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Systematics of *Calonectria*: a genus of root, shoot and foliar pathogens

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Cover: Top from left to right: Cylindrocladium leaf blight of a *Eucalyptus* sp. *Calonectria polizzii* conidiophore branches. Yellow perithecium of *Ca. colhounii*. Bottom from left to right: *Calonectria colombiana*, macroconidiophore. *Calonectria polizzii*, conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. *Calonectria eucalypti*, perithecium. Asci containing eight ascospores.

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Species concepts in *Calonectria* (*Cylindrocladium*)

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Abstract: Species of *Calonectria* and their *Cylindrocladium* anamorphs are important plant pathogens worldwide. At present 52 *Cylindrocladium* spp. and 37 *Calonectria* spp. are recognised based on sexual compatibility, morphology and phylogenetic inference. The polyphasic approach of integrating Biological, Morphological and Phylogenetic Species Concepts has revolutionised the taxonomy of fungi. This review aims to present an overview of published research on the genera *Calonectria* and *Cylindrocladium* as they pertain to their taxonomic history. The nomenclature as well as future research necessary for this group of fungi are also briefly discussed.

Key words: *Calonectria*, *Cylindrocladium*, species concepts, nomenclature, pathogenicity.

INTRODUCTION

The genus *Calonectria* (*Ca*.) was erected in 1867 by De Notaris, based on *Ca. daldiniana* collected on leaves of *Magnolia grandiflora* (*Magnoliaceae*), in Daldini, Italy (Rossman 1979a). Rossman (1979a) later reduced *Ca. daldiniana* to synonymy under *Ca. pyrochroa*, and defined this nectrioid fungus as having an ascocarp wall structure that is brightly coloured, changing to blood-red in 3 % KOH solution, warty to scaly and with a *Cylindrocladium* (*Cy.*) anamorph (Rossman 1993, Rossman *et al.* 1999). However, due to the restricted morphological characteristics of the teleomorph (Rossman 1979b, 1983), specimens can in many cases only be identified to species level if the anamorph is present (Schoch *et al.* 2000b, Crous 2002).

The anamorph genus *Cylindrocladium*, which is based on *Cy. scoparium,* was first described by Morgan (1892) in the U.S.A., where it was found growing as saprobe on a pod of *Gleditsia triacanthos*. Although Morgan (1892) failed to mention the stipe extension terminating in a vesicle of characteristic shape, he defined the genus as having branched conidiophores producing cylindrical conidia. This fungus has a wide distribution in sub-tropical and tropical regions of the world, and species are pathogenic to numerous plants (Crous 2002).

The aim of this review is to present an overview of published research on the genus *Calonectria* and their *Cylindrocladium* anamorphs. More specifically, the application of three types of species concepts is considered as they pertain to the taxonomic history of this genus. Although several species concepts (Mayden 1997) have been proposed, only the Morphological Species Concept (MSC), the Biological Species Concept (BSC) and the Phylogenetic Species Concept (PSC) are treated, as these have been most widely applied to *Calonectria*. Several reviews (Rossman 1996, Brasier 1997, Harrington & Rizzo 1999, Taylor *et al.* 1999, 2000, Seifert *et al.* 2000, Kohn 2005) have treated the various species concepts applied to the taxonomy of fungi and this topic is not treated other than in the manner in which it applies to *Calonectria.*

TAXONOMIC HISTORY

Calonectria resides in the *Nectriaceae*, one of three families in *Hypocreales*, an order that has been reviewed extensively (Rogerson 1970, Rossman 1983, Rossman *et al.* 1996, 1999). The *Nectriaceae* is circumscribed as having uniloculate ascomata that are orange to purple and not immersed in well-developed stromata (Rossman *et al.* 1999). The family includes approximately 20 genera of socio-economic importance and of these, *Calonectria* is most clearly distinguished from the others by its *Cylindrocladium* anamorphs and relevance as plant pathogens.

The first monograph of *Cylindrocladium* by Boedijn & Reitsma (1950), introduced seven *Cylindrocladium* species with one *Calonectria* connection. Later, in her treatment of *Calonectria*, Rossman (1983) recognised five species including the novel *Ca. ophiospora*. However, this species description did not include the anamorph state. The circumscribed type, *Ca. pyrochoa*, was also incorrectly reduced to synonymy with several other species based only on the teleomorph morphology. Peerally (1991a) highlighted this in a monograph of *Cylindrocladium*, where he regarded the anamorph morphology as important in distinguishing species of *Calonectria*. He subsequently recognised 10 *Calonectria* species with their *Cylindrocladium* anamorphs, including an additional 16 *Cylindrocladium* species not associated with a teleomorph. However, he mistakenly reduced *Cylindrocladiella*, a genus that accommodates *Cylindrocladium*-like species with small conidia (Boesewinkel 1982) and *Nectricladiella* teleomorphs, to synonymy with *Cylindrocladium* (Schoch *et al*. 2000b).

The monograph of *Cylindrocladium* by Crous & Wingfield (1994) entrenched the importance of anamorph characteristics in the taxonomy of *Calonectria* spp. In this monograph, 22

Cylindrocladium species and one variety were recognised, associated with 16 *Calonectria* species. Five species were assigned to the genus *Cylindrocladiella* based on morphological characters of the holomorph. The focus on anamorph characteristics is perpetuated in the most recent monograph (Crous 2002), which recognised 28 *Calonectria* species, all associated with *Cylindrocladium* anamorphs and an additional 18 *Cylindrocladium* species for which teleomorph states were not known. Of the latter group, seven taxa were of doubtful authenticity. Presently, 37 *Calonectria* and 52 *Cylindrocladium* species are recognised (Table 1; Crous 2002, Crous *et al.* 2004b, 2006a; Gadgil & Dick 2004, Lombard *et al.* 2009, 2010).

A general search on MycoBank (www.mycobank.org; Crous *et al.* 2004a, Robert *et al.* 2005) and Index Fungorum (www.indexfungorum.org) resulted in a total of 291 and 261 name records respectively for *Calonectria*. A similar search for *Cylindrocladium* species on both electronic databases indicated a total of 98 and 93 names respectively.

Table 1. List of recognised *Calonectria* species and their respective *Cylindrocladium* anamorphs.

Teleomorph	Reference	Anamorph	Reference
Calonectria acicola Gadgil & M.A. Dick	Gadgil & Dick 2004	*Cylindrocladium acicola* Gadgil & M.A. Dick	Gadgil & Dick 2004
Calonectria asiatica Crous & Hywel-Jones	Crous *et al.* 2004b	*Cylindrocladium asiaticum* Crous & Hywel-Jones	Crous *et al.* 2004b
Calonectria avesiculata T.S. Schub., Ell-Gholl, Alfieri & Schoult.	Schubert *et al.* 1989	*Cylindrocladium avesiculatum* D.L. Gill, Alfieri & Sobers	Gill *et al.* 1971
Calonectria brassicae (Panwar & Bohra) L. Lombard, M.J. Wingf. & Crous	Lombard *et al.* 2009		
Calonectria brachiatica L. Lombard, M.J. Wingf. & Crous	Lombard *et al.* 2009		
Calonectria cerciana L. Lombard, M.J. Wingf. & Crous	Lombard *et al.* 2010		
Calonectria clavata Alfieri, El-Gholl & E.L. Barnard	El-Gholl *et al.* 1993b	*Cylindrocladium flexuosum* Crous	Crous *et al.* 1995
Calonectria colhounii Peerally	Peerally 1973	*Cylindrocladium colhounii* Peerally	Peerally 1973
Calonectria colombiensis Crous	Crous *et al.* 2004b	*Cylindrocladium colombiense* Crous	Crous *et al.* 2004b
Calonectria gracilipes Crous & Mchau	Crous *et al.* 1997a	*Cylindrocladium graciloideum* Crous & Mchau	Crous *et al.* 1997a
Calonectria gracilis Crous, M.J. Wingf. & Alfenas	Crous *et al.* 1997b	*Cylindrocladium pseudogracile* Crous	Crous *et al.* 1997b
Calonectria hederae G. Arnaud ex C. Booth	Booth & Murray 1960	*Cylindrocladium hederae* G. Arnaud ex Peerally	Peerally 1991a
Calonectria hongkongensis Crous	Crous *et al.* 2004b	*Cylindrocladium hongkongense* Crous	Crous *et al.* 2004b
Calonectria ilicicola Boedijn & Reitsma	Boedijn & Reitsma 1950	*Cylindrocladium parasiticum* Crous, M.J. Wingf. & Alfenas	Crous *et al.* 1993d
Calonectria indusiata (Seaver) Crous	Crous 2002	*Cylindrocladium theae* (Petch) Subram	Alfieri *et al.* 1972
Calonectria insularis C.L. Schoch & Crous	Schoch *et al.* 1999	*Cylindrocladium insulare* C.L. Schoch & Crous	Schoch *et al.* 1999
Calonectria kyotensis Terash.	Terashita 1968	*Cylindrocladium floridanum* Sobers & C.P. Seym.	Sobers & Seymour 1967
Calonectria leguminum (Rehm) Crous	Crous 2002	*Cylindrocladium leguminum* Crous	Crous 2002
Calonectria macroconidialis (Crous, M.J. Wingf. & Alfenas) Crous	Crous *et al.* 1999	*Cylindrocladium macroconidiale* (Crous, M.J. Wingf. & Alfenas) Crous	Crous *et al.* 1999
Calonectria madagascariensis Crous	Crous 2002	*Cylindrocladium madagascariense* Crous	Crous 2002
Calonectria mexicana C.L. Schoch & Crous	Schoch *et al.* 1999	*Cylindrocladium mexicanum* C.L. Schoch & Crous	Schoch *et al.* 1999
Calonectria morganii Crous, Alfenas & M.J. Wingf.	Crous *et al.* 1993a	*Cylindrocladium scoparium* Morgan	Morgan 1892
Calonectria multiseptata Crous & M.J. Wingf.	Crous *et al.* 1998b	*Cylindrocladium multiseptatum* Crous & M.J. Wingf.	Crous *et al.* 1998b
Calonectria naviculata Crous & M.J. Wingf.	Crous *et al.* 1994	*Cylindrocladium naviculatum* Crous & M.J. Wingf.	Crous *et al.* 1994
Calonectria ovata D. Victor & Crous	Victor *et al.* 1997	*Cylindrocladium ovatum* El-Gholl , Alfenas, Crous & T.S. Schub.	El-Gholl *et al.* 1993a
Calonectria pauciramosa C.L. Schoch & Crous	Schoch *et al.* 1999	*Cylindrocladium pauciramosum* C.L. Schoch & Crous	Schoch *et al.* 1999
Calonectria pseudoreteaudii L. Lombard, M.J. Wingf. & Crous	Lombard *et al.* 2010		
Calonectria pseudospathiphylli J.C. Kang, Crous & C.L. Schoch	Kang *et al.* 2001b	*Cylindrocladium pseudospathiphylli* J.C. Kang, Crous & C.L. Schoch	Kang *et al.* 2001b
Calonectria pteridis Crous, M.J. Wingf. & Alfenas	Crous *et al.* 1993c	*Cylindrocladium pteridis* F.A. Wolf	Wolf 1926
Calonectria pyrochroa (Desm.) Sacc.	Rossman 1979a	*Cylindrocladium ilicicola* (Hawley) Boedijn & Reitsma	Boedjin & Reitsma 1950
Calonectria queenslandica L. Lombard, M.J. Wingf. & Crous	Lombard *et al.* 2010		
Calonectria reteaudii (Bugnic.) C. Booth	Booth 1966	*Cylindrocladium reteaudii* (Bugnic.) Boesew.	Boesewinkel 1982

Table 1. (Continued).

Teleomorph	Reference	Anamorph	Reference
Calonectria rumohrae El-Gholl & Alfenas	El-Gholl *et al.* 1997	*Cylindrocladium rumohrae* El-Gholl & Alfenas	El-Gholl *et al.* 1997
Calonectria scoparia Ribeiro & Matsuoka ex Peerally	Peerally 1991a	*Cylindrocladium candelabrum* Viégas	Crous 2002
Calonectria spathiphylli El-Gholl, J.Y. Uchida, Alfenas, T.S. Schub., Alfieri & A.R. Chase	El-Gholl *et al.* 1992	*Cylindrocladium spathiphylli* Schoult., El-Gholl & Alfieri	Schoulties *et al.* 1982
Calonectria spathulata El-Gholl, Kimbr., E.L. Barnard, Alfieri & Schoult.	Crous & Wingfield 1994	*Cylindrocladium spathulatum* El-Gholl, Kimbr., E.L. Barnard, Alfieri & Schoult.	Crous & Wingfield 1994
Calonectria terrae-reginae L. Lombard, M.J. Wingf. & Crous	Lombard *et al.* 2010		
Calonectria variabilis Crous, B.J.H. Janse, D. Victor, G.F. Marais & Alfenas	Crous *et al.* 1993b	*Cylindrocladium variabile* Crous, B.J.H. Janse, D. Victor, G.F. Marais & Alfenas	Crous *et al.* 1993b
		Cylindrocladium angustatum Crous & El-Gholl	Crous *et al.* 2000
		Cylindrocladium australiense Crous & K.D. Hyde	Crous *et al.* 2006a
		Cylindrocladium canadense J.C. Kang, Crous & C.L. Schoch	Kang *et al.* 2001b
		Cylindrocladium chinense Crous	Crous *et al.* 2004b
		Cylindrocladium citri (H.S. Fawc. & Klotz) Boedijn & Reitsma	Boedjin & Reitsma 1950
		Cylindrocladium curvatum Boedijn & Reitsma	Boedjin & Reitsma 1950
		Cylindrocladium curvisporum Crous & D. Victor	Victor *et al.* 1997
		Cylindrocladium ecuadoriae Crous & M.J. Wingf.	Crous *et al.* 2006a
		Cylindrocladium gordoniae Leahy, T.S. Schub. & El-Gholl	Leahy *et al.* 2000
		Cylindrocladium hawksworthii Peerally	Peerally 1991b
		Cylindrocladium hurae (Linder & Whetzel) Crous	Crous 2002
		Cylindrocladium indonesiae Crous	Crous *et al.* 2004b
		Cylindrocladium leucothoës El-Gholl, Leahy & T.S. Schub.	El-Gholl *et al.* 1989
		Cylindrocladium malesianum Crous	Crous *et al.* 2004b
		Cylindrocladium multiphialidicum Crous, Simoneau & Risède	Crous *et al.* 2004b
		Cylindrocladium pacificum J.C. Kang, Crous & C.L. Schoch	Kang *et al.* 2001b
		Cylindrocladium penicilloides (Tubaki) Tubaki	Tubaki 1958
		Cylindrocladium pseudonaviculatum Crous, J.Z. Groenew. & C.F. Hill	Crous *et al.* 2002
		Cylindrocladium sumatrense Crous	Crous *et al.* 2004b

NOMENCLATURE OF *CALONECTRIA*

The nomenclature of pleomorphic fungi has been a topic of substantial debate during the course of the past two decades (Gams 1991, Cannon & Kirk 2000, Hawksworth 2004, 2005). The separate naming of anamorphs (mitotic morphs) and teleomorphs (meiotic morphs) has resulted in confusion, especially for non-taxonomists (Cannon & Kirk 2000). This is especially evident where teleomorph species epithets are different to those of their anamorphs and also where more than one anamorph (synanamorph) is found. The naming of fungal morphs based on the International Code of Botanical Nomenclature (ICBN; McNeill *et al.* 2005) and in particular following strict interpretation of Article 59 of the Code has now been unsatisfactory for many fungal groups due to our ability to connect morphs using molecular evidence, and there are increasing calls for further changes to be made.

Recent alterations to the Code at the ICBN meeting in Vienna allows for anamorphic fungi to be named in teleomorph genera, but these are vulnerable to be superseded by a connected teleomorph name in the future (Hawksworth 2004, McNeill *et al.* 2005, P. Cannon pers. comm.). Although there are several *Cylindrocladium* species without *Calonectria* connections (Crous 2002, Crous *et al.* 2004b, 2006a), we believe that new species should be described in *Calonectria* irrespective of whether a teleomorph is known or not. This follows a clear view based on phylogenetic inference that *Cylindrocladium* spp. all have *Calonectria* states (Schoch *et al.* 1999, 2000a, 2000b, Crous 2002, Crous *et al.* 2004b, 2006a). Following the approach of Crous *et al.* (2006b, 2008, 2009a, b) with other fungal groups, Lombard *et al.* (2009, 2010) recently described five new species in the genus *Calonectria*, irrespective whether the teleomorph was observed or not. Thus, for taxonomic purposes, *Cylindrocladium* species with known teleomorph states are referred to as *Calonectria* in this review.

IMPORTANCE OF *CALONECTRIA*

The genus *Calonectria* was initially regarded as a saprobe as no disease symptoms could be induced by inoculating a suspected host (Graves 1915). The first proof of pathogenicity of these fungi was provided by Massey (1917), and subsequently by Anderson (1919), who proved pathogenicity of *Ca. morganii* (as *Cy. scoparium*). Subsequently, *Calonectria* species have been associated with a wide range of disease symptoms on a large number of hosts worldwide (Crous 2002; Table 2; Figs 1–2). In the past, several authors have indicated that *Calonectria* species cause disease on plants residing in approximately 30 plant families (Booth & Gibson 1973, French & Menge 1978, Peerally 1991a, Wiapara *et al.* 1996, Schoch *et al.* 1999). Upon closer inspection, the number of plant families is actually closer to 100 (Table 2) and approximately 335 plant host species (Crous 2002). The plant hosts include important forestry, agricultural and horticultural crops and the impact of these plant pathogens has likely been underestimated.

