asci uniformly thick-walled which is interpreted as a double wall (bitunicate) bounding an interior mucilaginous layer.
clavate and short-stalked; asci mature the stalk elongates, later spatulate to capitate, long-stalked, mature asci thin-walled which is interpreted as only the inner wall remaining after rupturing and passive loss of the outer wall and mucilaginous layer, mostly 8-spored or 3-spored, arranged in clusters. Ascospores at first hyaline, then becoming dark brown, unicellular, with thin outer wall and thick inner wall, smooth or warted.

*Corynelia africana* A.R. Wood, Van der Linde, Cheew. & Crous, *sp. nov.* — MycoBank MB814492; Fig. 4, 5, 6

*Etymology.* Named for the continent from where it has been collected.

*Type.* SOUTH AFRICA, Western Cape, Grootvadersbosch Nature Reserve, W of Heidelberg, on Podocarpus latifolius, 20 Nov. 2000, A.R. Wood 247 (holotype PREM 57242).

*Diagnosis.* Differs from *Corynelia uberata* by its larger (13–16(–18) µm) and prominently warted ascospores.

Colonies on attached green living leaves, occasionally stems, scattered, black, conspicuous, often on both sides of leaf, 1–13 colonies on each infected leaf; colonies breaking through leaf surface and producing erumpent stromata. Stromata subcircular or elongate, sometimes coalescing, black, few to more than 20 crowded fertile extensions mature to ascomata or spermogonia. *Spermogonia* usually preceding the ascomata, intermixed or developed on separate stromata. *Ascomata* vertical or, more often, irregularly arranged, pointing to sides from stromatic cushion, formed inside stromatic tissue and elongating as cylindrical extensions of the stromata, black, shiny, roughened, variable in shape, initially flask-shaped but tending to more or less distinctly dumbbell-shaped at maturity, rounded at base and apex, straight or slight constriction in middle, sometimes at maturity bending below the apex so that the apex is at an angle to the base and neck, 0.9–1.5 × 0.3–0.45 mm at base, 0.2–0.3 mm at neck and 0.23–0.45 mm at apex; immature ascomata present little indication of a dehiscence zone at the apex, not

Fig. 7 Paratype of *Corynelia fructigena* (PREM 59201). a. Infected podocarpium; b, c. flask-shaped ascomata; d. mid-line transverse sections through ascomata; e–i. asci with eight ascospores. — Scale bars: d = 1.5 mm; e–g = 50 µm; h–i = 30 µm.
processing a true ostiolum, tip becomes shaggy in appearance at dehiscence, dehiscence via a wide, deep, single, transverse cleft which separates the tip of each ascoma into two broad sections, light brown on interior of sections; interior a lobe, the lower portion globose containing the ascus and the distal portion free ascospores; ascomatal wall composed chiefly of interwoven hyphae of textura intricata. Paraphyses not observed. Ascii arising from basal cushion, maturing sequentially, young ascus uniformly thick-walled, clavate and short-stalked, then as ascus matures stalk elongates, mature ascus sp鄢ate to capitulate, pointed at the apex and long-stalked, the homogenous thick wall breaks and is lost during maturation of the asci so that mature ascii are thin-walled, (38–)47–55(–67) µm long, (38–)47–55(–67) µm wide, stalk up to 90 µm long, usually 8-spored, arranged in a cluster. Ascospores at first hyaline, then becoming dark brown, unicellular, globose, prominently warted, 13–16(–18) µm.

Other material examined. SOUTH AFRICA, Western Cape, Grootvadersbosch Nature Reserve, W of Heidelberg, on Podocarpus latifolius, 17 Oct. 2004, A.R. Wood 656 (PREM 59200); Krisjan-se-Nek picnic site, Goudveld Forest, Garden Route National Park, N of Knysna, on P. latifolius, 15 July 2006, A.R. Wood 683 (PREM 61206); Jubilee Creek picnic site, Goudveld Forest, Garden Route National Park, N of Knysna, on P. latifolius, 15 July 2006, A.R. Wood 681 (whole specimen used for molecular analysis); same locality, on P. latifolius, 15 July 2006, A.R. Wood 682 (PREM 61205); Woodcutters trail, Millwood, Goudveld Forest, Garden Route National Park, N of Knysna, on P. latifolius, 5 June 2000, A.R. Wood 194 (PREM 57235); Terblans walk, Gouna Forest, Garden Route National Park, N of Knysna, on P. latifolius, 30 Aug. 2000, A.R. Wood 225 (PREM 57244); same locality, on P. latifolius, 14 July 2006, A.R. Wood 678 (PREM 61201); Kranshoek picnic site, Harkerville Forest, Garden Route National Park, E of Knysna, on P. latifolius, 14 July 2006, A.R. Wood 675 (PREM 61198); Perdekop walk, Harkerville Forest, Garden Route National Park, E of Knysna, on P. latifolius, 14 July 2006, A.R. Wood 673 (PREM 61194); Garden of Eden, Garden Route National Park, E of Knysna, on P. latifolius, 14 July 2006, A.R. Wood 671 (PREM 61194); Eastern Cape, approx. 4 km S of Grahamstown, on P. latifolius, 27 Jan. 2000, A.R. Wood 164 (PREM 57235); same locality, on P. latifolius, 18 July 2006, A.R. Wood 688 (PREM 61204).