The majority of disease reports associated with *Calonectria* species in forestry include hosts in five plant families, of which the most important are associated with *Fabaceae* (*Acacia* spp.), *Myrtaceae* (*Eucalyptus* spp.) and *Pinaceae* (*Pinus* spp.). Disease symptoms (Figs 1–2) include cutting rot (Crous *et al.* 1991, Crous 2002, Lombard *et al.* 2009, 2010), damping-off (Batista 1951, Cox 1953, Terashita & Itô 1956, Sharma & Mohanan 1982, Sharma *et al.* 1984, Crous *et al.* 1991, Brown & Ferreira 2000, Crous 2002, Taniguchi *et al.* 2008) leaf diseases (Cox 1953, Hodges & May 1972, Barnard 1984, Sharma *et al.* 1984, El-Gholl *et al.* 1986, Peerally *et al.* 1991a, Crous *et al.* 1993b, Crous & Wingfield 1994, Crous *et al.* 1998b, Schoch & Crous 1999, Schoch *et al.* 1999, Booth *et al.* 2000, Park *et al.* 2000, Crous & Kang 2001, Gadgil & Dick 2004), shoot blight (Sharma *et al.* 1984, Crous *et al.* 1991, 1998b, Crous & Kang 2001), stem cankers (Cox 1953, Sharma *et al.* 1984, 1985, Crous *et al.* 1991, Lombard *et al.* 2009) and root rot (Cox 1953, Hodges & May 1972, Cordell & Skilling 1975, Mohanan & Sharma 1985, Crous *et al.* 1991, Lombard *et al.* 2009). The majority of these diseases is associated with seedling and cutting production in forestry nurseries, but in a few cases *Cylindrocladium* species have also been reported from older, established commercial plantations. In these cases the pathogens have been reported to cause leaf diseases and shoot blight resulting in defoliation of trees leading to loss of vigour (Hodges & May 1972, Sharma *et al.* 1985, Booth *et al.* 2000, Park *et al.* 2000, Crous & Kang 2001, Crous 2002, Old *et al.* 2003, Rodas *et al.* 2005).

In agriculture, *Calonectria* species have been reported to cause diseases on several economically important crops. Several plant families of agricultural importance are susceptible to *Calonectria* infections, including *Fabaceae* and *Solanaceae*. Important diseases in these families are Cylindrocladium black rot of *Arachis hypogea* (peanut) and red crown rot of *Glycine max* (soybean) caused by *Ca. ilicicola* and *Ca. pyrochroa* in the USA (Bell & Sobers 1966, Beute & Rowe 1973, Rowe *et al.* 1973, Sobers & Littrell 1974, Rowe & Beute 1975, Phipps *et al.* 1976, Johnson 1985, Dianese *et al.* 1986, Berner *et al.* 1988, 1991, Culbreath *et al.* 1991, Porter *et al.* 1991, de Varon 1991, Hollowell *et al.* 1998, Kim *et al* 1998) and

Table 2. Plant families that are host to *Calonectria* species and number of known plant host species in each family (Crous 2002).

Host Plant family	Host species	Host Plant family	Host species	Host Plant family	Host species	Host Plant family	Host species
Actinidiaceae	2	*Cornaceae*	1	*Malipighiaceae*	2	*Pteridaceae*	1
Altingiaceae	1	*Crassulaceae*	1	*Malvaceae*	6	*Rhamnaceae*	1
Anacardiaceae	3	*Cupressaceae*	4	*Meliaceae*	2	*Rhizophoraceae*	1
Annonaceae	4	*Curcurbitaceae*	3	*Moraceae*	2	*Rosaceae*	10
Aparagaceae	1	*Cycadaceae*	1	*Musaceae*	2	*Rubiaceae*	2
Apiaceae	1	*Davalliaceae*	1	*Myristicaceae*	1	*Ruscaceae*	1
Apocynaceae	2	*Dennstaedtiaceae*	1	*Myrsinaceae*	1	*Rutaceae*	3
Aquifoliaceae	4	*Dilleniaceae*	1	*Myrtaceae*	31	*Salicaceae*	3
Araceae	5	*Dipterocarpaceae*	1	*Nelumbonaceae*	1	*Sapindaceae*	4
Araliaceae	2	*Dryopteridaceae*	2	*Nepenthaceae*	1	*Sapotaceae*	3
Arecaceae	21	*Ebenaceae*	1	*Nothofagaceae*	1	*Sarraceniaceae*	1
Araucariaceae	2	*Ericaceae*	14	*Nymphaeaceae*	1	*Saxifragaceae*	1
Aspleniaceae	1	*Euphorbiaceae*	6	*Oleaceae*	1	*Solanaceae*	4
Asteraceae	5	*Fabaceae*	57	*Onagraceae*	2	*Sterculiaceae*	2
Berberidaceae	2	*Fagaceae*	4	*Orchidaceae*	1	*Strelilziaceae*	2
Betulaceae	1	*Ginkgoaceae*	1	*Phytolaccaceae*	1	*Theaceae*	1
Bixaceae	1	*Juglandaceae*	2	*Pinaceae*	17	*Ulmaceae*	1
Bromeliaceae	3	*Lauraceae*	6	*Piperaceae*	1	*Verbenaceae*	1
Buxaceae	1	*Laxmanniaceae*	1	*Platanaceae*	1	*Vitaceae*	2
Caricaceae	2	*Lecythidaceae*	1	*Plumbaginaceae*	1	*Vochysiaceae*	1
Caryophyllaceae	1	*Leeaceae*	1	*Poaceae*	6	*Xanthorrhoeaceae*	1
Celastraceae	1	*Linaceae*	1	*Polygalaceae*	1	*Zingiberaceae*	1
Chenopodiaceae	1	*Lomariopsidaceae*	1	*Polygonaceae*	3		
Combretaceae	3	*Lythraceae*	1	*Polypodiaceae*	1		
Convolvulaceae	1	*Magnoliaceae*	2	*Proteaceae*	7		

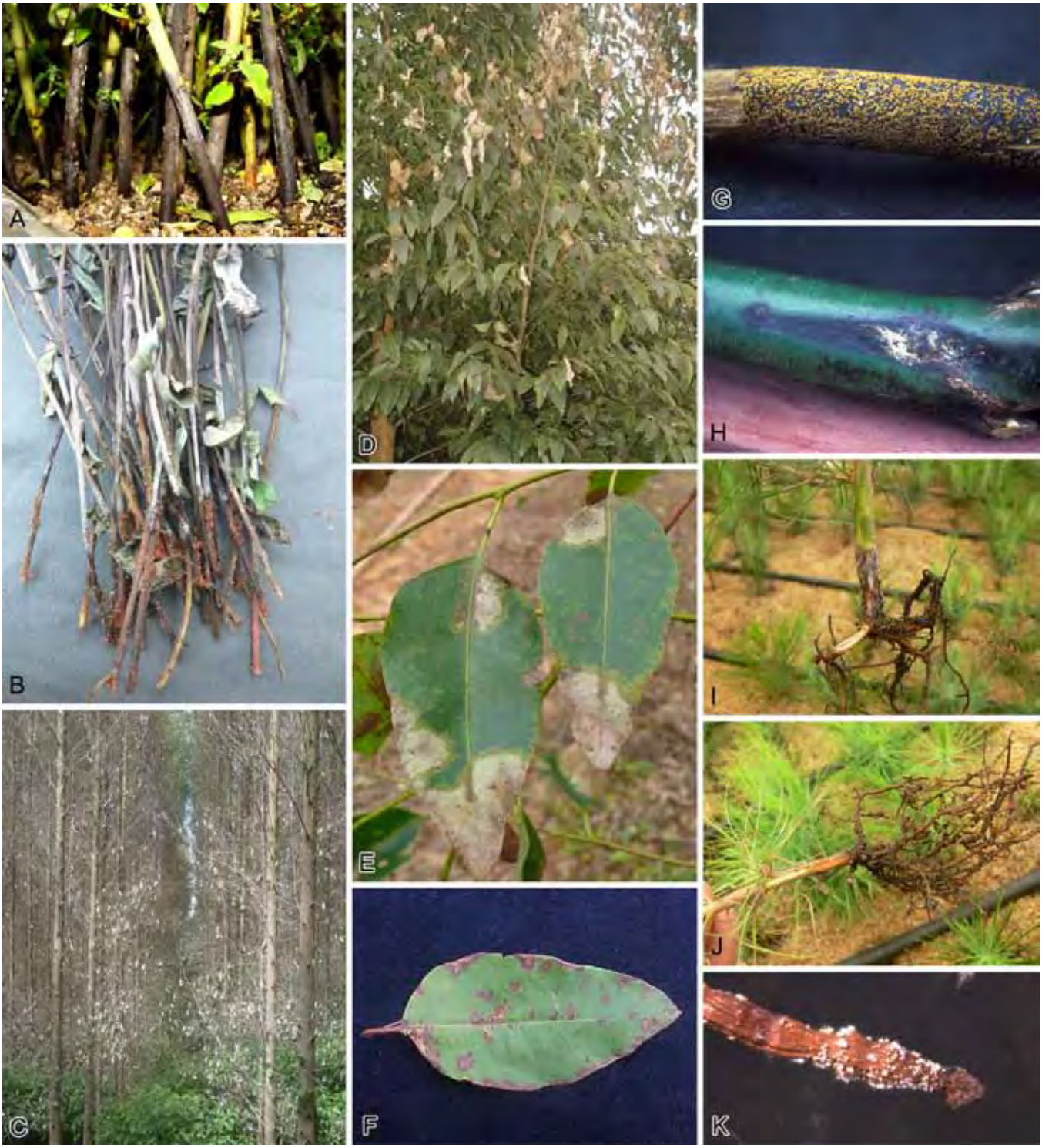

Fig. 1. Disease symptoms associated with *Calonectria* (*Cylindrocladium*). A. Cutting rot of *Vallea stipolaris*. B. Cutting rot of *Eucalyptus* sp. C. Defoliated *Eucalyptus* trees in a plantation. D. Leaf and shoot blight of a *Eucalyptus* sp. E. Cylindrocladium leaf blight of a *Eucalyptus* sp. F. Leaf spots on a *Eucalyptus* sp. G–H. Stem cankers on twigs of a *Eucalyptus* sp. I–J. Root and collar rot of *Pinus* spp. K. Root rot of *Eucalyptus* sp. with conidiophores on the root surface.

Cylindrocladium tuber rot of *Solanum tuberosum* (potato) (Boedijn & Reitsma 1950, Bolkan *et al.* 1980, 1981) by *Ca. brassicae* (as *Cy. gracile*) in Brazil. Other diseases associated with *Calonectria* species on agricultural crops include root rot and leaf diseases of fruit bearing and spice plants (Jauch 1943, Wormald 1944, Sobers & Seymour 1967, Nishijima & Aragaki 1973, Milholland 1974, Krausz & Caldwell 1987, Hutton & Sanewski 1989, Anandaraj & Sarma 1992, Risède 1994, Jayasinghe & Wijesundera 1996, Risède & Simoneau 2001, Vitale & Polizzi 2008), post-harvest diseases of fruits (Fawcett & Klotz 1937, Boedijn & Reitsma 1950, Sepiah 1990, Fitzell & Peak 1992, Vaidya & Roa 1992, Sivapalan *et al.* 1998), root and crown rot of *Medicago sativa* (alfalfa) (Ooka & Uchida 1982, Hwang & Flores 1987), and sheath net blotch of *Oryza sativa* (rice) (Crous 2002).

On horticultural crops, *Calonectria* species have been reported mostly from the Northern Hemisphere, especially in gardens and ornamental commercial nurseries in Europe and Asia (Polizzi & Crous 1999, Polizzi 2000, Crous 2002, Henricot & Culham 2002, Pérez-Sierra *et al.* 2007, Polizzi *et al.* 2007a, b, Hirooka *et al.* 2008, Polizzi *et al.* 2009, Vitale *et al.* 2009). Hosts in this sector

include ornamental trees, shrubs and cut-flowers in several plant families, most commonly in *Arecaceae*, *Asteraceae*, *Ericaceae* and *Rosaceae*. A wide range of disease symptoms are recorded including crown-, collar- and root rot, leaf spots, and cutting rot (Massey 1917, Anderson 1919, Aragaki *et al.* 1972, 1988, Peerally 1991b, Uchida & Kadooka 1997, Polizzi & Crous 1999, Polizzi 2000, Crous 2002, Henricot & Culham 2002, Henricot & Beales 2003, Poltronieri *et al.* 2004, Lane *et al.* 2006, Pérez-Sierra *et al.* 2006, 2007, Polizzi *et al.* 2006a, b, 2007a, b, Vitale & Polizzi 2007, Aghajani *et al.* 2008, Hirooka *et al.* 2008, Vitale *et al*. 2008, Polizzi *et al.* 2009, Vitale *et al.* 2009).

MORPHOLOGY

Morphological or phenotypic characters have played a major role in the description of fungal species (Brasier 1997, Taylor *et. al.* 2000) and form the basis of new fungal descriptions as required by the ICBN (McNeill *et al.* 2005). In recent years, the use of morphological characters alone to delimit new species has been set aside to a large extent, with more focus being placed on biological and phylogenetic characters (Rossman 1996, Brasier 1997, Taylor *et al.* 2000). This trend is also evident in recent studies on *Calonectria* species (Crous *et al.* 2004b, 2006a).

The morphology of *Calonectria* and to a greater extent its anamorph, *Cylindrocladium*, has been important in the taxonomic history of these fungi. Prior to the 1990s, identification of species was based on morphological characteristics and to a lesser extent on sexual compatibility using standardised media (Boedijn & Reitsma 1950, Peerally 1991a, Crous *et al.* 1992, Crous & Wingfield 1994, Crous 2002). This resulted in the establishment of several species complexes, as many *Cylindrocladium* species are morphologically very similar. These include the *Ca. scoparia* complex (Schoch *et al.* 1999), *Ca. brassicae* (as *Cy. gracile*) complex (Crous *et al.* 2004b) and *Ca. kyotensis* complex (Crous *et al.* 2006a). Characteristics of the anamorphs that are extensively employed in identifications include vesicle shape, stipe extension length and macroconidial septation and dimensions (Fig. 3) (Boesewinkel 1982, Peerally 1991a, Crous & Wingfield 1994, Crous 2002). The morphological characteristics of the teleomorph (Fig. 4) that are important for identifications are ascospore septation and dimensions, ascospore number within the asci and perithecial colour. Perithecia of *Calonectria* species are morphologically very similar and these are not typically useful in identifications (Crous & Wingfield 1994, Crous 2002).

The use of biochemical techniques can also be used in phenotypic characterisation. These include substrate utilisation and cell wall polysaccharide analysis. The use of aminopeptidase specificity (Stevens *et al*. 1990) and utilisation of nitrogen and carbon (Hunter & Barnett 1978, Sharma *et al*. 1992) have been used successfully to separate several *Cylindrocladium* species. The use of polysaccharides obtained from cell walls of *Cylindrocladium* positively identified linkages between asexual species and their respective *Calonectria* teleomorphs (Ahrazem *et al*. 1997). However, this method has been found to have limited value as some species in complexes could not be distinguished (Crous 2002).

MATING COMPATIBILITY

Mating strategies have been employed in the taxonomy of *Calonectria* and have played an important role in identifying new species of the genus (Schoch *et al.* 1999, Crous 2002). Based on these studies, there are approximately 18 homothallic and 34 heterothallic species of *Calonectria* (Crous 2002, Crous *et al.* 2004b, Gadgil & Dick 2004, Crous *et al.* 2006a), with the heterothallic species showing a biallelic mating system (Schoch *et al.* 1999). Studies in the female fertility of *Cylindrocladium* by Schoch *et al.* (1999, 2000a, 2001a) have also shown that several species are self-sterile hermaphrodites requiring fertilisation from an opposite mating type. This is typical of heterothallic ascomycetes (Leslie & Klein 1996).

Several difficulties associated with applying the BSC have been highlighted (Brasier 1997, Taylor *et al.* 1999, 2000, Kohn 2005). The most relevant underlying problem occurs where genetically isolated fungal strains retain the ancestral ability to recombine to produce viable progeny (Brasier 1997). This phenomenon has also been found with several phylogenetic species that are closely related in *Calonectria*. Crous (2002), for example, showed that *Cy. hawksworthii*, *Ca. insularis* and *Ca. morganii* were capable of recombining, but that the progeny had low levels of fertility. Other mating studies done by Overmeyer *et al.* (1996) and Neubauer & Zinkernagel (1995) have found that induction of fertile perithecia requires the presence of an additional isolate that, however, does not contribute to the genetic make-up of the progeny. This clearly highlights the need for further studies regarding the mechanism of perithecial formation and recombination in *Calonectria*.

PHYLOGENY

Phylogenetic studies on *Calonectria* and its *Cylindrocladium* anamorphs have substantially influenced the taxonomy of these genera. Application of molecular techniques and particularly DNA sequence comparisons to distinguish between species has resulted in the recognition of numerous cryptic species. Several molecular approaches have been employed that include total protein electrophoresis (Crous *et al.* 1993a, El-Gholl *et al.* 1993a), isozyme electrophoresis (El-Gholl *et al.* 1992, 1997, Crous *et al.* 1998a), random amplification of polymorphic DNA (RAPD) (Overmeyer *et al.* 1996, Victor *et al.* 1997, Schoch *et al.* 2000a, Risède & Simoneau 2004) restriction fragment length polymorphisms (RFLP) (Crous *et al.* 1993b, 1995, 1997b, Jeng *et al.* 1997, Victor *et al.* 1997; Risède & Simoneau 2001) and DNA hybridisation (Crous *et al.* 1993b, 1995, 1997a, Victor *et al.* 1997). Although the above-mentioned techniques have been useful, DNA sequence comparisons and associated phylogenetic inference have had the most dramatic impact on the taxonomy of *Calonectria* and are most widely applied today.

In the first study using 5.8S ribosomal RNA gene and flanking internally transcribed spacers (ITS) sequences Jeng *et al.* (1997) were able to distinguish between *Cy. scoparium* and *Cy. floridanum* isolates. Subsequently, it was found that this gene region contains few informative characters (Crous *et al.* 1999, Schoch *et al.* 1999, Risède & Simoneau 2001, Schoch *et al.* 2001b). Therefore, the β-tubulin (Schoch *et al.* 2001b) and histone H3 (Kang *et al.* 2001a) gene regions have been applied in order to allow for improved resolution in separating species.

The first complete DNA sequence-based phylogenetic study using partial β-tubulin gene sequences (Schoch *et al.* 2001b)

Fig. 2. Disease symptoms associated with *Calonectria* (*Cylindrocladium*). A–D. Defoliation and yellowing associated with *Calonectria pseudonaviculata* infection on *Buxus* sp. at Paleis Het Loo in the Netherlands (upper part of hedge in A, arrows). B–D. Leaf yellowing and defoliation (note detaching leaves in D, arrows). E–H. *Calonectria ilicicola* causing Cylindrocladium black rot (CBR) on *Arachis hypogaea* in Georgia, U.S.A. F. Perithecia forming at the basal plant parts. G. Pods infected with tomato spotted wilt virus (left), healthy pods (middle), and pods infected with CBR (right). H. Field symptoms associated with CBR (photos with permission of T. Brenneman). I. Avocado roots infected with *Ca. ilicicola* (photo with permission of L. Forsberg). J. Seeding blight of *Callistemon citrinus* associated with *Ca. morganii* (photo with permission of G. Polizzi). K. Seedling rot of *Drosera* sp. associated with *Ca. pteridis* infection. L. Leaf spots of *Callistemon citrinus* associated with *Ca. pauciramosa* (photo with permission of G. Polizzi). M. *Arbutus unedo* associated with *Ca. pauciramosa* infection (photo with permission of G. Polizzi). N–O. Root rot and petiole lesions of *Spathiphyllum* sp. associated with *Ca. spathiphylli* infection (photo with permission from the late N.E. El-Gholl). P. Potato tuber infected with *Ca. brassicae*. Q–R. Leaf blight of *Eucalyptus* sp. associated with a mixed infection of *Ca. pteridis* and *Ca. ovata*.

Fig. 3. Anamorph structures of *Calonectria*. A. Macroconidiophore of *Ca. pauciramosa*. B. Macroconidiophore of *Ca. hongkongensis*. C. Macroconidiophore of *Ca. brassicae*. D. Clavate vesicle of *Ca. reteaudii*. E. Obpyriform vesicle of *Ca. pauciramosa*. F. Sphaeropedunculate vesicle of *Ca. hongkongensis*. G. Pyriform vesicle of *Ca. morganii*. H. Fertile branches of *Ca. pauciramosa* with doliiform to reniform phialides. I. Fertile branches of a *Calonectria* sp. with elongate-doliiform to reniform phialides. J. Fertile branches of *Ca. reteaudii* with cylindrical to allantoid phialides. K. One-septate macroconidia of *Ca. pauciramosa*. L. Three-septate macroconidia of *Ca. colhounii*. M–N. Five to eight-septate macroconidia of *Ca. reteaudii*. O–P. Microconidiophores of *Ca. reteaudii*. Q. three-septate microconidium of *Ca. reteaudii*. Scale bars: B–C, M = 50 µm; A, O–P = 20 µm; D–L, N, Q = 10 µm.

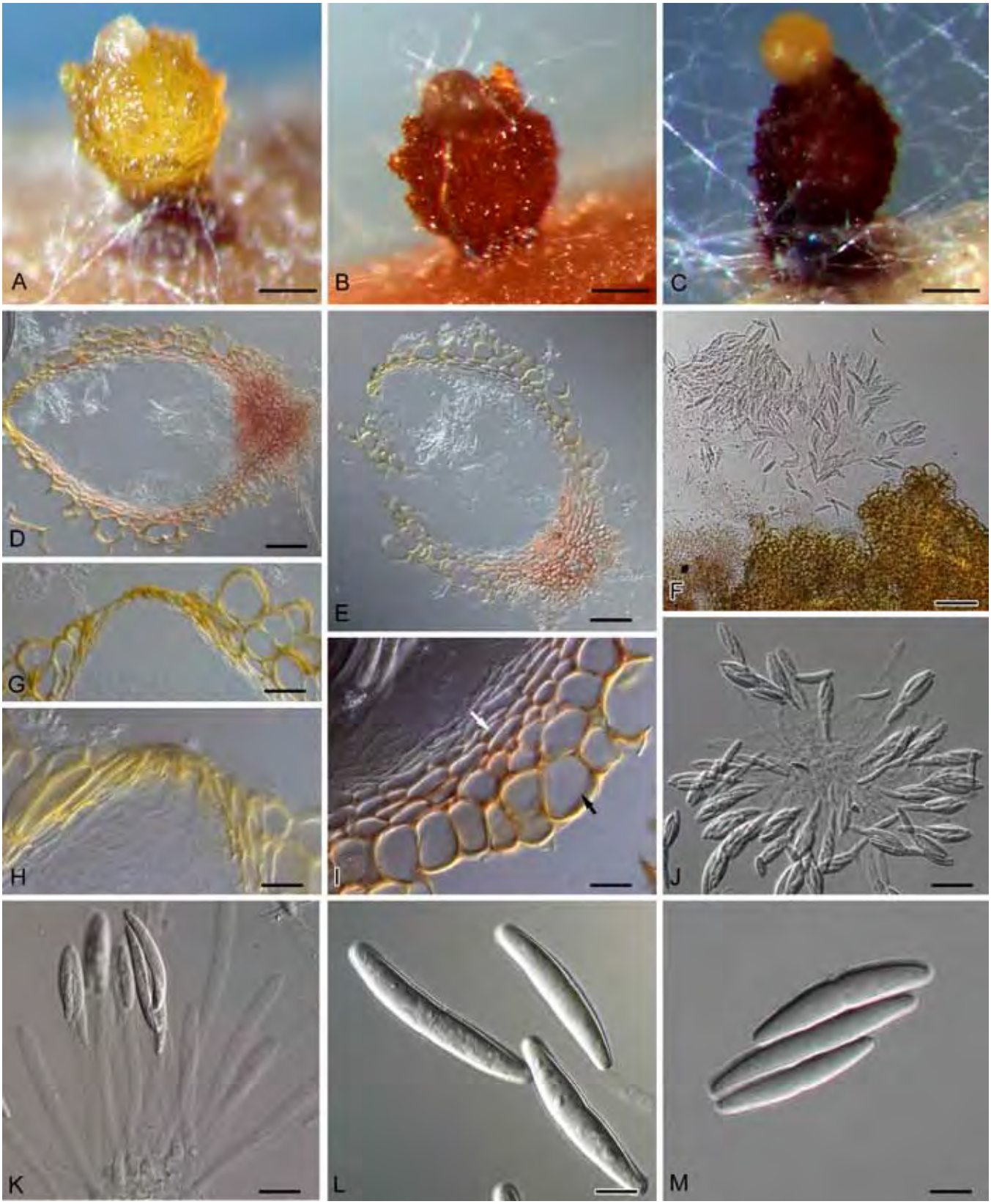

Fig. 4. Teleomorph structures of *Calonectria* spp. A. Yellow perithecium of *Ca. colhounii*. B. Orange to red perithecium of *Ca. pauciramosa*. C. Dark red perithecium of *Calonectria* sp. D–E. Vertical sections through perithecia. F. Squashed perithecium exuding ascospores. G–H. Ostiolar regions of perithecia. I. Vertical section through the wall of a perithecium showing the *textura globulosa* (black arrow) and *textura angularis* (white arrow) wall layers. J. Asci containing eight ascospores. K. Asci containing four ascospores. L–M. One-septate ascospores. Scale bars: A–C = 100 µm; F = 50 µm; J–K = 20 µm; D–E, G–I, L–M = 10 µm.

compared phenotypic, biological and phylogenetic concepts used in the taxonomy of *Cylindrocladium*. This also highlighted the fact that *Calonectria* represents a monophyletic lineage (Schoch *et al*. 2000b, 2001b). Subsequently, combined DNA sequence data for the ITS, β-tubulin and histone H3 gene regions have been widely used in studies relating to taxonomic issues surrounding *Cylindrocladium* and *Calonectria* (Crous *et al.* 1999, Schoch *et al*. 2000a, 2000b, Crous & Kang 2001, Kang *et al.* 2001a, 2001b, Henricot & Culham 2002, Crous *et al.* 2004b, 2006a, Lombard *et al.* 2009, 2010). Other partial gene sequences recently used include

translation elongation 1-alpha (TEF-1α) and calmodulin (Crous *et al.* 2004b, Lombard *et al.* 2010). However, insufficient data are currently available for these gene regions on GenBank (www.ncbi.nlm.nih.gov) to make them particularly valuable for comparative analysis.

A recent search in GenBank (March 2010) revealed a total of 734 partial gene sequences for *Calonectria* and *Cylindrocladium*. These include 311 for β-tubulin, 177 for histone H3, 159 for ITS, 39 for calmodulin, 36 for TEF-1α, five for large subunit RNA gene (LSU), three each for the high mobility group (HMG) box and peptidase synthetase and one for the small subunit RNA (SSU) gene. For *Cylindrocladium* and *Calonectria*, there are only six studies (Kang *et al.* 2001a, 2001b; Crous *et al.* 2004b, 2006a, Lombard *et al.* 2009, 2010) that provide files on TreeBase (www.treebase.org).

FUTURE RESEARCH

Population biology

Most studies on *Calonectria* have focused on the taxonomy, phylogeny and pathology of species. There have in contrast been relatively few studies treating the population biology of these fungi. This is unfortunate as population dynamics contributes considerable knowledge to a better understanding of population structure, distribution of genetic diversity, gene flow, centres of origin and mating strategies (McDonald 1997, Linde *et al.* 2002, Grünwald *et al.* 2003). An understanding of the population dynamics of *Calonectria* would contribute in determining the natural spread of these fungi as well as assist in phytosanitary and quarantine regulations. Another important aspect surrounding knowledge of *Calonectria* population dynamics is that this would contribute to plant breeding programmes and thus control of the many diseases that are caused by these fungi (McDonald 1997, Wright *et al.* 2006, 2007).

Limited research has been conducted on the population dynamics of *Calonectria*. To date only two studies (Wright *et al.* 2006, 2007) have reported on the development of polymorphic markers to characterise simple sequence repeats (SSRs) in loci of *Ca. ilicicola* (Wright *et al.* 2006) and *Ca. pauciramosa* (Wright *et al.* 2007). However, no study has yet been published on the population biology of either of these important pathogens using these markers. There is clearly a gap in this area of research concerning *Calonectria* spp. and future research on this topic should be encouraged.

Whole genome sequences

A relatively new and innovative technology employed in fungal genetics is the use of whole genome sequences of filamentous fungi. Whole genome sequencing has become relatively inexpensive and thus common in recent years. This revolutionary technology will promote our understanding of the mechanisms of gene function, conidiation, pathogenesis and sexual reproduction at the genotype level (Kupfer *et al.* 1997, Prade 1998, Yoder & Turgeon 2001, Foster *et al.* 2006, Cuomo *et al.* 2007). It is estimated that most filamentous fungi have a genome size of 30 to 40 Mb, containing approximately 8000 to 9000 genes (Kupfer *et al.* 1997, Prade 1998, Foster *et al.* 2006). There are currently several completed fungal genome sequences (http://www.broad.mit.edu/annotation/fungi/fgi/, Foster *et al.* 2006, Baker *et al.* 2008), including the model yeast *Saccharomyces cerevisiae* (Goffeau *et al.* 1996), plant pathogens and spoilage fungi such as *Aspergillus flavus* (Payne *et al.* 2006), *Fusarium graminearum* (http://www.broad.mit.edu, Cuomo *et al.* 2007), *Magnaporthe grisea* (Dean *et al.* 2005) and the model filamentous fungus *Neurospora crassa* (Galagan *et al.* 2003). Although there are currently over 300 ongoing filamentous fungal genome sequencing projects (http://www.genomesonline.org, Baker *et al.* 2008, Liolios *et al.* 2008), none include species of *Calonectria*.

The most closely related plant pathogen to *Calonectria* species currently being sequenced is *Haematonectria haematococca* (http://www.ncbi.nlm.gov). When the first *Calonectria* species is selected for whole genome sequencing, comparisons with *H. haematococca* could help to identify important genes in pathogenesis and sexual reproduction. Some *Calonectria* species that could be considered for genome sequencing include *Ca. pauciramosa*, based on its pathogenicity and importance on several plant hosts worldwide (Crous 2002), and *Ca. reteaudii*, one of the most important forest pathogens of South East Asia (Booth *et al.* 2000, Old *et al.* 2003).

CONCLUSIONS

Early studies on the taxonomy of *Calonectria* and *Cylindrocladium* focused on the use of MSC in combination with BSC. More recently, the wide availability of molecular techniques and particularly DNA sequence data have revolutionised the taxonomy of *Calonectria* and *Cylindrocladium.* Today, it is well accepted that the morphology of the *Cylindrocladium* state contributes most information to naming species and that these fungi all reside in *Calonectria*.

The first study to combine MSC, BSC and PSC concepts by Schoch *et al.* (1999) resulted in the identification of four species within a single species complex. Subsequently, several studies including the MSC, BSC and PSC have elucidated cryptic species in the genus (Kang *et al.* 2001a, 2001b, Henricot & Culham 2002, Crous *et al.* 2004b, 2006a, Lombard *et al.* 2009, 2010). Application of the BSC in the taxonomy of *Calonectria* has been found to be unreliable in some instances (Crous 2002). However, the implementation of MSC and PSC in combination provides powerful tool for taxonomic studies of these genera and it is likely that this will continue in future studies. Although several species complexes have been identified in *Calonectria*, more research is needed on the population level in order to study the gene flow between populations. Additional to this, more gene regions need to be identified and widely used in PSC. With the identification of several new species since 2002, an updated monograph is required to facilitate ease of identification.

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Multigene phylogeny and mating tests reveal three cryptic species related to *Calonectria pauciramosa*

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Abstract: *Calonectria pauciramosa* is a pathogen of numerous plant hosts worldwide. Recent studies have indicated that it included cryptic species, some of which are identified in this study. Isolates from various geographical origins were collected and compared based on morphology, DNA sequence data of the β-tubulin, histone H3 and translation elongation factor-1α regions and mating compatibility. Comparisons of the DNA sequence data and mating compatibility revealed three new species. These included *Ca. colombiana* sp. nov. from Colombia, *Ca. polizzii* sp. nov. from Italy and *Ca. zuluensis* sp. nov. from South Africa, all of which had distinguishing morphological features. Based on DNA sequence data, *Ca. brasiliensis* is also elevated to species level.

Key words: *Calonectria*, plant pathogens, sexual compatibility, systematics.
Taxonomic novelties: *Calonectria brasiliensis* (Bat. & Cif.) L. Lombard, M.J. Wingf. & Crous, comb. nov., *Calonectria colombiana* L. Lombard, Crous & M.J. Wingf., sp. nov., *Calonectria polizzii* L. Lombard, Crous & M.J. Wingf., sp. nov., *Calonectria zuluensis* L. Lombard, Crous & M.J. Wingf., sp. nov.

INTRODUCTION

Several past studies have focused on the taxonomy of *Calonectria* spp. with small, 1-septate macroconidia and ellipsoidal to obpyriform vesicles (Crous *et al.* 1993, Overmeyer *et al.* 1996, Schoch *et al.* 1999, 2000). These *Calonectria* spp. were initially regarded as either *Ca. morganii* (= *Cylindrocladium scoparium*) or *Ca. scoparia* (= *Cy. candelabrum*) based on their morphological similarities. However, the anamorph state of *Ca. morganii* was circumscribed as having ellipsoidal to pyriform vesicles and *Ca. scoparia* having ellipsoidal to obpyriform vesicles by Crous *et al.* (1993). Later studies, incorporating DNA sequence data, have shown that *Ca. morganii* is restricted to the Northern Hemisphere and Brazil (Crous *et al.* 1993, Overmeyer *et al.* 1996, Schoch *et al.* 2000). In contrast, *Ca. scoparia* is found worldwide and forms part of a species complex consisting of four mating groups, each representing a different *Calonectria* species that includes *Ca. pauciramosa* (anamorph: *Cy. pauciramosum*), *Ca. scoparia*, *Ca. mexicana* (anamorph: *Cy. mexicanum*) and *Ca. insularis* (anamorph: *Cy. insulare*) (Schoch *et al.* 1999).

Calonectria pauciramosa has been reported worldwide on numerous plant hosts (Schoch *et al.* 1999, Koike *et al.* 1999, Koike & Crous 2001, Polizzi & Crous 1999, Polizzi 2000, Polizzi & Catara 2001, Polizzi & Vitale 2001, Crous 2002, Polizzi *et al.* 2006, 2007, 2009, Vitale *et al.* 2009), where it causes diseases such as cutting rot, damping-off, root rot and leaf blight. In South Africa and Australia, *Ca. pauciramosa* is regarded as the dominant pathogen in commercial forest nurseries (Crous 2002) and it is also found on various horticultural crops in commercial nurseries in Italy and the U.S.A. (Schoch *et al.* 2001, Crous 2002, Polizzi *et al.* 2006, 2007, 2009, Vitale *et al.* 2009).