Notes — Corynelia africana has the largest ascospores (13–16(–18) µm) of the three species considered here, as well as the most prominently warted ascospores. Its ascocarps are the most variable in shape, ranging from flask-shaped (especially when still immature) to prominently dumbbell-shaped (usually when the apices have dehisced). However, the width of the apex is never as broad as C. uberata can become. The apex tends to be the shaggiest in appearance, has the deepest dehiscence cleft, and in some specimens the opening ascomata curve over below their apices approximating a right angle. The majority of specimens collected on leaves and stems of P. latifolius and other species of African and Madagascan Podocarpus spp. (African subclade fide Knopf et al. 2012), and previously morphologically identified as C. uberata, likely belong to this species. These hosts include P. elongatus, P. henkelii, P. madagascariensis, and P. milanjianus (Winter 2006a).

Corynelia fructigena A.R. Wood, Van der Linde, Cheew. & Crous, sp. nov. — MycoBank MB814493; Fig. 4, 5, 7

Etymology. Named for its preference to infect the fruit of its hosts.

Type. SOUTH AFRICA, Western Cape Province, at information centre, Grootvadersbosch Nature Reserve, W of Heidelberg, on fruit of Podocarpus latifolius, 20 Nov. 2000, A.R. Wood 250 (holotype PREM 57240).

Diagnosis. Differs from Corynelia uberata by smooth flask-shaped ascomata and slightly larger ascospores (11.5–13.5 µm).

Colonies on attached fruits or sometimes leaves, scattered, black, conspicuous, not found on stems, colonies breaking through fruit surface and producing erumpent stromata. Stroma clavate, coalescing to form longer colonies, often crowded, black, with fertile extensions which mature into ascoma that are vertical or irregularly arranged. Spermogonia not observed. Ascomata formed inside stromatic tissue and elongating as cylindrical extensions of the stromata, black, shiny, smooth, rather uniform and distinctly flask-shaped, only slightly constricted in the middle, straight or slightly curved, rounded at tip, 0.7–1.1 mm × 0.28–0.42 mm at base, 0.2–0.25 mm at neck and 0.16–0.3 mm at apex; immature ascomata present no indication of a dehiscence zone at the apex, both possessing a true ostiolum, ascomata remain closed for long periods, opening via an inconspicuous, shallow, single transverse cleft which separates into two sections; interior a lobe, the lower portion globose containing the young ascus and the distal portion free ascospores; ascomatal wall is composed mainly of interwoven hyphae of textura intricata. Paraphyses not observed. Ascii arising from basal cushion, maturing sequentially, young ascus uniformly thick-walled, clavate and short-stalked, stalk elongates as ascus mature, later spatulate to capitulate, pointed at the apex and long-stalked; thin-walled in mature ascus, 33–38(–44) × 50–58 µm, stalk up to 200 µm, mostly 8-spored, arranged in a cluster. Ascospores at first hyaline, then becoming dark brown, unicellular, with thin outer wall and thick inner wall, minutely warted, 11.5–13.5 µm.

Other material examined. SOUTH AFRICA, Western Cape Province, at information centre, Grootvadersbosch Nature Reserve, W of Heidelberg, on fruit of Podocarpus latifolius, 17 Oct. 2004, A.R. Wood 657 (PREM 59201); Krisjan-se-Nek picnic site, Goudveld Forest, Garden Route National Park, N of Knysna, on one leaf of P. latifolius, 14 July 2006, A.R. Wood 684 (whole specimen used for molecular analysis); Garden of Eden, Garden Route National Park, E of Knysna, on fruit of P. latifolius, 14 July 2006, A.R. Wood 682 (PREM 61195).

Notes — Corynelia fructigena differs from C. uberata (ascospores 9–12 µm, ascomata rough dumbbell-shaped) in having slightly larger ascospores (11.5–13.5 µm), and smooth flask-shaped ascomata. It differs from C. africana (ascospores 13–16(–18) µm, ascomata shaggy flask to dumbbell-shaped) in the slightly smaller ascomata and ascomatal shape. It is possible that the majority of specimens collected on fruit of P. latifolius and other species of African and Madagascan Podocarpus species (African subclade fide Knopf et al. 2012), and previously morphologically identified as C. uberata, represent this species, though it may apparently also occasionally infect leaves. Unfortunately, specimens on fruit were infrequently observed and sequences were not obtained from some of the few specimens collected. This is in contrast to the observation by Phillips (1927) at the beginning of the 20th century that in infection was, at least in some years, abundant and widespread.