Schoch *et al.* (2001) considered female fertility in populations of *Ca. pauciramosa* from various geographical regions to determine the ratio of mating types present, and based on these data suggested that *Ca. pauciramosa* was endemic to South America given that the ratio of both mating types approached 1:1. Furthermore, the study also indicated that *Ca. pauciramosa* isolates from California were represented by only one mating type, supporting the view that this represented an introduced pathogen. Isolates from Italy showed higher ratios of hermaphrodites and some variation was observed in the β-tubulin sequences. In contrast, South African isolates had close to a 1:1 mating type ratio and showed variation in β-tubulin sequence data (Schoch *et al.* 1999, 2001), indicating that this was either a native pathogen or that there had been multiple introductions into the country.

Initial investigations using DNA sequence comparisons and mating studies on *Ca. pauciramosa* isolates from South Africa and Colombia showed some variation amongst isolates. These findings and those of Schoch *et al.* (2001) suggested that *Ca. pauciramosa* might accommodate a number of cryptic species. The aim of this study was to consider the phylogenetic relationships, morphological characters and mating compatibility of available isolates of *Ca. pauciramosa* and to determine whether this species represented an assemblage of cryptic taxa.

MATERIALS AND METHODS

Isolates

Isolates of *Ca. pauciramosa* were obtained from culture collections (Table 1) or were isolated from infected plant material and soil

Table 1. Isolates of *Calonectria pauciramosa* and other *Calonectria* species studied.

			GenBank accession no.					
Species	**Isolate**	**Mating type**	**β-tubulin**	**Histone H3**	**Translation elongation factor-1α**	**Host**	**Country**	**Collector**
Ca. brasiliensis	CBS 230.51^{T} (= IMI 299576)		GQ267241	GQ267259	GQ267328	*Eucalyptus* sp.	Brazil	T.R. Ciferri
	CBS 114257		GQ267242	GQ267260	GQ267329	Leaf litter	Brazil	A.C. Alfenas
	CBS 116078 (= UFO 202)		GQ421772	GQ421780	GQ421788	*E. citriodora*	Brazil	A.O. Carvalo
	CMW 31505 (= CPC 2581)		GQ421775	GQ421783	GQ421791	*Prunus* sp.	South Africa	C. Linde
	CMW 31507 (= CPC 602)		GQ421773	GQ421781	GQ421789	*Eucalyptus* sp.	Brazil	P.W. Crous
	CMW 31508 (= CPC 1943)		GQ421774	GQ421782	GQ421790	Leaf litter	Brazil	A.C. Alfenas
Ca. colombiana sp. nov.	CBS 111136	Homothallic	FJ972424	FJ972443	FJ972493	Soil	Colombia	M.J. Wingfield
	CBS 115127^{T}	Homothallic	FJ972423	FJ972442	FJ972492	Soil	Colombia	M.J. Wingfield
	CBS 115638	Homothallic	FJ972422	FJ972441	FJ972491	Soil	Colombia	M.J. Wingfield
	CBS 115694	Homothallic	FJ972425	FJ972444	FJ972494	Soil	Colombia	M.J. Wingfield
	CMW 9058	Homothallic	FJ972420	FJ972439	FJ972489	Soil	Colombia	M.J. Wingfield
Ca. colombiensis	CBS 112221		AY725620	AY725663	AY725712	Soil	Colombia	M.J. Wingfield
Ca. insularis	CBS 114558		AF210861	FJ918526	FJ918556	Soil	Madagascar	P.W. Crous
	CBS 114559		AF210862	FJ918525	FJ918555	Soil	Madagascar	C.L. Schoch
Ca. mexicana	CBS 110918^{T}		AF210863	FJ972460	FJ972526	Soil	Mexico	M.J. Wingfield
Ca. morganii	CBS 110666		FJ918509	FJ918527	FJ918557	*Ilex vomitoria*	U.S.A.	N.E. El-Gholl
	CBS 119669		DQ521599	DQ521601	GQ421796	*Pistacia lentiscus*	Italy	G. Polizzi
	CBS 119670		DQ521600	DQ521602	GQ421797	*Pistacia lentiscus*	Italy	G. Polizzi
	CMW 31506 (= CPC1722 = P94-4359)		AF210875	GQ421787	GQ421795	*Dodenaea vicosa*	U.S.A.	N.E. El-Gholl
Ca. pauciramosa	CMW 1786	Unknown	FJ972378	FJ972445	FJ972495	*Eucalyptus smithii*	South Africa	M.J. Wingfield
	CMW 2151	Mat1-2	FJ972400	FJ972468	FJ972517	*E. nitens*	South Africa	M.J. Wingfield
	CMW 5683^{T}	Mat1-2	FJ918514	FJ918531	FJ918565	*E. grandis*	South Africa	P.W. Crous
	CMW 7592	Mat1-1	FJ972380	FJ972447	FJ972497	*E. grandis*	Uruguay	M.J. Wingfield
	CMW 7597	Mat1-1	FJ972406	FJ972474	FJ972523	*E. grandis*	Uruguay	M.J. Wingfield
	CMW 7600	Mat1-1	FJ972405	FJ972473	FJ972522	*E. grandis*	Uruguay	M.J. Wingfield
	CMW 7826	Mat1-2	FJ972392	FJ972459	FJ972509	Soil	Australia	P.W. Crous
	CMW 7827	Mat1-2	FJ972385	FJ972452	FJ972502	Soil	Australia	P.W. Crous
	CMW 7828	Mat1-2	FJ972391	FJ972458	FJ972508	Soil	Australia	P.W. Crous
	CMW 7849	Mat1-2	FJ972383	FJ972450	FJ972500	*Erica* sp.	U.S.A.	S.T. Koike
	CMW 7851	Mat1-2	FJ972382	FJ972449	FJ972499	*Mytrus communis*	U.S.A.	S.T. Koike
	CMW 7852	Mat1-2	FJ972381	FJ972448	FJ972498	*M. communis*	U.S.A.	S.T. Koike
	CMW 8061	Mat1-2	FJ972386	FJ972453	FJ972503	Soil	Australia	P.W. Crous
	CMW 9151	Mat1-2	FJ972384	FJ972451	FJ972501	*Acacia mearnsii*	South Africa	L. Lombard
	CMW 9172	Mat1-2	FJ972379	FJ972446	FJ972496	*A. mearnsii*	South Africa	L. Lombard
	CMW 10148	Mat1-2	FJ972387	FJ972454	FJ972504	*Erica* sp.	U.S.A.	S.T. Koike
	CBS 102296	Mat1-2	FJ972404	FJ972472	FJ972521	*Vriessea* sp.	New Zealand	H.M. Dance
	CBS 110945	Mat1-1	FJ972389	FJ972456	FJ972506	*Podocarpus* sp.	South Africa	P.W. Crous
	CBS 111873	Mat1-1	FJ972399	FJ972467	FJ972516	*Prunus* sp.	South Africa	C. Linde
	CBS 114861	Mat1-1	FJ972403	FJ972471	FJ972520	*Eucalyptus* sp.	South Africa	P.W. Crous

Table 1. (Continued).

Species	Isolate	Mating type	GenBank accession no. β-tubulin	Histone H3	Translation elongation factor-1α	Host	Country	Collector
	CBS 115670	Mat1-1	FJ972393	FJ972461	FJ972510	*Pinus* sp.	South Africa	P.W. Crous
	CBS 115893	Unknown	FJ972411	FJ972430	FJ972480			
	CMW 30819	Mat1-2	FJ972402	FJ972470	FJ972519	*E. grandis*	South Africa	P.W. Crous
	CMW 30875	Mat1-1	FJ972390	FJ972457	FJ972507	*Eucalyptus* sp.	South Africa	P.W. Crous
	CMW 30823	Mat1-1	FJ918515	FJ918532	FJ918566	*E. grandis*	South Africa	P.W. Crous
	CMW 30814	Unknown	FJ972408	FJ972427	FJ972477	*Eucalyptus* sp.	Kenya	J. Roux
	CMW 30822	Unknown	FJ972409	FJ972428	FJ972478	*Eucalyptus* sp.	Kenya	J. Roux
	CMW30873	Mat1-2	FJ972388	FJ972455	FJ972505	*Eucalyptus* sp.	South Africa	L. Lombard
	CMW 27203	Mat1-2	FJ972398	FJ972466	FJ972515	*Eucalyptus* sp.	China	S. Chen
	CMW 27206	Mat1-2	FJ972396	FJ972464	FJ972513	*Eucalyptus* sp.	China	S. Chen
	CMW 27283	Mat1-2	FJ972397	FJ972465	FJ972514	*Eucalyptus* sp.	China	S. Chen
	CMW 30878	Mat1-1	FJ972401	FJ972469	FJ972518	*Prunus* sp.	South Africa	C. Linde
	CMW 30818	Mat1-2	FJ972395	FJ972463	FJ972512	*Limonium* sp.	New Zealand	I. Brice
	CMW 30817	Unknown	FJ972394	FJ972462	FJ972511	*Rhododendron* sp.	New Zealand	R.A.J. White
	CMW 30879	Mat1-2	FJ972407	FJ972475	FJ972524	*Azalea* sp.	Germany	G. Hagedorn
	CMW 30815	Unknown	FJ972410	FJ972429	FJ972479	*Eucalyptus* sp.	South Africa	P.W. Crous
Ca. polizzii sp. nov.	CBS 123402^{T}		FJ972419	FJ972438	FJ972488	*Arbutus unedo*	Italy	G. Polizzi
	CMW 7804		FJ972417	FJ972436	FJ972486	*Callistemon citrinus*	Italy	G. Polizzi
	CMW 10151		FJ972418	FJ972437	FJ972487	*A. unedo*	Italy	G. Polizzi
Ca. scoparia	CMW 31000		FJ972426	FJ972476	FJ97252	*Eucalyptus* sp.	Brazil	A.C. Alfenas
	CMW 31001		GQ421779	GQ267246	GQ267246	*Eucalyptus* sp.	Brazil	A.C. Alfenas
	CBS 116076		GQ421776	GQ421784	GQ421792	*Eucalyptus* sp.	Brazil	P.W. Crous
	CBS 116081		GQ421777	GQ421785	GQ421793	Soil	Brazil	M.J. Wingfield
	CMW 7578		GQ421778	GQ421786	GQ421794	*E. grandis*	Argentina	L. Lombard
Ca. spathulata	CBS 112689		AF308463	FJ918524	FJ918554	*E. viminalis*	Brazil	N.E. El-Gholl
	CBS555.92^{T}		GQ267215	GQ267261	GQ267331	*Araucaria angustifolia*	Brazil	C. Hodges
Ca. zuluensis sp. nov.	CMW 9115	Homothallic	FJ972413	FJ972432	FJ972482	*Eucalyptus* sp.	South Africa	L. Lombard
	CMW 9188^{T}	Homothallic	FJ972414	FJ972433	FJ972483	*Eucalyptus* sp.	South Africa	L. Lombard
	CMW 9208	Homothallic	FJ972412	FJ972431	FJ972481	*Eucalyptus* sp.	South Africa	L. Lombard
	CMW 9215	Homothallic	FJ972416	FJ972435	FJ972485	*Eucalyptus* sp.	South Africa	L. Lombard
	CMW 9896	Homothallic	FJ972415	FJ972434	FJ972484	*Eucalyptus* sp.	South Africa	L. Lombard
Cy chinense	CBS 112744		AY725618	AY725660	AY725709	Soil	China	M.J. Wingfield
Cy. hawksworthii	CBS 111870^{T}		AF333407	DQ190649	FJ918558	*Nelumbo nucifera*	Mauritius	A. Peerally
Cy. leucothoës	CBS 109166^{T}		FJ918508	FJ918523	FJ918553	*Leucothoë axillaris*	U.S.A.	N.E. El-Gholl

CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMW: culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; ^{T} Ex-type cultures.

samples following the methods of Crous (2002). For each isolate, single conidial cultures were prepared on 2 % (w/v) malt extract agar (MEA, Biolab, Midrand, South Africa). Representative strains are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa and the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

Sexual compatibility

A total of 57 single conidial *Ca. pauciramosa*-like isolates (Table 1), originating from various geographic regions and hosts were crossed in all possible combinations. Mating-tester strains CMW 30823 (= STE-U 416) and CMW 5683 (= STE-U 971) for *Ca. pauciramosa* defined by Schoch *et al.* (2001) were also crossed with these isolates. Matings were done as described in Schoch *et al.* (1999) on carnation leaf agar (CLA; Fisher *et al.* 1982, Crous *et al.* 1993) and on minimal salt agar (MSA; Guerber & Correll 2001, Halleen

et al. 2006) with sterile toothpicks placed on the surface of the agar. Control tests, where isolates were crossed with themselves, were undertaken to determine whether strains had a heterothallic or homothallic mating system. The plates were stacked in plastic containers and incubated at 22 °C for 6 wk. Matings were regarded as successful when isolate combinations produced perithecia extruding viable ascospores.

DNA sequence comparisons

Calonectria pauciramosa-like isolates were grown on MEA for 7 d. Mycelium was then scraped from the surface of the cultures, freeze-dried, and ground to a powder in liquid nitrogen, using a mortar and pestle. DNA was extracted from the powdered mycelium as described by Lombard *et al.* (2008). Three loci including fragments of the β-tubulin (BT), histone H3 (HIS3) and translation elongation factor-1 alpha (TEF-1α) gene regions were sequenced. Primers used to sequence these regions were T1 (O'Donnell & Cigelnik 1997) and CYLTUB1R (Crous *et al.* 2004b) for the BT region, CYLH3F and CYLH3R (Crous *et al*. 2004b) for the HIS3 region and EF1-728F (Carbone & Kohn 1999) and EF2 (O'Donnell *et al.* 1998) for the TEF-1α region. The PCR reaction mixture used to amplify the different loci consisted of 2.5 units FastStart *Taq* polymerase (Roche Applied Science, U.S.A.), 1× PCR buffer, 1–1.5 mM $MgCl_2$, 0.25 mM of each dNTP, 0.5 µm of each primer and approximately 30 ng of fungal genomic DNA, made up to a total reaction volume of 25 µL with sterile distilled water.

Amplified fragments were purified using High Pure PCR Product Purification Kit (Roche, U.S.A.) and sequenced in both directions. For this purpose, the BigDye terminator sequencing kit v. 3.1 (Applied Biosystems, U.S.A.) and an ABI PRISM™ 3100 DNA sequencer (Applied Biosystems) were used. All PCRs and sequencing reactions were performed on an Eppendorf Mastercycler Personal PCR (Eppendorf AG, Germany) with cycling conditions as described in Crous *et al.* (2006) for BT and HIS3. The same cycling conditions for HIS3 were used for TEF-1α amplifications.

The generated sequences were added to other sequences of closely related *Calonectria* spp. obtained from GenBank (http://www.ncbi.nlm.nih.gov) and these were assembled and aligned using Sequence Navigator v. 1.0.1 (Applied Biosystems) and MAFFT v. 5.11 (Katoh *et al.* 2005), respectively. The aligned sequences were then manually corrected where needed. Single nucleotide polymorphisms (SNP'S) were determined for each gene region analysed using DnaSP v. 5.00.07 (Librado & Rozas 2009).

To determine whether the DNA sequence datasets for the three gene regions were congruent, a 70 % reciprocal bootstrap method using Neighbour-Joining with Maximum Likelihood distance was employed (Mason-Gamer & Kellogg 1996, Gueidan *et al.* 2007). Models of evolution were estimated in Modeltest v. 3.7 (Posada & Crandall 1998) using the Akaike Information Criterion for each separate gene region. The bootstrap analyses were run in PAUP (Phylogenetic Analysis Using Parsimony v. 4.0b10, Swofford 2002) for 10 000 replicates. Resulting tree topologies were compared visually for conflicts between the separate gene regions. Phylogenetic relationships were estimated in PAUP, by heuristic searches based on 1 000 random addition sequences and tree bisection-reconnection was used, with the branch swapping option set on "best trees" only.

All characters were weighted equally and alignment gaps were treated as missing data. Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC). Bootstrap analysis (Hillis & Bull 1993) was based on 1 000 replications. All sequences for the isolates studied were analysed using the Basic Local Alignment Search Tool for Nucleotide sequences (BLASTN, Altschul *et al.* 1990). The phylogenetic analysis included 73 partial gene sequences per gene, representing 11 *Calonectria* and *Cylindrocladium* species (Table 1). *Calonectria colombiensis* (CBS 112221) and *Cy. chinense* (CBS 112744) were used as outgroup taxa (Lombard *et al*. 2009). Novel sequences were deposited in GenBank and all alignments in TreeBASE (http://www.treebase.org).

A Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities using MrBayes v. 3.1.1 (Ronquist & Huelsenbeck 2003). Models of nucleotide substitution for each gene were determined using Mrmodeltest (Nylander 2004) and included for each gene partition. Two analyses of four MCMC chains were run from random trees for 1 000 000 generations and sampled every 100 generations. Both runs converged on the same likelihood score and tree topology. Therefore, the first 1 000 trees were discarded as the burn-in phase of each analysis and posterior probabilities were determined from the remaining trees.