H. Rehm distributed specimens under the name C. clavata f. fructicola (Ascomyceten no. 1326a) collected by P. MacOwan near Somerset East (South Africa, Eastern Cape; S F205134, S F205137/8/9) (Rehm 1900), however this name was never validated. The name C. fructicola cannot be used as this binomial is pre-occupied by C. fructicola (Pat.) Höhn. (current name for this fungus is Coryneliospora fructicola (Pat.) Fitzk.).

Corynelia uberata Fr., Observ. Mycol. (Havniae) 2: 343. 1818

— Fig. 4, 5, 8, 9

(binomial sanctioned in Syst. Mycol. 2: 535. 1823)

non Corynelia clavata (L.f.) Sac., in Berl. & Voglino, Sylf. Fung. Addit. I–IV (Abellini): 193 (1886). illegitimate name, the basionym used (Mcuor clavatus L.f.) is a synonym of Sphinctrina turbinata (Pers.) De Not.

Type. SOUTH AFRICA, Western Cape Province, `in Sylvis Houthuiquis' (in forests on the Outeniqua mountains), on Afrocarpus falcatus, 1772, C.P. Thunberg s.n. (holotype UPS:BOT-F-005148 ex Herb E. Fries; consisting of one small piece of leaf with two stromata, which was taken from Thunberg's original collection UPS-Thunb 27440 & UPS:BOT-F-118557 (both numbers given to the same specimen)); isotype S F38997; Krisjan-se-Nek picnic site, Goudveld forest, Garden Route National Park, N of Knysna, on Afrocarpus falcatus, 15 July 2006, A.R. Wood 686 (epitype PREM 61207, MBT202696).
Colonies on green living leaves, scattered, black, conspicuous, often on only one side of leaf, sometimes on both sides, also often found on stems and fruit, 1–18 colonies on each infected leaf, colonies breaking through leaf surface and producing erumpent stromata. Stromata subcircular or elongated, black, with crowded fertile extensions which mature to ascomata or spermogonia, basal part of stroma composed of brown thick-walled cells. Spermogonia globose, usually preceding the ascomata. Ascomata formed inside stromatic tissue and elongating as cylindrical extensions of the stromata, distinctly dumbbell-shaped with upper and lower portions rounded and constricted in the middle, black, shiny, roughened, vertical or irregularly arranged, 0.7–1.1 × 0.3–0.36 mm at base, 0.19–0.23 mm at neck and 0.27–0.65 mm at apex; walls undifferentiated, composed of textura angularis or prismatica along the inner layer surrounding an internal locule in which asci are produced at the base and distal to which is a funnel-shaped chamber where released ascospores accumulate leading to the opening; lacking a true perithecial wall, immature ascomata present little indication of a dehiscence zone on the apex, a true ostiolum absent, dehiscence by a single transverse cleft which separates into two sections, the cleft deepening slowly and not opening outwards. Paraphyses not observed. Asci arising from basal cushion, maturing sequentially, young asci uniformly thick-walled, clavate and short-stalked, then the stalk elongates so that the asci become spatulate to capitate, pointed at the apex and long-stalked, the apparently homogenous thick wall breaks and is lost during elongation and maturation so that mature asci are uniformly thin-walled, 33–36(–39) × 50–55(–57) µm, stalk up to 220 µm, usually 8-spored, occasionally fewer ascospores in asci, arranged in a cluster. Ascospores at first hyaline, then becoming dark brown, unicellular, with thin minutely warted outer wall and thick smooth inner wall, ascospores of specimen S F38997 (isotype) measure (8–)9–11 µm diam (Ibai Olariaga Ibarguren, Naturhistoriska Riksmuseet, Stockholm (S), pers. comm.; 10–11 µm diam fide Saccardo 1886), those of PREM 61207 (epitype) measure 11–12 µm diam.

Other material examined. SOUTH AFRICA, Western Cape Province, Grootvadersbosch Nature Reserve, W of Heidelberg, on Afrocarpus falcatus, 20 Nov. 2000, A.R. Wood 246 (PREM 57243); Jubilee creek picnic site, Goudveld forest, Garden Route National Park, N of Knysna, on A. falcatus, 15 July 2006, A.R. Wood 680 (PREM 61203); Ysterhoutrug picnic site, Diepwalle forest, Garden Route National Park, N of Knysna, on A. falcatus, 14 July 2006, A.R. Wood 676 (PREM 61199); Terblans trail, Gouna forest, Garden Route National Park, N of Knysna, on A. falcatus, 30 Aug. 2000, A.R. Wood 224 (PREM 57239); Woodcutter trail, Millwood, Goudveld Forest, Garden Route National Park, N of Knysna, on A. falcatus, 6 June 2000, A.R. Wood 187 (PREM 57237).