Taxonomy

For morphological identification of the anamorphs, single conidial cultures were prepared on synthetic nutrient-poor agar (SNA; Nirenburg 1981, Lombard *et al.* 2009, 2010). Inoculated plates were incubated at room temperature and examined after 7d. Gross morphological characteristics were determined by mounting fungal structures in lactic acid and 30 measurements at ×1 000 magnification were made for each isolate. Teleomorph morphology was determined by mounting perithecia obtained from the sexual compatibility tests in Leica mountant (Setpoint Premier, Johannesburg, South Africa) and hand-sectioned with a Leica CM1100 cryostat (Setpoint Technologies) at -20 ˚C. The 10 µm sections were mounted in lactophenol or 3 % KOH. Gross morphological characteristics were observed as above. The 95 % confidence levels were calculated and extreme measurements of conidia are given in parentheses. For other structures, only the extremes are indicated. Optimal growth temperatures were determined for each isolate on MEA at 5–35 °C in 5 °C intervals in the dark. Colony colours were determined after 7 d on MEA at 25 °C in the dark, using the colour charts of Rayner (1970) for comparison. Descriptions, nomenclature, and illustrations were deposited in MycoBank (Crous *et al.* 2004a).

RESULTS

Sexual compatibility

Protoperithecia formed within 3 wk and successful matings produced perithecia with viable ascospores within 6 wk on both CLA and MSA. A total of 1 649 crosses were made using the 57 putative *Ca. pauciramosa* isolates and mating tester strains for *Ca. pauciramosa*. This resulted in 642 tests where perithecia produced viable ascospores. Self-self crosses indicated that 11 of the 57 isolates were self-fertile (homothallic). These included the Colombian isolates CBS 111041, CBS 111136, CBS 115127, CBS 115638, CBS 115694 and CMW 9058, and South African isolates CMW 9115, CMW 9188, CMW 9208, CMW 9215 and CMW 9896. Sixteen of the 57 putative *Ca. pauciramosa* did not cross with

the mating tester strains for that species or with any other isolate included in this study. These included isolates CMW 7578 from Argentina; CBS 114257, CBS 116078, CBS 116076, CBS 116081, CMW 31505, CMW 31507 and CMW 31508, from Brazil; CMW 7804, CMW 10151 and CBS 123402 from Italy, CMW 30814 and CMW 30815 from Kenya; CMW 30817 from New Zealand; CMW 1786 and CMW 30815 from South Africa. The remaining 30 isolates produced perithecia containing viable ascospores when crossed with the *Ca. pauciramosa* mating tester strains and between them. This resulted in 203 successful heterothallic matings (Table 2).

DNA sequence comparisons

Amplicons of approx. 500 bp were generated for the BT and TEF-1α gene regions and those for the HIS3 region were approx. 450 bp. Comparing the tree topologies of the 70 % reciprocal bootstrap trees indicated no conflicts. Subsequently, the datasets were combined and this resulted in a data set consisting of 1 529 characters including gaps. Of these characters, 1 151 were constant and parsimony-uninformative. The 378 parsimony-informative characters included in the parsimony analyses yielded eight most parsimonious trees (TL = 993, CI = 0.732, RI = 0.903, RC = 0.661), one of which is presented (Fig. 1). For Bayesian analyses, a HKY+I model was selected for BT, GTR+I+G model for HIS3 and a GTR+G model for TEF-1α and incorporated into the analyses. The consensus tree obtained for the Bayesian analyses confirmed the tree topology obtained with parsimony as well as bootstrap support (Fig. 1).

The majority of the *Ca. pauciramosa* isolates grouped together to form a monophyletic cluster with a bootstrap (BP) value of 100 and a Bayesian posterior probability (PP) value of 1.00. Within this cluster, two separate clades could be distinguished. The first (BP = 66, PP = 0.92) represented isolates obtained from South Africa (Table 1) and analyses of the SNP's (Table 3) showed one fixed allele for BT, two for HIS3 and one indel for TEF-1α. The second clade (BP = 97, PP = 1.00) represented isolates from Italy (Table 1) that were closely related to *Ca. pauciramosa* and have a number of shared fixed polymorphisms; five BT and two HIS3 (Table 3). Isolates from Colombia (Table 1) grouped together (BP = 100, PP = 1.00), separate from the *Ca. pauciramosa* cluster and SNP analyses show that six BT, 13 HIS3 and nine TEF-1α shared fixed alleles including three indels are characteristic for this group (Table 3). These isolates were closely related to *Ca. spathulata*. Isolates from Brazil grouped together with isolate CBS 230.51 (ex-type of *Cy. brasiliensis*; BP = 100, PP = 1.00), closely related to *Ca. morganii* and *Ca. insularis*, but separate from both of these species. Analyses of the SNP's for the isolates from Brazil compared to *Ca. morganii* and *Ca. insularis* also show several fixed alleles for these isolates, which include the ex-type culture of *Cy. brasiliensis* (CBS 230.51) (Table 4). The DNA sequence data for the three gene regions used in the present study showed 16 fixed alleles between *Cy. brasiliensis*, *Ca. insularis* and *Ca. morganii* (Table 4). An additional 10 fixed alleles were shared between *Cy. brasiliensis* and *Ca. insularis* and distinguished both species from *Ca. morganii*.

Taxonomy

Isolates CMW 9115, CMW 9188, CMW 9208, CMW 9215 and CMW 9896 represent a distinct species closely related to *Ca. pauciramosa*, based on phylogenetic inference. Mating studies also showed that these isolates have a homothallic mating system, distinguishing them from *Ca. pauciramosa*. A similar situation was found for the isolates CBS 111136, CBS 115127, CBS 115638 and CBS 115694 from Colombia and they are also treated as a new species based on their homothallic mating system and phylogenetic inference. Furthermore, isolates CBS 123402, CMW 7804 and CMW 10151 from Italy are closely related to *Ca. pauciramosa* and failed to cross with the mating tester strains of that species. Morphological observations and DNA sequence data indicate that these isolates represent an undescribed taxon.

Species of *Cylindrocladium* (1892) represent anamorph states of *Calonectria* (1867) (Rossman *et al.* 1999). In this study, these fungi are described as new species of *Calonectria,* which represents the older generic name. This is irrespective whether the teleomorph states of these fungi have been found or not and follows the approach of Lombard *et al.* (2009, 2010).

Calonectria brasiliensis (Bat. & Cif.) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB 515110. Fig. 2.

Basionym: *Cylindrocladium brasiliensis* (Bat. & Cif.) Peerally, (as *braziliensis*) CMI Descriptions of Pathogenic Fungi and Bacteria 427. 1974.

≡ *Cylindrocladium scoparium* var. *brasiliensis* Bat. & Cif., (as *brasiliense*) Boletim de SA.I.C. Pernambuco 18: 188–191. 1951.

Teleomorph unknown. *Conidiophores* with a stipe bearing a penicillate suite of fertile branches, stipe extensions, and terminal vesicles. *Stipe* septate, hyaline, smooth, 63–103 × 7–14 µm; stipe extensions septate, straight to flexuous, 204–266 µm long, 6–7 µm wide at the apical septum, terminating in an ellipsoidal to obpyriform vesicle, 7–11 µm diam. *Conidiogenous apparatus* 58–90 µm long, and 81–103 µm wide; primary branches aseptate or 1-septate, 25–34 × 5–8 µm; secondary branches aseptate, 14–25 × 4–7 µm; tertiary branches aseptate, 8–20 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 8–12 × 2–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (35–)36–40(–41) × 3–5 µm (av. = 38 × 3.5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Megaconidia* and *microconidia* not seen.

Specimens examined: **Brazil**, Ceara State, *Eucalyptus* sp., Sept. 1948, T.R. Ciferri, ex-type culture CBS 230.51 = IMI 299576 = CMW 23671; Aracruz, *Eucalyptus* sp., June 1998, A.C. Alfenas, CBS 114257 = CMW 32949; Rio de Janeiro, *Corymbia citriodora* sub. sp. *citriodora*, A.O. Carvalho, CBS 116078 = CMW 32950; Champion nursery, *Eucalyptus* sp., P.W. Crous, CPC 602 = CMW 31507; Aracruz, *Eucalyptus* sp., P.W. Crous, CPC 1943 = CMW 31508.

Culture characteristics: Colonies fast growing (30–45 mm diam after 7 d) with optimal growth temperature at 25 ºC (growth at 10–30 ºC) on MEA, reverse amber to sepia-brown after 7 d; sparse white aerial mycelium with sparse sporulation; chlamydospores moderate throughout the medium, forming microsclerotia.

Substrate: *Eucalyptus* spp.

Distribution: Brazil.

Notes: Based on morphological observations, Crous & Wingfield (1994) reduced *Ca. brasiliensis* to synonymy with *Ca. morganii*. However, phylogenetic inference in this study has shown that the ex-type culture of *Ca. brasiliensis* (CBS 230.51) is distinct from

Table 2. Results of mating studies between isolates of *Calonectria pauciramosa* from various geographic regions.

	CBS 102296	CBS 110945	CBS 111873	CBS 114861	CBS 115670	CMW 2151	**CMW 5683**	CMW 7592	CMW 7597	CMW 7600	CMW 7826	CMW 7827	CMW 7828	CMW 7849	CMW 7851	CMW 7852	CMW 8061	CMW 9151	CMW 9172	CMW 10148	CMW 27203	CMW 27206	CMW 27283	CMW 30817	CMW 30818	CMW 30819	**CMW 30823**	CMW 30873	CMW 30875	CMW 30878	CMW 30879
CBS 102296	-																													
CBS 110945	+	-																												
CBS 111873	+	-	-																											
CBS 114861	+	-	-	-																										
CBS 115670	-	+	+	+	-																									
CMW 2151	-	+	+	+	-	-																								
CMW 5683	-	+	+	+	-	+	-																							
CMW 7592	+	-	-	-	+	-	+	-																						
CMW 7597	+	-	-	-	+	-	+	-	-																					
CMW 7600	+	-	-	-	+	-	+	-	-	-																				
CMW 7826	-	+	+	+	-	+	-	+	+	+	-																			
CMW 7827	-	+	+	+	-	+	-	+	+	+	-	-																		
CMW 7828	-	+	+	+	-	+	-	+	+	+	-	-	-																	
CMW 7849	-	+	+	+	-	+	-	+	+	+	-	-	-	-																
CMW 7851	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-															
CMW 7852	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-														
CMW 8061	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-													
CMW 9151	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-												
CMW 9172	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-											
CMW 10148	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-										
CMW 27203	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-									
CMW 27206	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-								
CMW 27283	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-							
CMW 30817	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
CMW 30818	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
CMW 30819	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
CMW 30823	+	-	-	-	+	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-				
CMW 30873	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-			
CMW 30875	+	-	-	-	+	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-		
CMW 30878	+	-	-	-	+	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	
CMW 30879	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-

Isolates in bold indicate *Ca. pauciramosa* mating tester strains. + indicates formation of perithecia with viable ascospores; - indicates no perithecial formation.

Table 3. Single nucleotide polymorphisms (SNP's)[1] from the β-tubulin, histone H3 and translation elongation factor-1α sequence data of *Calonectria* isolates from Colombia, Italy and South Africa.

Species	Isolate no.	β-tubulin													Histone H3														Translation elongation factor-1α												
		87	187	200	201	208	336	375	382	385	401	406	496	514	36	39	76	209	251	257	266	274	276	281	282	292	329	350	53	86	94	95	103	126	127	128	269	423	426	515
Ca. pauciramosa	CMW 5683	A	A	C	G	A	C	C	C	G	C	C	T	T	G	C	G	C	A	T	G	C	A	C	T	C	C	T	T	G	C	A	A	T	T	-	A	C	C	T
	CMW 30823	A	A	C	G	A	C	C	C	G	C	C	T	T	G	C	G	C	A	T	G	C	A	C	T	C	C	T	T	G	C	A	A	T	T	-	A	C	C	T
Ca. colombiana	CBS 111136	G	C	C	A	G	C	C	C	A	C	A	C	T	C	T	A	T	G	C	A	T	G	T	A	T	T	C	C	T	-	-	G	A	A	G	T	T	T	-
	CBS 115127	G	C	C	A	G	C	C	C	A	C	A	C	T	C	T	A	T	G	C	A	T	G	T	A	T	T	C	C	T	-	-	G	A	A	G	T	T	T	-
	CBS 115638	G	C	C	A	G	C	C	C	A	C	A	C	T	C	T	A	T	G	C	A	T	G	T	A	T	T	C	C	T	-	-	G	A	A	G	T	T	T	-
	CBS 115694	G	C	C	A	G	C	C	C	A	C	A	C	T	C	T	A	T	G	C	A	T	G	T	A	T	T	C	C	T	-	-	G	A	A	G	T	T	T	-
	CMW 9058	G	C	C	A	G	C	C	C	A	C	A	C	T	C	T	A	T	G	C	A	T	G	T	A	T	T	C	C	T	-	-	G	A	A	G	T	T	T	-
Ca. polizzii	CBS 123402	G	A	C	G	A	T	G	T	G	G	C	T	C	C	C	G	G	A	T	G	C	A	C	T	C	C	T	T	G	C	A	A	T	T	-	A	C	C	-
	CMW 7804	G	A	C	G	A	T	G	T	G	G	C	T	C	C	C	G	G	A	T	G	C	A	C	T	C	C	T	T	G	C	A	A	T	T	-	A	C	C	-
	CMW 10151	G	A	C	G	A	T	G	T	G	G	C	T	C	C	C	G	G	A	T	G	C	A	C	T	C	C	T	T	G	C	A	A	T	T	-	A	C	C	-
Ca. zuluensis	CMW 9115	G	A	T	G	A	C	C	C	G	C	C	T	T	C	C	G	G	A	T	G	C	A	C	T	C	C	T	T	G	C	A	A	T	T	-	A	C	C	-
	CMW 9188	G	A	T	G	A	C	C	C	G	C	C	T	T	C	C	G	G	A	T	G	C	A	C	T	C	C	T	T	G	C	A	A	T	T	-	A	C	C	-
	CMW 9208	G	A	T	G	A	C	C	C	G	C	C	T	T	C	C	G	G	A	T	G	C	A	C	T	C	C	T	T	G	C	A	A	T	T	-	A	C	C	-
	CMW 9215	G	A	T	G	A	C	C	C	G	C	C	T	T	C	C	G	G	A	T	G	C	A	C	T	C	C	T	T	G	C	A	A	T	T	-	A	C	C	-
	CMW 9896	G	A	T	G	A	C	C	C	G	C	C	T	T	C	C	G	G	A	T	G	C	A	C	T	C	C	T	T	G	C	A	A	T	T	-	A	C	C	-

[1]Polymorphisms are highlighted; Yellow = unique fixed alleles, Blue = shared fixed alleles.

Table 4. Single nucleotide polymorphisms (SNP's)[1] from the sequence data of β-tubulin, histone H3 and translation elongation factor-1α of *Ca. brasiliensis*, *Ca. insulare* and *Ca. morganii* used in this study.

Species	Isolate no.	β-tubulin					Histone H3														Transation elongation factor-1α																				
		53	61	117	360	472	9	10	68	69	70	94	204	252	257	359	389	416	451	460	9	10	20	47	48	102	110	112	113	114	115	142	280	378	406	407	408	414	437	479
Ca. brasiliensis	CBS 230.51	C	C	C	A	T	T	G	-	-	-	G	T	G	T	C	T	T	A	T	A	G	T	A	C	G	C	-	-	-	-	T	T	T	T	C	C	T	G	T
	CBS 114257	C	C	C	A	T	T	G	-	-	-	G	T	G	T	C	T	T	A	T	A	G	T	A	C	G	C	-	-	-	-	T	T	T	T	C	C	T	G	T
	CBS 116078	C	C	C	A	T	T	G	-	-	-	G	T	G	T	C	T	T	A	T	A	G	T	A	C	G	C	-	-	-	-	T	T	T	T	C	C	T	G	T
	CPC1943	C	C	C	A	T	T	G	-	-	-	G	T	G	T	C	T	T	A	T	A	G	T	A	C	G	C	-	-	-	-	T	T	T	T	C	C	T	G	T
	CPC602	C	C	C	A	T	T	G	-	-	-	G	T	G	T	C	T	T	A	T	A	G	T	A	C	G	C	-	-	-	-	T	T	T	T	C	C	T	G	T
	CPC2581	C	C	C	A	T	T	G	T	T	C	A	T	G	T	C	T	T	A	T	A	G	T	A	C	G	C	-	-	-	-	T	T	T	T	C	C	T	G	T
Ca. insulare	CBS114558	C	C	T	G	C	T	G	T	T	C	A	T	G	T	C	C	C	A	C	G	T	G	C	C	A	A	C	A	C	C	-	C	C	G	A	T	C	A	C
	CBS 114559	C	C	T	G	C	T	G	T	T	C	A	C	G	T	C	C	C	A	C	G	T	G	C	C	A	A	C	A	C	C	-	C	C	G	A	T	C	A	C
Ca. morganii	CBS 110666	A	A	T	A	T	C	T	-	-	-	A	C	T	C	T	C	C	C	C	G	T	T	A	G	G	A	-	-	-	C	-	T	C	G	A	T	C	A	C
	CBS 119669	A	A	T	A	T	C	T	-	-	-	A	C	T	C	T	C	C	C	C	G	T	T	A	G	G	A	-	-	-	C	-	T	C	G	A	T	C	A	C
	CBS 119670	A	A	T	A	T	C	T	-	-	-	A	C	T	C	T	C	C	C	C	G	T	T	A	G	G	A	-	-	-	C	-	T	C	G	A	T	C	A	C
	CPC 1722	A	A	T	A	T	C	T	-	-	-	A	C	T	C	T	C	C	C	C	G	T	T	A	G	G	A	-	-	-	C	-	T	C	G	A	T	C	A	C

[1]Polymorphisms are highlighted; Yellow = unique fixed alleles, Blue = shared fixed alleles.l