Notes — The identity of the host plant of the Thunberg specimen is A. falcatus (Fig. 6), C. uberata is thus confirmed as occurring on A. falcatus in southern Africa and on A. gracilior in northern Africa (Assefa et al. 2014, 2015), but is likely to also occur on other species of Afrocarpus in Africa. Specimens previously identified as C. uberata but occurring on other host
genera and on continents other than Africa are likely to be other as yet undescribed species. The comprehensive description provided by Minter (2006a) refers to this species as well as the two described above. All three have similar morphology, the distinguishing characteristics are discussed under each.

*Neodevriesia corynelia* is frequently associated with stromata of this fungus (Fig. 4) (Crous et al. 2014), but has not yet been observed as occurring on stromata of the new species described above.

When establishing the family *Coryneliaceae*, Saccardo used the earliest epithet of the names associated with this fungus by various authors, namely *Mucor clavatus*. Thus this fungus was referred to as *C. clavata* for many years. However, *M. clavatus* is now recognised as a synonym of *Sphinctrina turbinata*, a lichen (www.mycobank.org). Thus the first epithet given which has both a valid description and a type specimen is *C. uberata* (Fitzpatrick 1942b).

**Lagenulopsis** Fitzp., Mycologia 34: 487. 1942

Type species: *Lagenulopsis bispora* (Fitzp.) Fitzp. (1942).

Phytopathogenic on *Podocarpus*. Stromata subcircular, sometimes coalescing, black, fertile extensions become ascomata or spermospores. *Spermospora* sessile, ovoid, loculate, with a minute apical perforation; spermatia unicellular, oblong to fusiform, hyaline. *Ascomata* formed inside stromatic tissue then extending beyond the stroma, black, smooth, shiny, crowded, narrowly flask-shaped, with a flat to slightly rounded tip, dehis-cence by an apical pore through which ascospores are extruded appearing as a reddish brown knob, interior a locule, the lower rounded portion containing the developing asci and a distal long narrow neck with free ascospores; ascomatal wall composed mainly of interwoven hyphae of *textura intricata*. Para-physes not observed. Asci arising from a basal cushion, maturing sequentially, young asci uniformly thick-walled, clavate and short-stalked, later spatulate to capitulate and long-stalked, pointed at the apex and long-stalked, mature asci thin-walled, mostly 2-spored at maturity. Ascospores at first hyaline, then becoming dark brown, unicellular, with thin outer wall and thick inner wall.

**Lagenulopsis bispora** (Fitzp.) Fitzp., Mycologia 34: 488. 1942 — Fig. 10, 11

Basionym. *Corynelia bispora* Fitzp., Mycologia 12: 242. 1920. ≡ *Corynelia clavata* (L.f.) Sacc. f. *macrospora* Syd., Wiss. Ergebn. Deut. Zentr.-Afr.-Exped. (1907–1908), Bot. 2: 10. 1910.

Type. **UGANDA**, Ruwenzi, west Butaguz Tali., on *Podocarpus milanjianus*, Feb. 1908, J. Mildbraed 2547 (holotype S F51449). – **SOUTH AFRICA**, Western Cape Province, Grootvadersbosch Nature Reserve, Heidelberg, on *P. latifolius*, 20 Nov. 2000, A.R. Wood 249 (epitype PREM 57232, MBT202697).

Colonies on attached green living leaves, often associated with an angular chlorotic area of leaf, colonies breaking through leaf surface and producing an erumpent stroma, often on only one side of leaf, 1–10 colonies on each infected leaf, not found on stems. *Stromata* subcircular, small, several apparently originating from a single infection, sometimes coalescing, forming longer colonies aligned with the main axis of substratum, black, 2–5(–8) × (2–)5–12 mm, with few to crowded fertile exten-

![Fig. 9 Epitype of Corynelia uberata (PREM 61207). a–c. Stromata with dumbbell-shaped ascomata; d. mid-line transverse sections through ascomata; e–g. asci with 8 ascospores; h. minutely warty ascospores. — Scale bars: d = 2.5 mm; e–g = 50 μm; h = 30 μm.](image-url)
sections which mature to ascomata or spermogonia. Spermogonia usually preceding the ascomata, intermixed, sessile, with a minute apical perforation; spermatia unicellular, minute, oblong to fusiform, hyaline. Ascomata formed inside stromatic tissue and elongating as cylindrical extensions of the stroma, 1–29 extensions per stromatum, black, smooth, shiny, flask-shaped with a glabrous, shiny, narrow neck, usually more than twice as long as it is wide, straight or slightly curved, with a flat to slightly rounded tip, 0.9–1.7 × 0.23–0.4 mm at base, 0.12–0.19 mm at middle of the neck and 0.17–0.24 mm at apex, dehiscence by a pore, the spore mass forming a powdery, reddish brown knob at the tip of the ascomata; interior a locule, the lower portion globose containing the asci and the long narrow distal portion the free ascospores; the ascomatal wall is composed mainly of interwoven hyphae of textura intricata. Paraphyses not observed. Asci arising from a basal cushion, maturing sequentially, young asci uniformly thick-walled, clavate and short-stalked, the stalk elongates, becoming spatulate to capitate, pointed at the apex and long-stalked, mature asci thin-walled, 15–19 × 35–55 μm, stalk up to 150 μm long, 2-spored when mature but sometimes up to 5-spored when immature. Ascospores at first hyaline, then becoming dark brown, unicellular, with thin outer wall and thick inner wall, minutely warted or smooth once released, 10–15 μm, wall 1–2.5 μm thick.