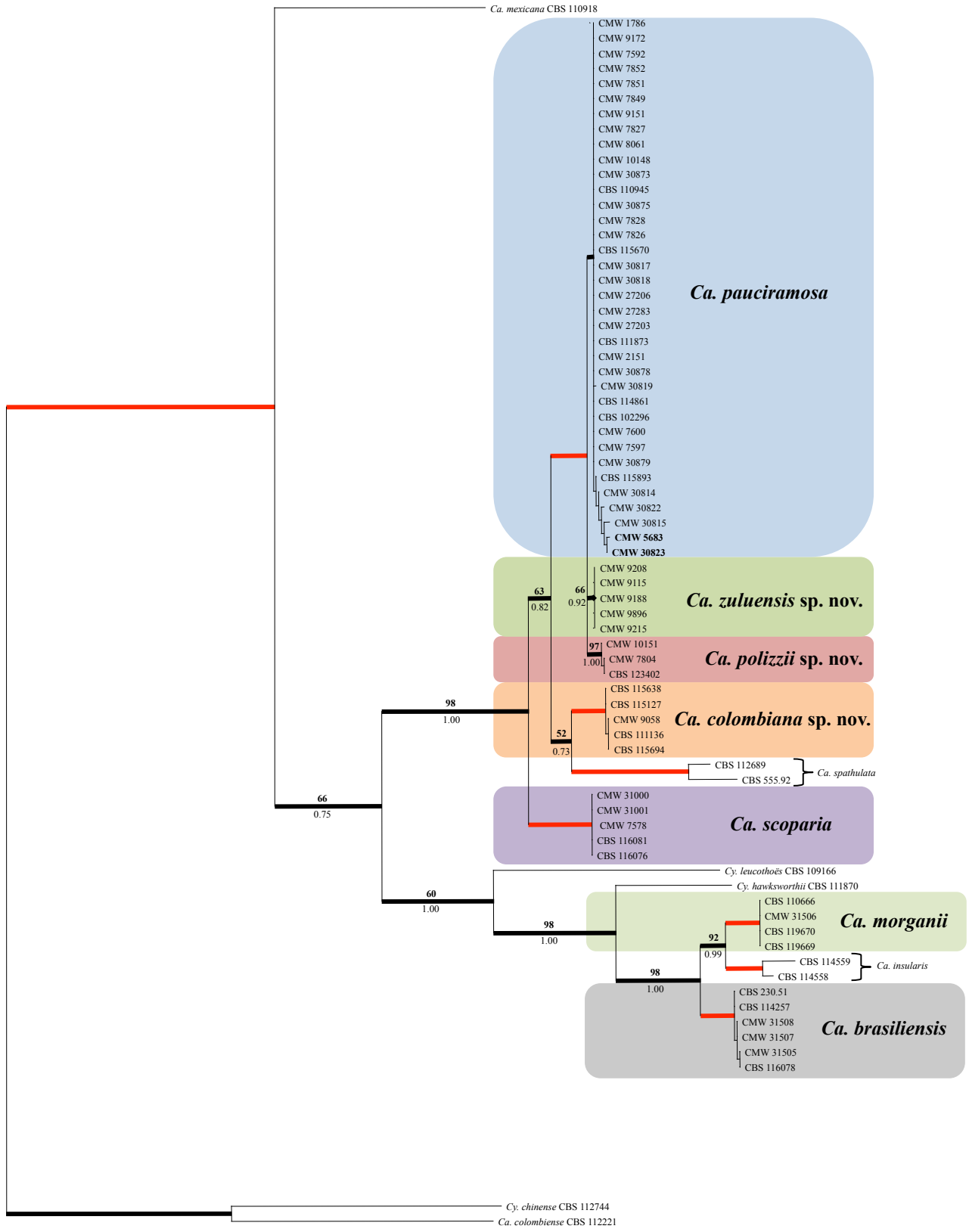

Fig. 1. One of eight most parsimonious trees obtained from a heuristic search with 1 000 random addition of the combined BT, HIS3 and TEF-1α sequence alignments. Scale bar shows 10 changes and bootstrap support values from 1 000 replicates are shown above the nodes in bold. Bayesian posterior probability values are indicated below the nodes. Red lines indicate bootstrap support values of 100 and posterior probability values of 1.00. Thickened lines indicate branches in the strict consensus and Bayesian consensus tree. The tree was rooted to *Ca. colombiensis* (CBS 112221) and *Ca. chinensis* (CBS 112744). Mating tester strains of *Ca. pauciramosa* used in this study are indicated in bold.

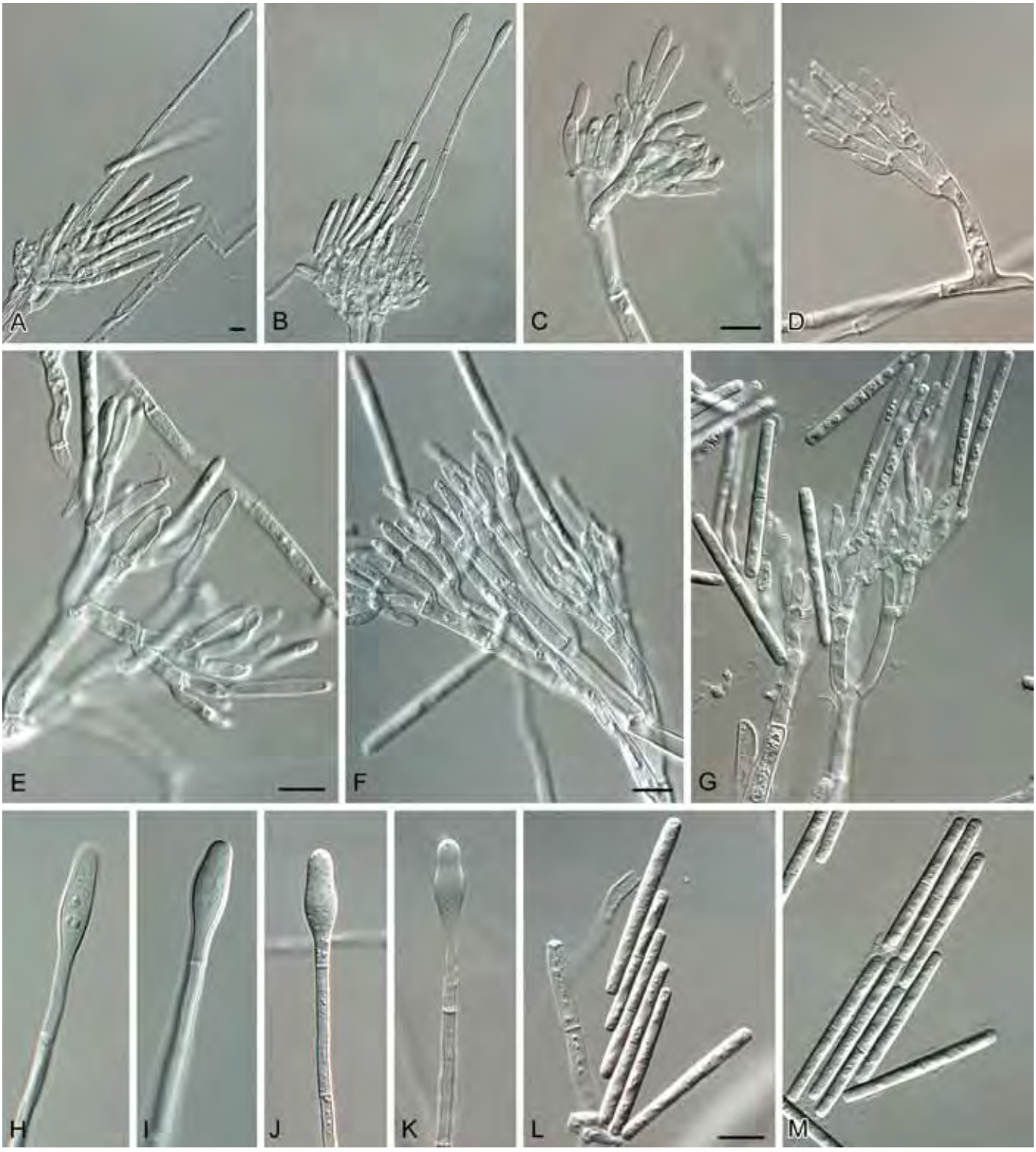

Fig. 2. *Calonectria brasiliensis*. A–B. Macroconidiophores. C–G. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. H–K. Ellipsoidal to obpyriform vesicles. L–M. One-septate macroconidia. Scale bars = 10 µm.

Ca. morganii (CBS 110666, CBS 119669, CBS 119670 and CMW 31506). Morphological observations in this study also indicated that conidia of *Ca. brasiliensis* (av. 38 × 3.5 µm) are smaller than those of *Ca. morganii* (av. 45 × 4 µm). *Calonectria brasiliensis* only produces up to three branches per conidiophore, whereas *Ca. morganii* can have up to six branches per conidiophore.

Calonectria colombiana L. Lombard, Crous & M.J. Wingf., **sp. nov.** MycoBank MB515065, Fig. 3.

Etymology: Name refers to Colombia, the country this fungus was isolated from.

Telomorpha *Calonectriae pauciramosa* similis, sed ascosporis brevioribus, (28–)31–36(–40) × 3–5 µm (in medio 34 × 4 µm). Culturae homothallicae. Anamorpha *Cylindrocladio pauciramoso* simile, sed vesiculis obpyriforme vel fusiforme (8–12 µm diam.) et conidiis maioribus (33–)35–39(–40) × 3–4 µm, in medio 37 × 3 µm.

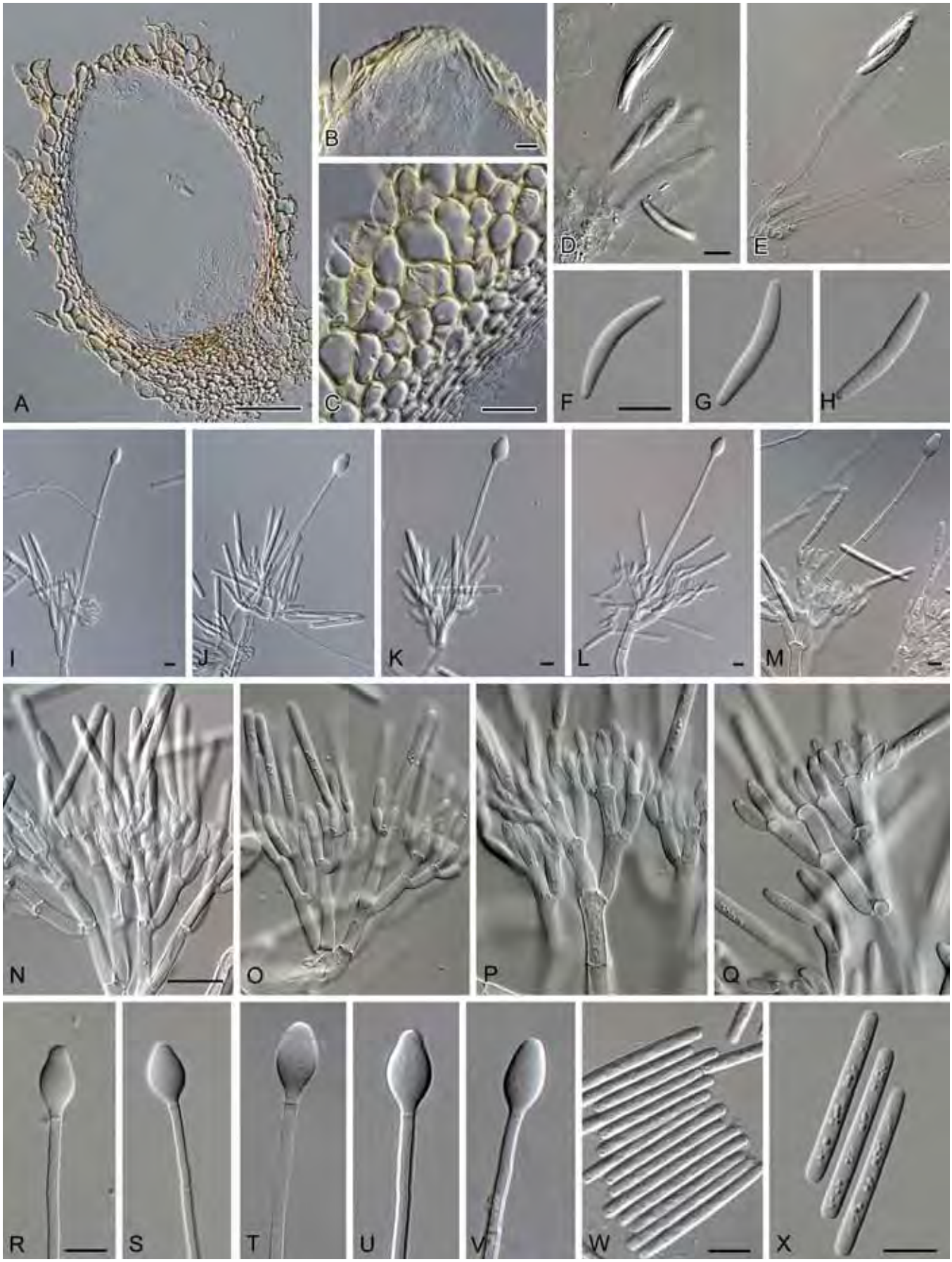

Fig. 3. *Calonectria colombiana*. A. Perithecium. B. Ostiolar region of perithecium. C. Vertical section through perithecium, showing wall structure. D–E. Asci. F–H. Ascospores. I–M. Macroconidiophores. N–Q. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. R–V. Obpyriform to ellipsoid vesicles. W–X. One-septate macroconidia. Scale bars: A = 70 µm, B–C = 30 µm, other scale bars = 10 µm.

Perithecia solitary or in groups, orange to red, becoming red-brown with age; in section, apex and body yellow to orange, base red-brown, sub-globose to ovoid, 270–410 µm high, 175–285 µm diam, body turning dark red, and base dark red-brown (KOH+). Perithecial walls rough, consisting of 2 thick-walled layers: outside layer of *textura globulosa*, 24–90 µm wide; becoming more compressed towards inner layer of *textura angularis,* 18–22 µm wide; becoming thin-walled and hyaline towards the center, outer cells, 38–55 × 16–40 µm; inner cells, 3–12 × 3–7 µm: perithecial base up to 114 µm wide; consisting of dark red, angular cells; merging with an erumpent stroma, cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. *Asci* 8-spored, clavate, 87–162 × 12–18 µm, tapering to a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, gluttulate, fusoid with rounded ends, straight to slightly curved, 1-septate, not or slightly constricted at the septum, (28–)31–36(–40) × 3–5 µm (av. = 34 × 4 µm). Cultures homothallic. *Conidiophores* with a stipe bearing a penicillate suite of fertile branches, stipe extensions, and terminal vesicles. *Stipe* septate, hyaline, smooth, 45–126 × 6–9 µm; stipe extensions septate, straight to flexuous, 143–173 µm long, 5–7 µm wide at the apical septum, terminating in an obpyriform to ellipsoid vesicle, 8–12 µm diam. *Conidiogenous apparatus* 38–115 µm long, and 35–91 µm wide; primary branches aseptate or 1-septate, 19–37 × 5–8 µm; secondary branches aseptate, 9–17 × 4–5 µm; tertiary and additional branches (–4), aseptate, 8–13 × 3–4 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 9–12 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (33–)35–39(–40) × 3–4 µm (av. = 37 × 3 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Megaconidia* and *microconidia* not seen.

Specimens examined: **Colombia**, La Selva, from soil, June 1995, M.J. Wingfield, Herb. PREM 60295, **holotype** of *Calonectria colombiana*, cultures ex-type CBS 115127 = CMW 30871 = CPC 1160; La Selva, June 1995, M.J. Wingfield, CBS 111041 = CMW 30767 = CPC 1163; La Selva, June 1995, M.J. Wingfield, CBS 111136 = CMW 30812 = CPC 1151; La Selva, June 1995, M.J. Wingfield, CBS 115638 = CMW 30766 = CPC 1161 (Herb. PREM 60296); La Selva, June 1995, M.J. Wingfield, CBS 115694 = CMW 30813 = CPC 1162, CMW 9058.

Culture characteristics: Colonies fast growing (35–55 mm diam after 7 d) with optimal growth temperature at 25 ºC (growth at 10–30 ºC) on MEA, reverse amber to sepia-brown after 7 d; abundant white aerial mycelium with sparse sporulation; chlamydospores extensive throughout the medium, forming microsclerotia.

Substrate: Soil.

Distribution: Colombia.

Notes: Isolates of *Ca. colombiana* were previously regarded as either *Ca. pauciramosa* or *Ca. scoparia* (Crous 2002) based on the morphological similarity of the anamorph states of these species. Based on macroconidial dimensions, *Ca. colombiana* (av. 37 × 3 µm) can be distinguished from *Ca. pauciramosa* (av. 50 × 4.5 µm) and *Ca. scoparia* (av. 60 × 4.5 µm) in having smaller, 1-septate macroconidia. Both *Ca. pauciramosa* and *Ca. scoparia* have a biallelic, heterothallic mating system (Schoch *et al.* 1999, 2001), whereas *Ca. colombiana* is homothallic.

Calonectria polizzii L. Lombard, Crous & M.J. Wingf., **sp. nov.** MycoBank MB515066, Fig. 4.

Etymology: The name honours Prof. dr. Giancarlo Polizzi, who isolated the fungus in Italy.

Teleomorpha ignota. *Cylindrocladio pauciramoso* simile, sed vesiculis clavato vel obpyriforme (6–9 µm diam.) et conidiis maioribus (31–)32–42(–49) × 3–5 µm, in medio 37 × 4 µm.