Other material examined. CAMEROON, Western Province, Bafut-Ngem F.R., on P. latifolius (as P. milanjiana), no date, M. Brunt 827 (K(M) 154329). – FJI, Nausori highlands, 2000 ft altitude, on P. neriifolius, together with Corynelia braziliensis, 3 July 1973, de Laubenfels s.n. (FLAS-F53225). – JAMAICA, Newhaven Gap, Cinchona, on P. urbani, 6 Mar. 1906, W. Harris 9199 (CUP 31753). – SOUTH AFRICA, Eastern Cape Province, ± 4 km SW of Grahamstown, on P. latifolius, 27 Jan. 2000, A.R. Wood 163 (PREM 57331); Western Cape Province, Terblans trail, Gouna forest station, Knysna, on P. latifolius, 30 Aug. 2000, A.R. Wood 223 (PREM 57230); Redwood trail, Grootvadersbosch nature reserve, Heidelberg, on P. latifolius, 17 Oct. 2004, A.R. Wood 655 (PREM 59202); Krijan-se-Nek picnic site, Goudveld Forest, Garden Route National Park, N of Knysna, on P. latifolius, 15 July 2006, A.R. Wood 685 (whole specimen used for molecular analysis); Kranshoek picnic site, Harkerville Forest, on P. latifolius, 14 July 2006, A.R. Wood 674 (PREM 61197). – TANZANIA, Mbulu, Masai District, on P. latifolius (as P. milanjiana), Jan. 1953, Procter 133 (K(M) 154330).

Fig. 10 Lagenulopsis bispora. — a–g. Holotype of Lagenulopsis bispora (S F51449). a. Stroma; b–e. narrow flask-shaped ascomata; f, g. immature stroma. — h–j. Epitype of Lagenulopsis bispora (PREM 57232). h. Asci, some with more than 2 ascospores (arrows); i. immature asci with thick bitunicate walls (arrows); j. asci, some with more than two ascospores (arrows) and a thin-walled mature ascus (thick arrow). — Scale bars: h = 30 μm; i–j = 10 μm.
A collection from Fiji was also examined (FLAS-F53225); however, on this specimen an unidentified conidial fungus was also present with flask-shaped pycnidia which was similar looking to the ascomata of *L. bispora*, though smaller. Ascospores observed on the apex of the ascomata of the *L. bispora* present were significantly smaller than all other specimens examined, being 8–10 × 6–8 µm and with 1 µm thick walls (n = 10) which were more prominently warted (verrucose). Benny et al. (1985c: f. 7) illustrated this specimen as having encrusted walls. The identity of this specimen is therefore uncertain. Based on these observations, we suspect that the genus *Lagenulopsis* is not
monotypic, but additional specimens and sequences from the Americas and Melanesia are required to resolve this issue. Young asci typically had two ascospores developing within, though one or three to five ascospores were occasionally observed. Mature asci observed only had two ascospores, or very occasionally one ascospore. Some released ascospores appeared to have a bilaminate wall, and on others the outer thinner wall was observed to be breaking away from the inner wall and disintegrating. Most released ascospores appeared as having a single smooth wall. On the other hand a bilaminate wall was readily visible on ascospores still within the asci in the ascomatal locules.

*Tripospora* Sacc., Syll. Fung., Addit. I–IV (Abellini): 194. 1886

*Type species.* *Tripospora tripos* (Cooke) Lindau (1897).

Phytopathogenic on *Podocarpaceae.* Stromata subcircular to elongate, coalescing to form longer colonies parallel to the main axis of substratum, black, often crowded, bearing fertile extensions as ascomata or spermogonia. Spermogonia sessile, semi-

![Fig. 12](image)

Fig. 12 Epitype of *Tripospora tripos* (PREM 61202). a. Stromata; b, c. ascomata; d. immature stromata; e, f. transverse section through a spermogonium; g. mid-line transverse sections through ascomata; h–k. asci with ascospores at various stages of maturity; n. ascospores. — Scale bars: e = 1 mm; f, h–j = 50 μm; g = 2 mm; k–l = 30 μm.
globose, with a minute, apical perforation; spermata unilocular, minute, oblong to fusiform, hyaline. *Ascomata* formed inside stromatic tissue then extending beyond the stromata, black, shiny, roughened below, rather uniform, long slender flask or short dumbbell shaped, with glabrous shiny necks, apex rounded before dehiscence, dehiscence by an apical pore, the apex opening widely to become shallow funnel-shaped; interior a locale, the lower portion containing the asci and the distal portion free ascospores; the ascomatal wall is composed mainly of interwoven hyphae of *textura intricata*. *Paraphyses* not observed. Asci arising from basal cushion, maturing successively, young asci uniformly thick-walled, clavate and short-stalked, stalk elongates as ascus matures, becoming subglobose, pointed at the apex and long-stalked, mature asci thin-walled, usually 8-spored, arranged in cluster. *Ascospores* at first hyaline, then becoming dark brown, unilocular, characteristically star-shaped, thick-walled, with 4 short or long conical sharp-pointed projections radiating from a rounded central portion.

**Tripospora tripus** (Cooke) Lindau in Engler & Prantl., Nat. Pflanzenfam., Teil. I (Leipzig) 1: 413. 1897 — Fig. 12

*Basionym*. Corynelia tripus Cooke, Grevillea 8: 34. 1879.

Type. SOUTH AFRICA, Eastern Cape Province, near Somerset East, Cape of Good Hope, on leaves of Afrocarpus falcatus (as Podocarpus elongatus), P. Mac-Owan 1253 (holotype K(M) 137581 ex. herb. M.C. Cooke;, syntypes on Province, at Gouna forest station, Garden Route National Park, N of Knysna, 14 July 2006, A.R. Wood 677 (PREM 61200); KwaZulu-Natal, Natal National Botanical Garden, Pietermartizburg, on *A. falcatus*, 21 Jan. 1999, A.R. Wood s.n. (PREM 57234); Natal National Botanical Garden, Pietermartizburg, on *A. falcatus*, 24 June 2006, F.H.J. Rijkenberg s.n. (PREM 61190).

Notes — The only host on which this species was observed in South Africa during the course of this study was *Afrocarpus falcatus* as well as all specimens examined in K, S, and PREM. Therefore records on *P. latifolius* and *P. elongatus* are based on misidentification of the host plant, for example in Doidge (1950), Benny et al. (1985c), and Minter (2006b). This is due to P. MacOwan, the original collector of this species, having incorrectly identified the host of the type specimen as *P. elongatus*, whereas the host plant was in fact *A. falcatus* as determined by examining original specimens (K(M) 137581/2, K(M) 198323/4/5). For some time *P. elongatus* was considered to be a synonym of *P. latifolius*. This fungus has also been recorded on *A. gracilior* and *A. usambariensis* (Minter 2006b), and therefore is restricted to members of *Afrocarpus*. Minter (2006b) and Tim (1971) provide comprehensive descriptions.

In erecting the genus *Tripospora*, P.A. Saccardo renamed Corynelia tripus as *T. cookii* (Saccardo 1886), however the epithet published earlier by M.C. Cooke (Cooke 1879) remains valid, and therefore G. Lindau’s recombination is retained.

**DISCUSSION**

There have been several studies that have focused on monographic or morphological studies of the *Coryneliaceae* (Fitpatrick 1920, 1942a, b, Funk 1963, Tim 1971, Benny et al. 1985a—d, Johnston & Minter 1989). In spite of this, however, the correct taxonomic placement of the *Coryneliales* remains debatable, due to the contradictory characters of having apparently unitunicate ascii but ascolocular development (Johnston & Minter 1989). The latter type of ascocarp formation was considered characteristic for fungi with bitunicate ascii, whereas fungi with unitunicate ascii typically have ascohmymenal development. This prompted Tim (1971) to propose a new centrum type, the Corynelioid type, to contrast this group from other fungi within the then recognised pyrenomycetes. This situation was resolved when it was shown that the asci of the *Coryneliales* are in fact bitunicate, but differ from all other types of bitunicate asci in that no modification of the ascus apex is present as a release mechanism, and that the outer wall layer usually breaks during ascus elongation. Between the two wall layers is a thick mucilaginous layer (Johnston & Minter 1989). The unmodified ascus apex results in passive ascospore release typical of the *Coryneliales*. Thus Johnston & Minter (1989) placed the family in the Loculoascomycetes. This class is now no longer recognised and the order was subsequently placed in the Dothideomycetes (Kirk et al. 2001). Sequence analyses of the SSU gene, however, revealed the *Coryneliales* to belong to the Eurotiomycetes where they clustered with the Chaetothyriales (now Coleotthyriomycetidae) (Winka 2000). While the LSU phylogeny in the present study corresponds to this analysis, the LSU phylogeny supports recent multi-gene-analyses that place the *Coryneliales* as a basal clade within, or alternatively interpreted as a sister clade to, the Eurotiomycetidae (Geiser et al. 2006, Gueidan et al. 2014, Chen et al. 2015), where the
**Table 2** Historical sequence of the taxonomic placement of the Coryneliaceae.