Teleomorph unknown. *Conidiophores* with a stipe bearing a penicillate suite of fertile branches, stipe extensions, and terminal vesicles. *Stipe* septate, hyaline, smooth, 58–108 × 5–7 µm; stipe extensions septate, straight to flexuous, 111–167 µm long, 5–6 µm wide at the apical septum, terminating in an obpyriform to ellipsoid vesicle, 6–9 µm diam. *Conidiogenous apparatus* 27–57 µm long, and 28–51 µm wide; primary branches aseptate or 1-septate, 15–35 × 4–6 µm; secondary branches aseptate, 12–26 × 3–5 µm; tertiary branches aseptate, 10–15 × 4–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 8–13 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (31–)32–42(–49) × 3–5 µm (av. = 37 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Megaconidia* and *microconidia* not seen.

Specimens examined: **Italy**, Sicily, Carrubba, on *Arbutus unedo*, 1997, G. Polizzi, Herb. PREM 60297, **holotype** of *Calonectria polizzii*, cultures ex-type CBS 123402 = CMW 30872; Sicily, on *Callistemon citrinus*, 1997, G. Polizzi, CMW 7804 = CPC 2681 = CBS 125270; Sicily, on *Callistemon citrinus*, 1997, G. Polizzi, CMW 10151 = CPC 2771 = CBS 125271 (Herb. PREM 60298).

Culture characteristics: Colonies fast growing (35–40 mm diam after 7 d) with optimal growth temperature at 25 ºC (growth at 10–30 ºC) on MEA, reverse amber to sepia-brown after 7 d; abundant white aerial mycelium with sparse sporulation; chlamydospores extensive throughout the medium, forming microsclerotia.

Substrates: *Arbutus unedo, Callistemon citrinus.*

Distribution: Italy.

Notes: *Calonectria polizzii* is morphologically similar to *Ca. pauciramosa* and *Ca. zuluensis.* The macroconidia of *Ca. polizzii* (av. 37 × 4 µm) are smaller to those of *Ca. pauciramosa* (av. 50 × 4.5 µm). Mating tests also showed that *Ca. polizzii* does not mate with either of the tester strains of *Ca. pauciramosa* (Schoch *et al.* 2001) used in this study. However, the isolates of *Ca. polizzii* tested might represent a single mating type, or might have lost their ability to mate, and further studies incorporating more isolates will be required to confirm this.

Calonectria zuluensis L. Lombard, Crous & M.J. Wingf., **sp. nov.** MycoBank MB515067, Fig. 5.

Etymology: Name refers to KwaZulu-Natal, South Africa, the province were the fungus was isolated.

Telomorpha *Calonectriae pauciramosa* similis, sed ascosporis brevioribus, (26–)29–34(–38) × 4–5 µm (in medio 32 × 4 µm). Culturae homothallicae. Anamorpha *Cylindrocladio pauciramoso* simile, sed vesiculis clavato vel obpyriforme (6–10 µm diam) et conidiis maioribus (31–)34–38(–40) × 3–5 µm, in medio 36 × 4 µm.

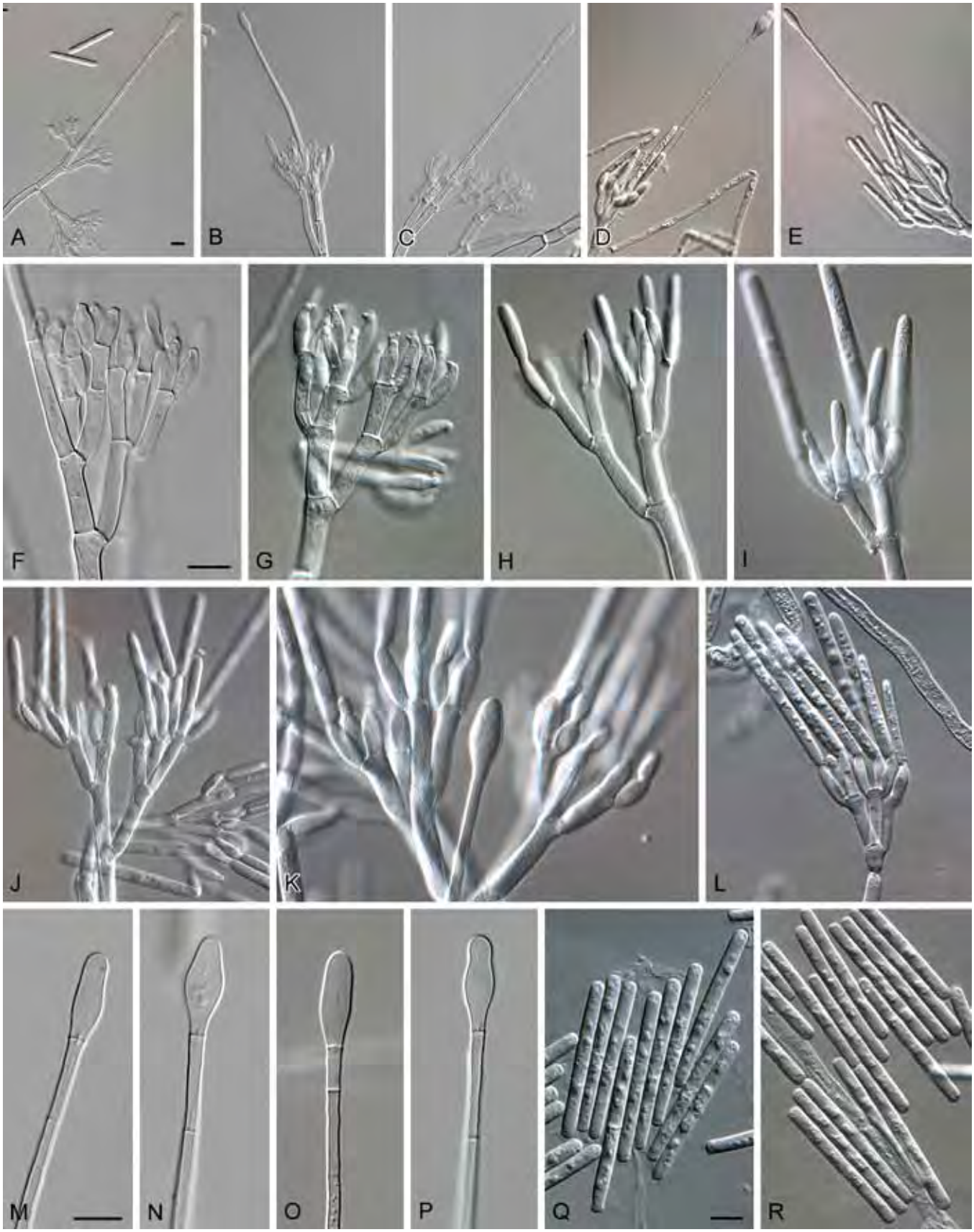

Fig. 4. *Calonectria polizzii*. A–E. Macroconidiophores. F–L. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. M–P. Obpyriform to ellipsoid vesicles. Q–R. One-septate macroconidia. Scale bars = 10 µm.

Fig. 5. *Calonectria zuluensis*. A. Perithecium. B. A vertical section through a perithecium, showing the wall layers. C–D. Asci. E–G. Ascospores. H–L. Macroconidiophores. M–P. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. Q–U. Ellipsoid to obpyriform vesicles. V–W. One-septate macroconidia. Scale bars: A = 70 µm, B = 30 µm, other scale bars = 10 µm.

Perithecia solitary or in groups, orange to red, becoming red-brown with age; in section apex and body yellow to orange, base red-brown, sub-globose to ovoid, 292–394 µm high, 170–285 µm diam, body turning dark red, and base dark red-brown (KOH+). Perithecial walls rough, consisting of 2 thick-walled layers: outside layer of *textura globulosa*, 30–80 µm wide; becoming more compressed towards inner layer of *textura angularis,* 20–22 µm wide; becoming thin-walled and hyaline towards the center, outer cells, 40–50 × 18–40 µm; inner cells, 4–12 × 3–5 µm: perithecial base up to 116 µm wide; consisting of dark red, angular cells; merging with an erumpent stroma, cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. *Asci* 8-spored, clavate, 92–140 × 10–16 µm, tapering to a long thin stalk. *Ascospores* aggregate in the upper third of the ascus, hyaline, gluttulate, fusoid with rounded ends, straight to slightly curved, 1-septate, not or slightly constricted at the septum, (26–)29–34(–38) × 4–5 µm (av. = 32 × 4 µm). Cultures homothallic. *Conidiophores* with a stipe bearing penicillate clusters of fertile branches, stipe extensions, and terminal vesicles. *Stipe* septate, hyaline, smooth, 57–84 × 6–9 µm; stipe extensions septate, straight to flexuous, 110–171 µm long, 5–8 µm wide at the apical septum, terminating in ellipsoid to obpyriform vesicles, 6–10 µm diam. *Conidiogenous apparatus* 35–67 µm long, and 37–70 µm wide; primary branches aseptate or 1-septate, 16–28 × 4–6 µm; secondary branches aseptate, 11–20 × 3–5 µm; tertiary branches aseptate, 8–13 × 3–4 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 10–13 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (31–)34–38(–40) × 3–5 µm (av. = 36 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Megaconidia* and *microconidia* not seen.

Specimens examined: **South Africa**, KwaZulu-Natal, Kwambonambi, from *Eucalyptus grandis* clonal cutting, Feb. 2001, L. Lombard, Herb. PREM 60292, **holotype** of *Calonectria zuluensis*, cultures ex-type CBS 125268 = CMW 9188; KwaZulu-Natal, Kwambonambi, *E. grandis* × *urophylla* hybrid cutting, Feb. 2001, L. Lombard, CMW 9115, CMW 9208 (Herb. PREM 60293), CMW 9215, Pietermarizburg, *E. grandis* × *urophylla* hybrid cutting, Mar. 2001, L. Lombard, CMW 9896 = CBS 125272.

Culture characteristics: Colonies fast growing (35–40 mm diam after 7 d) with optimal growth temperature at 25 ºC (growth at 10–30 ºC) on MEA, reverse amber to sepia-brown after 7 d; abundant white aerial mycelium with sparse sporulation; chlamydospores extensive throughout the medium, forming microsclerotia.

Substrate: *Eucalyptus grandis* and *E. grandis* × *urophylla* rooted cuttings.

Distribution: South Africa.

Notes: *Calonectria zuluensis* can be distinguished from *Ca. pauciramosa* and *Ca. scoparia* based on its homothallic mating system. Macroconidia of *Ca. zuluensis* (av. 36 × 4 µm) are also smaller than those of *Ca. pauciramosa* (av. 50 × 4.5 µm) and *Ca. scoparia* (av. 60 × 4.5 µm). This species is morphologically very similar to *Ca. colombiana*. However, *Ca. zuluensis* can be distinguished from *Ca. colombiana* based on the fact that it has broadly clavate to obpyriform vesicles as compared with the obpyriform to fusiform vesicles in *Ca. colombiana*. Furthermore, *Ca. zuluensis* can easily be distinguished based on phylogenetic inference.

DISCUSSION

Considerable variation observed amongst isolates of "*Ca. pauciramosa*" from different geographical localities was illustrated in this study. Morphological characteristics, phylogenetic inference and mating studies revealed the presence of three cryptic species accommodated in cultures that have collectively been treated as *Ca. pauciramosa*. This is consistent with the results of previous studies (Schoch *et al.* 1999, 2001), which noted variation within *Ca. pauciramosa,* although at that time the sample size was inordinately small to consider the matter further. Schoch *et al.* (2001) also noted a high level of variation among isolates from South America, but concluded that this most likely reflected diversity consistent with an endemic population.

Crous (2002) suggested that mating isolates with recognised mating tester strains represented an important step in identifying isolates of *Ca. pauciramosa*. Various studies (Crous *et al.* 1993, Crous & Wingfield 1994, Crous *et al.* 1998, Schoch *et al.* 1999, 2001, Crous 2002) have used CLA as standardised medium to study sexual compatibility amongst isolates of *Cylindrocladium*. However, CLA has its limitations in that carnation leaf pieces are not always available and the present study used both CLA and MSA amended with sterile tooth picks, which proved to be very successful. Effective application of the latter technique to induce teleomorphs in culture has also been achieved for various other plant pathogenic genera, including *Glomerella* (Geurber & Correll 2001) and *Neonectria* (Halleen *et al.* 2006).

The descriptions of *Ca. colombiana*, *Ca. zuluensis* and *Ca. polizzii* add three new species to the *Ca. scoparia* species complex. This complex is characterised by species having ellipsoidal to obpyriform vesicles and producing 1-septate macroconidia (Schoch *et al.* 1999, Crous 2002). The complex was previously regarded as having a biallelic, heterothallic mating system (Schoch *et al*. 1999, 2001). However, both the newly described *Ca. colombiana* and *Ca. zuluensis* are homothallic. The occurrence of both heterothallic and homothallic *Calonectria* species in a single complex is not unique, having previously been found in the *Ca. kyotensis* species complex (Crous *et al.* 2004b).

Schoch *et al.* (2001) considered female fertility of *Ca. pauciramosa*, and found variation in BT sequence data for isolates from Italy. This variation has most likely been captured in the description of *Ca. polizzii* in the present study. This new species has thus been shown as unique based on morphological, phylogenetic inference and biological characteristics, separating it from *Ca. pauciramosa*. Morphologically, *Ca. polizzii* can be distinguished from *Ca. pauciramosa* by its smaller 1-septate macroconidia. Isolates of *Ca. polizzii* were also not capable of mating with the *Ca. pauciramosa* mating-tester strains or other *Ca. pauciramosa* isolates from different geographic regions.

Schoch *et al.* (2001), noted variation amongst isolates of *Ca. pauciramosa* from South America, and suggested that the fungus could be native to that continent. Results of the present study, including isolates from Colombia, led to the description of *Ca. colombiana*. This fungus is distinct from *Ca. pauciramosa* in having a homothallic mating system, smaller macroconidia and quaternary branches on the conidiophores. Although *Ca. insularis* also forms conidiophores with quaternary branches (Schoch *et al.* 1999), *Ca. colombiana* can easily be distinguished from it based on DNA sequence comparisons and its homothallic mating system.

More than eight species of *Calonectria* have been recorded from South Africa (Crous *et al.* 1991, Crous *et al.* 1993, Schoch

et al. 1999, Crous 2002) and the description of *Ca. zuluensis* adds another species to those already reported from the country. *Calonectria zuluensis* has a homothallic mating system, which is different from *Ca. pauciramosa* with a biallelic, heterothallic mating system (Schoch *et al.* 2001). The two species can also easily be distinguished from each other based on DNA sequence comparisons.

In the analyses of the SNP's for the three gene regions used in this study, several fixed and shared SNP alleles were found for *Ca. colombiana*, *Ca. polizzii* and *Ca. zuluensis*. The majority of the fixed SNPs are shared between *Ca. polizzii* and *Ca. zuluensis*, indicating that these are sibling species, and that genetic isolation between them occurred recently (Taylor *et al.* 2000). For *Ca. colombiana*, fewer of the fixed SNPs are shared with *Ca. polizzii* and *Ca. zuluensis,* indicating that speciation occurred less recently than that of *Ca. polizzii* and *Ca. zuluensis*. These three species do not share the same alleles with *Ca. pauciramosa*, clearly distinguishing it from them.

Calonectria brasiliensis has been elevated to species level based on phylogenetic inference. Although Peerally (1974) indicated that the macroconidia of *Ca. brasiliensis* (24–38 × 2–3 µm) are smaller than those of *Ca. morganii* (av. 45 × 4 µm), Crous & Wingfield (1994) reduced *Ca. brasiliensis* to synonymy under *Ca. morganii*, based on similar conidial dimensions and vesicle morphology observed in culture. It is possible, however, that the original ex-type strain of *Ca. brasiliensis* was in fact morphologically degenerated, appearing atypical for the species. Several isolates from Brazil, previously identified as *Ca. pauciramosa*, grouped with the ex-type strain of *Ca. brasiliensis* (CBS 230.51). Previous DNA sequence comparisons and mating studies with *Ca. morganii* (Crous *et al.* 1993, Overmeyer *et al.* 1996, Schoch *et al.* 2000, 2001) failed to include the ex-type strain CBS 230.51 of *Ca. brasiliensis*, as this species was seen as a synonym of *Ca. morganii* (Crous 2002).

This study has shown the importance of combining morphological, biological and phylogenetic data to identify cryptic species of *Calonectria*. Although the biological species concept is regarded as insufficient for this purpose and needs to be clearly defined in *Calonectria* (Crous 2002), this study has shown that it has some use in identifying cryptic species within *Ca. pauciramosa*. The presence of homothallic and heterothallic mating strategies in closely related fungi is interesting and could well provide another opportunity to analyse the genetics of mating systems in ascomycetes. This study has shown, however, that morphology in combination with phylogenetic inference provides the most useful approach to identify cryptic species in *Calonectria* (Lombard *et al.* 2009). The present study has also shown the importance of the multi-gene approach in studying the phylogenetic relationships of phenotypic closely related *Calonectria* spp.

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Phylogeny and systematics of the genus *Calonectria*

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Abstract: Species of *Calonectria* are important plant pathogens, several of which have a worldwide distribution. Contemporary taxonomic studies on these fungi have chiefly relied on DNA sequence comparisons of the β-tubulin gene region. Despite many new species being described, there has been no phylogenetic synthesis for the group since the last monographic study almost a decade ago. In the present study, the identity of a large collection of *Calonectria* isolates from various geographic regions was determined using morphological and DNA sequence comparisons. This resulted in the discovery of seven new species; *Ca. densa*, *Ca. eucalypti*, *Ca. humicola*, *Ca. orientalis*, *Ca. pini*, *Ca. pseudoscoparia* and *Ca. sulawesiensis*, bringing the total number of currently accepted *Calonectria* species to 68. A multigene phylogeny was subsequently constructed for all available *Calonectria* spp., employing seven gene regions, namely actin, β-tubulin, calmodulin, histone H3, the internal transcribed spacer regions 1 and 2 and the 5.8S gene of the ribosomal RNA, 28S large subunit RNA gene and translation elongation 1-alpha. Based on these data 13 phylogenetic groups could be distinguished within the genus *Calonectria* that correlated with morphological features. Dichotomous and synoptic keys to all *Calonectria* spp. currently recognised are also provided.