| Date | Event | References |
|------|-------|------------|
| 1886 | Coryneliaceae Sacc. ex Berl. & Voglino [as 'Coryneliaceae'] | Saccardo (1886) |
| 1891 | Family established, first spelt correctly; placed close to Cucurbitariaceae, Sphaeriales; including Corynelia and Tripospora | Saccardo (1891) |
| 1892 | Corynelia placed in Perisporiales with Capnodium and Antennaria; ostiole absent, irregularly split | Cooke (1992) |
| 1895 | Coryneliella (Co. consimilis) added, family related to Cucurbitariaceae, Sphaeriales | Saccardo (1895) |
| 1897 | Acknowledged monotypic genera (C. uberata, T. cookei, Coryneliella consimilis); related to Cucurbitariaceae | Lindau (1897) |
| 1920 | Typical Sphaeriales ostiole lacking, not supporting placement either in Sphaeriales or in Perisporiales | Fitzpatrick (1920) |
| 1926 | Include the family in an order of its own, Coryneliales | Seaver & Chardon (1926) |
| 1931 | Included Coryneliaceae in the Erysiphales | Clements & Shear (1931) |
| 1936 | Asccarp a loculoascomycetous pseudothecium, ascii long stipitate, deliquescent | McCormack (1936) |
| 1942 | Established Lagenulopsis (type species *L. bispora*), as well as a detailed study of Calicipsis | Fitzpatrick (1942a, b) |
| 1946 | Included the Coryneliaceae in the Sphaeriales | Hansford (1946) |
| 1951 | Centrum not typical Dothidiales; placed in Pyrenomycetes even though the ascocarp is ascomastromatic | Luttrell (1951) |
| 1963 | Coryneliaceae pseudoprototunicate, uncertain position; single-layered ascii lacking apical pore, deliquescent | Funk (1963) |
| 1969 | Fourth genus, Calicipsis (*C. podocarpus*) on two species of Podocarpus | Huguenin (1969) |
| 1973 | Corynelioid type of centrum proposed, unique to Coryneliaceae | Tim (1971) |
| 1974 | Coryneliopsis described | Butin (1971) |
| 1973 | Coryneliopsis and Coryneliospora either textura angularis or textura prismatica; Acrospermum in Ostropales | Korf (1973) |
| 1973 | Included the Coryneliaceae in the Sphaeriales | Muller & Von Anx (1973) |
| 1976 | Included the Coryneliaceae in the Etaphycomycetidae | Barr (1976) |
| 1982 | Coryneliaceae similar to Loculoascomycetes but lacking of bitunicate asci; centrum apaphysate, resembling capnodiaceous fungi and Hysteriales | Bezares & Kimbrough (1982) |
| 1982–1983 | Coryneliaceae pseudoprototunicate, producing an ascostromatic ascocarp; of uncertain position | Eriksson (1982a, b, 1983) |
| 1983 | Included the Coryneliaceae in the Parenchymaomycetidae | Barr (1983) |
| 1985 | Fitzpatrickella described | Benny et al. (1985a) |
| 1985 | Centrum of Calicipsis, Coryneliopsis, and Coryneliospora similar to other Coryneliaceae | Benny et al. (1985d) |
| 1989 | Asci different from other Loculoascomycetes, ascus apex elongation resulting in passive ascospore release | Johnston & Minter (1989) |
| 2000 | SSU sequence analyses placing Coryneales in Eurotiomycetes, clustering with the Chaetothyriales | Winka (2000) |
| 2001 | Class Loculoascomycetes no longer recognized; family placed in the Dothideomycetes | Kirk et al. (2001) |
| 2004 | Positioned in Eurotiomycetes based on SSU evidence, sister group Chaetothyriomycetes | Inderbitzin et al. (2004) |
| 2006 | Multigene phylogeny supporting placement of Coryneliaceae, basal clade within Eurotiomycetidae | Geiser et al. (2006) |
| 2016 | Coryneliaceae placed in new sub-class, Coryneliomycetidae | This publication |

_Coryneliales_ form a transition stage between the prototunicate _Eurotiomycetidae_ and the bitunicate _Chaetothyriomycetidae_. Based on the results obtained in this study we placed the _Coryneliaceae/Coryneliales_ in a separate subclass, the _Coryneliomycetidae_. Because of their unique morphology, the early nomenclatural history of the _Coryneliaceae_ was complicated, a summary of which is presented in Table 2.

Benny et al. (1985b) noted that immature asci of _C. uberata_ s.l. had a uniform thick wall. Johnston & Minter (1989) observed that this was the case for immature asci of most species in the _Coryneliaceae_, the only exception being _Coryneliopsis_. SEM observations of asci of _C. uberata_ and _C. tropica_ revealed an inner wall surrounded by a layer they interpreted as composed of mucilage, although this differentiation into wall layers could not be observed using a light microscope. As the ascus matured the thick wall broke and sloughed off in sections, so that fully developed ascii were thin-walled. A basal frill, the remnant of the bitunicate wall, may occur at the base of mature ascii (Johnston & Minter 1989). This same ascus morphological development was observed for all species examined in this study, and was also observed in the recently described _Calicipsisbeckhausii_ and _C. valentina_ (Garrido-Benavent & Pérez-Ortega 2015). In some genera of the _Sordariales_ (Jattaea, Pleurostoma, and Togninia) that are not closely related to typical bitunicate fungi, so-called remnant bases remain on the ascogenous hyphae after detachment of mature, undamaged ascii, or hair-like structures were observed at the bases of ascii that are reminiscent of the frills at the ascus base of _Coryneliomycetidae_ (Réblóvá et al. 2004, Mostert et al. 2006, Damm et al. 2008a, b). This suggests the existence of additional outer wall layers in earlier stages of the ascus development as well. In _Calosphaeria africana_ (_Calosphaeriaceae, Sordariomycetes_) two wall layers detach from each other during ascus development and are involved in changes of the ascus shape (Damm et al. 2008b) similar to those observed in _Coryneliomycetidae_.

_Corynelia uberata_ is the type species of the _Coryneliaceae_, and before this study it was considered to be a wide-ranging species with a distribution through Africa to Asia and Australasia, occurring on many different species of _Podocarpus_ (Minter 2006a). However, here we consider it to be restricted to Africa only, on members of the segregate genus _Afrocapsa_, e.g. _A. falcatus_ and _A. gracillor_ (Assefa et al. 2014), and most likely also _A. usambarensis_ (Minter 2006a). To stabilise the application of the name, an epitope was selected for this taxon. Furthermore, two new species of _Corynelia_ are newly described from _P. latifolius_ in South Africa, and are likely to be the species present on other members of the African subclade of the subgenus _Podocarpus_ (fide Knopf et al. 2012), from which _C.uberata_ has been recorded (_P. elongatus, P. henkeli, P. madagascarenaes, and P. milanjianus_ (usually considered a synonym of _P. latifolius_), Minter 2006a). _Corynelia africana_ was a common species typically occurring on leaves, whereas _Corynelia fructigena_ was infrequently found and then usually on fruit. Although previously considered as belonging to _Corynelia uberata_ by all previous workers (Fitzpatrick 1920, 1942a, Benny et al. 1985b, Minter 2006a), the two new species described herein could be distinguished from _C.uberata_ s.str. by consistent differences in host preference, morphology, and nrDNA...
sequences. Collections from other parts of the world on other host species identified as C. iberata need to be re-examined as it is likely that they belong to as yet undescribed species. Their hosts (Minter 2006a) belong to various subclades within Podocarpus and the segregate genus Nageia from Asia, which is a sister clade to Afrocarpus, and the distantly related genus Falcatusiulus (Biffin et al. 2011, Knopf et al. 2012). Lagenulopsis bispora is assumed to be rare in South Africa, though its presence may be masked by the more abundant C. africana. Previously only a single specimen of L. bispora from South Africa was known (Benny et al. 1985c, K(M) 187631). In this study, the presence of L. bispora in South Africa is confirmed. Some young asci were observed to have more than two, or sometimes only one developing ascospore, although mature asci observed appeared to have only two spores. Corynelia jamaicensis and C. portoricensis are typically 3-spored (Fitzpatrick 1942a), suggesting that the programmed spore death (Raju & Perkins 2000) may occur in these species. Lagenulopsis bispora as currently delimited has a disjunct distribution, being recorded from Africa (on P. latifolius), the Neotropics (Jamaica and Mexico) and Micronesia (Fiji). Considering the differences in spore dimensions noted, as well as the hosts in this latter region belonging to the Tropical American subclade of the subgenus Podocarpus (P. matudai and P. urbani; Benny et al. 1985c) and the Fijian subclade of the subgenus Foliolatus (P. degenerii; Benny et al. 1985c) of Knopf et al. (2012), it is possible that one or more separate species await discovery.

The Coryneliales make ideal subjects for studies of the evolution of the Ascomycetes, biogeography of fungi, and co-evolution with their hosts. Corynelia iberata and T. tripos were only recorded on Afrocarpus, whereas C. africana, C. fructigena, and L. bispora were only recorded on African Podocarpus in this study. The present investigation indicates that more diversity within this group awaits discovery, particularly following molecular analyses. Increased knowledge of the true diversity of this group will allow a more detailed analysis of their co-evolution with the Podocarpaceae. Unfortunately this group of plants is under threat from over exploitation, which therefore also raises concerns about the continued existence of these associated obligate pathogens.

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