Key words: *Cylindrocladium*, DNA phylogeny, sexual compatibility, taxonomy.
Taxonomic novelties: New combinations - *Calonectria angustata* (Crous & El-Gholl) L. Lombard, M.J. Wingf. & Crous, *Ca. australiensis* (Crous & H.D. Hyde) L. Lombard, M.J. Wingf. & Crous, *Ca. canadensis* (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous, *Ca. chinensis* (Crous) L. Lombard, M.J. Wingf. & Crous, *Ca. citri* (H.S. Fawc. & Klotz) L. Lombard, M.J. Wingf. & Crous, *Ca. curvata* (Boedijn & Reitsma) L. Lombard, M.J. Wingf. & Crous, *Ca. curvispora* (Crous & D. Victor) L. Lombard, M.J. Wingf. & Crous, *Ca. ecuadoriae* (Crous & M.J. Wingf.) L. Lombard, M.J. Wingf. & Crous, *Ca. gordoniae* (Leahy, T.S. Schub. & El-Gholl) L. Lombard, M.J. Wingf. & Crous, *Calonectria hawksworthii* (Peerally) L. Lombard, M.J. Wingf. & Crous, *Calonectria hurae* (Crous) L. Lombard, M.J. Wingf. & Crous, *Calonectria indonesiae* (Crous) L. Lombard, M.J. Wingf. & Crous, *Ca. leucothoës* (El-Gholl, Leahy & T.S. Schub.) L. Lombard, M.J. Wingf. & Crous, *Ca. malesiana* (Crous) L. Lombard, M.J. Wingf. & Crous, *Ca. multiphialidica* (Crous, Simoneau & Risède) L. Lombard, M.J. Wingf. & Crous, *Ca. pacifica* (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous, *Ca. penicilloides* (Tubaki) L. Lombard, M.J. Wingf. & Crous, *Ca. pseudonaviculata* (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M.J. Wingf. & Crous, *Ca. sumatrensis* (Crous) L. Lombard, M.J. Wingf. & Crous. **New species -** *Ca. densa* L. Lombard, M.J. Wingf. & Crous, *Ca. eucalypti* L. Lombard, M.J. Wingf. & Crous, *Ca. humicola* L. Lombard, M.J. Wingf. & Crous, *Ca. orientalis* L. Lombard, M.J. Wingf. & Crous, *Ca. pini* L. Lombard, M.J. Wingf. & Crous, *Ca. pseudoscoparia* L. Lombard, M.J. Wingf. & Crous, *Ca. sulawesiensis* L. Lombard, M.J. Wingf. & Crous.

INTRODUCTION

The genus *Calonectria* (*Ca.*) was first described in 1867, with *Ca. daldiniana* as the type. This species was later reduced to synonymy with *Ca. pyrochroa* based on morphological comparisons by Rossman (1979). *Calonectria* spp. are *Euascomycetes* in the order *Hypocreales* (Hibbett *et al.* 2007, Schoch *et al.* 2009) and are characterised by their yellow to dark red perithecia, with scaly to warty ascocarp walls giving rise to long-stalked, clavate asci with 1–multi-septate ascospores and *Cylindrocladium* (*Cy.*) anamorphs (Rossman 1993, Crous 2002, Lombard *et al*. 2010b). The genus *Cylindrocladium* was described by Morgan (1892), and is characterised by branched conidiophores with stipe extensions terminating in characteristic vesicles and producing cylindrical, 1–multi-septate conidia (Crous & Wingfield 1994, Crous 2002). Morphologically, the anamorph provides the greatest number of distinguishing characters for *Calonectria* and it is also the state most frequently encountered in nature (Peerally 1991, Crous & Wingfield 1994, Schoch *et al.* 2001b, Crous 2002). Consequently, species of *Calonectria* are primarily distinguished by their anamorph characters, especially vesicle shape, stipe extension length, conidial septation, and dimensions on a standardised medium under defined growth conditions (Boesewinkel 1982, Peerally 1991, Crous & Wingfield 1994, Crous 2002). Despite, the use of standardised conditions, taxonomic confusion can result because some intraspecific variation in vesicle shape and conidial dimension is common (Crous & Peerally 1996, Crous *et al.* 1998a).

The reliability of vesicle shape as a distinguishing morphological character has been questioned (Sober & Alfieri 1972, Hunter & Barnett 1978, Rossman 1983), although Crous *et al.* (1992) demonstrated experimentally that the shape of this structure can be influenced by the osmotic potential of the medium and the age of the culture, but that it remains a reliable morphological feature if these aspects are standardised. In the original description of *Ca. morganii* (= *Cy. scoparium*), the type of the anamorph, Morgan (1892) failed to include details of the stipe extension and terminal vesicle, which is a defining characteristic in distinguishing anamorphs of *Calonectria* (Boesewinkel 1982, Peerally 1991, Crous & Wingfield 1994, Crous 2002).

Calonectria spp. produce three different morphological forms of conidia, of which the macroconidia are present in all but *Ca. multiseptata* (Peerally 1991, Crous & Wingfield 1994, Crous *et al.* 1998b, Crous 2002). Mega- and microconidia are less frequently encountered and these are not regarded as important characters to distinguish between species (Sober 1971, Crous & Wingfield 1994, Crous & Seifert 1998, Crous 2002). Similar to vesicle shape,

significant variability can occur in the production of all conidial types, so that this feature alone is not always a reliable taxonomic character to define species.

Both homothallic and heterothallic mating systems are found amongst species of *Calonectria* (Alfieri *et al.* 1982, Schubert *et al.* 1989, Crous & Wingfield 1994, Crous 2002). Heterothallic *Calonectria* spp. have a biallelic heterothallic mating system with the female structures (protoperithecia) spermatised by conidia or hyphae of an opposite mating type strain (Schoch *et al.* 1999, 2000a, 2001a). Some *Calonectria* spp. have retained the ability to recombine with other closely related *Calonectria* spp., although the progeny from these crosses have low levels of fertility (Crous 2002). This has complicated the application of the biological species concept for *Calonectria,* although it has been useful for some species (Schoch *et al.* 1999, Lombard *et al.* 2010a).

Difficulties experienced in morphological identification, have led to several molecular approaches being employed to identify *Calonectria* spp. These include total protein electrophoresis (Crous *et al.* 1993a, El-Gholl *et al.* 1993), isozyme electrophoresis (El-Gholl *et al.* 1992, El-Gholl *et al.* 1997, Crous *et al.* 1998a), random amplification of polymorphic DNA (RAPD) (Overmeyer *et al.* 1996, Victor *et al.* 1997, Schoch *et al.* 2000a, Risède & Simoneau 2004), restriction fragment length polymorphisms (RFLP) (Crous *et al.* 1993b, Crous *et al.* 1995, Crous *et al.* 1997, Jeng *et al.* 1997, Victor *et al.* 1997, Risède & Simoneau 2001) and DNA hybridisation (Crous *et al.* 1993a, 1995, 1997, Victor *et al.* 1997). However, DNA sequence comparisons and associated phylogenetic inference has had the most significant impact on the taxonomy of the group. It is also most widely applied in contemporary species descriptions. The 5.8S ribosomal RNA gene and flanking internally transcribed spacer (ITS) sequences made it possible for Jeng *et al.* (1997) to distinguish between *Cy. scoparium* and *Cy. floridanum* isolates. Subsequently, it was found that this gene region contains few informative characters for members of the genus (Crous *et al.* 1999, Schoch *et al.* 1999, Risède & Simoneau 2001, Schoch *et al.* 2001b). As a consequence, this resulted in the β-tubulin (BT) (Schoch *et al.* 2001b) and histone H3 (HIS3) (Kang *et al.* 2001b) gene regions being widely employed to improve the resolution of phylogenetic trees for species of *Calonectria*.

The first complete DNA sequence-based phylogenetic study using partial BT gene sequences (Schoch *et al.* 2001b) compared phenotypic, biological and phylogenetic species concepts used in the taxonomy of *Calonectria.* Results showed that the genus represents a well resolved monophyletic lineage. Subsequently, combined DNA sequence data for the ITS, BT and HIS3 gene regions have been used to resolve taxonomic questions for *Calonectria* (Schoch *et al.* 2000a, Henricot & Culham 2002, Crous *et al.* 2004b, 2006). Other DNA sequences recently used to distinguish between species include the translation elongation factor 1–alpha (TEF-1α) and calmodulin (CAL) gene regions (Crous *et al.* 2004b, Lombard *et al.* 2009, 2010a, b). However, sequence data for these regions on GenBank (www.ncbi.nlm.nih.gov) are incomplete for the group, substantially reducing their value.

The aim of this study was to consider the identity of a large collection of previously unidentified *Calonectria* isolates collected over a five year period from various parts of the world. Morphological characteristics, phylogenetic inference and mating compatibility were employed for this purpose. Subsequently, the phylogenetic relationships between *Calonectria* spp. were re-evaluated by constructing a multigene phylogeny for seven gene regions and considering these results together with morphological features for all species in the genus.

MATERIALS AND METHODS

Isolates

Plant material showing symptoms of *Calonectria* infections as well as soil samples were collected from various geographical regions over a period of five years. Diseased plant material was placed in moist chambers and incubated for 48 h at room temperature to induce sporulation. Direct isolations were made onto malt extract agar (2 % w/v; MEA; Biolab, Midrand, South Africa) and cultures were incubated for 7 d at 25 °C under continuous near-ultraviolet light. Baiting, using seeds of *Medicago sativa*, was applied for the soil samples following the technique of Crous (2002). For each isolate, single conidial cultures were prepared on MEA. Representative strains are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa and the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands (Table 1).

DNA extraction and amplification

Identification of unknown *Calonectria* isolates

Total genomic DNA was extracted from 7 d old *Calonectria* cultures using the methods presented in Lombard *et al.* (2008). Three loci were amplified and sequenced. These included a fragment of the BT gene region using primers T1 (O'Donnell & Cigelnik 1997) and CYLTUB1R (Crous *et al.* 2004b), a fragment of the HIS3 gene region using primers CYLH3F and CYLH3R (Crous *et al.* 2004b) and a fragment of the TEF-1α gene region using primers EF1-728F (Carbone & Kohn 1999) and EF2 (O'Donnell *et al.* 1998).

Phylogenetic relationships amongst *Calonectria* spp.

Total genomic DNA was extracted as above. Seven loci were amplified including the ITS gene region using primers V9G (De Hoog & van den Ende 1998) and ITS4 (White *et al.* 1990); the 28S large subunit RNA gene (LSU) using primers LR0R (Moncalvo *et al.* 1995) and LR5 (Vilgalys & Hester 1990); and parts of the TEF-1α gene region; the BT gene region; the HIS3 gene region with the same primer sets mentioned previously, the actin (ACT) gene region using primers ACT-512F and ACT-783R (Carbone & Kohn 1999) and CAL gene region using primers CAL-228F and CAL-737R (Carbone & Kohn 1999).

The PCR reaction mixture used to amplify the different loci consisted of 2.5 units FastStart *Taq* polymerase (Roche Applied Science, USA), 1× PCR buffer, 1–1.5 mM MgCl2, 0.25 mM of each dNTP, 0.5 µm of each primer and approximately 30 ng of fungal genomic DNA, made up to a total reaction volume of 25 µL with sterile deionised water. Amplified fragments were purified using High Pure PCR Product Purification Kit (Roche, U.S.A.).

DNA sequencing and analysis

Amplified fragments were sequenced in both directions using the same primer pairs used for amplification. For this purpose, the BigDye terminator sequencing kit v. 3.1 (Applied Biosystems, U.S.A.) and an ABI PRISM™ 3100 DNA sequencer (Applied Biosystems) were used. All PCRs and sequencing reactions were performed on an Eppendorf Mastercycler Personal PCR (Eppendorf AG, Germany) with cycling conditions as described in Crous *et al.* (2006) for all loci amplified.

In addition to the sequences generated in this study, *Calonectria* spp. sequences were obtained from GenBank. All sequences were assembled and aligned using Sequence Navigator v. 1.0.1 (Applied Biosystems) and MAFFT v. 5.11 (Katoh *et al.* 2005), respectively. The aligned sequences were then manually corrected where necessary. Single nucleotide polymorphisms (SNP's) were determined for the aligned DNA sequences of each gene region using DnaSP v. 5.00.06 (Librado & Rozas 2009)

To determine whether the DNA sequence data sets were congruent, a partition homogeneity test (PHT; Farris *et al.* 1994) of all possible combinations, with 1 000 replications on all informative characters was conducted in PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Swofford 2002). A 70 % reciprocal bootstrap method using Neighbour-Joining with Maximum Likelihood distance (Mason-Gamer & Kellogg 1996; Gueidan *et al.* 2007) was also employed. Models of evolution were estimated in Modeltest v. 3.7 (Posada & Crandall 1998) using the Akaike Information Criterion (AIC) for each gene region. The bootstrap analyses were run in PAUP for 10 000 replicates. Resulting tree topologies were compared visually for conflict between the separate gene regions.

Maximum-parsimony genealogies, for single genes and the combined genes were estimated in PAUP, by heuristic searches based on 1 000 random addition sequences and tree bisection-reconnection, with the branch swapping option set on "best trees" only. All characters were weighted equally and alignment gaps were treated as missing data. Statistics calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC). Bootstrap analysis (Hillis & Bull 1993) was based on 1 000 replications. All sequences for the isolates studied were analysed using the Basic Local Alignment Search Tool for Nucleotide sequences (BLASTN, Altschul *et al.* 1990).

A Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees for each gene region and combined sequence data subsets with Bayesian probabilities using MrBayes v. 3.1.1 (Ronquist & Huelsenbeck 2003). Models of nucleotide substitution for each gene were determined using MrModeltest (Nylander 2004) and included for each gene partition. Four MCMC chains were run simultaneously from random trees for one million generations, sampled every 100 generations and repeated twice. Both runs converged on the same likelihood score and tree topology for each gene. The first 1 000 trees were, therefore, discarded as the burn-in phase of each analysis and posterior probabilities were determined from the remaining trees.

Sexual compatibility

Based on the results of the DNA sequence analyses, single conidial isolates of *Calonectria* spp. of unknown identity were crossed with closely related species in all possible combinations. Where available, mating tester strains defined in previous studies were also used. Crosses were made as described in Schoch *et al.* (1999) on carnation leaf agar (CLA; Fisher *et al.* 1982, Crous *et al.* 1993a) and minimal salt agar (MSA; Guerber & Correll 2001, Halleen *et al.* 2006) with sterile toothpicks placed on the surface of the agar (Lombard *et al.* 2010a). Controls consisted of isolates self-crossed, making it possible to distinguish between those having heterothallic or homothallic mating systems. Isolates CBS 125273–125276 from Indonesia were mated with *Ca. macroconidialis* (CBS 114880). Colombian isolates CBS 123698 and CMW 31210 and Indonesian isolates CBS 125258–125260 were crossed with *Ca. brachiatica* (CBS 123700 and CMW 25302) and *Ca. brassicae* (CBS 111478 and CBS 111869) in all possible combinations. Isolates CBS 125248, CBS 125253, CBS 125277 and CMW 14883 were crossed with *Ca. cerciana* (CBS 123693 and CBS 123695), *Ca. brasiliensis* (CBS 230.51 and CBS 114257) and mating tester strains of *Ca. insularis* (CBS 114558 and CBS 114559; Schoch *et al.* 1999). Similarly, isolates CBS 125249–125252, CBS 125261 and CBS 125269 were crossed with mating tester strains of *Ca. spathiphylli* (CBS 114540 and CBS 116168; Crous 2002). Isolates CBS 125254–125257 were crossed with mating tester strains of *Ca. scoparia* (CMW 31000 and CMW 31001; Lombard *et al.* 2010a) and *Ca. pauciramosa* (CMW 5683 and CMW 30823; Schoch *et al.* 2001a). The plates were stacked in plastic containers and incubated at 22 °C for 6–8 wk. Crosses were regarded as successful when isolate combinations produced numerous perithecia extruding viable ascospores.

Taxonomy

For identification of *Calonectria* isolates based on morphology, single conidial cultures were prepared on MEA and synthetic nutrient-poor agar (SNA; Nirenburg 1981, Lombard *et al.* 2009, 2010a, c). Inoculated plates were incubated at room temperature and examined after 7 d. Gross morphological characteristics of the anamorph structures were determined by mounting fungal structures in lactic acid and 30 measurements at ×1 000 magnification were made for all taxonomically informative characters for each isolate. Teleomorph morphology was determined by mounting perithecia resulting from the sexual compatibility tests in Leica mountant (Setpoint Premier, Johannesburg, South Africa) and making sections using a Leica CM1100 cryostat (Setpoint Technologies) at -20 °C. The 10 µm sections were mounted in lactophenol or 3 % KOH. Gross morphological characteristics were determined in the same manner as for the anamorph states. The 95 % confidence levels were determined and extremes of conidial measurements are given in parentheses. For other structures, only extremes are presented in the descriptions. Optimal growth conditions for cultures were determined in the dark on MEA for each isolate, at temperatures ranging from 5–35 °C at 5 °C intervals with three replicate plates for each temperature tested. Two measurements of culture diameter perpendicular to each other were made daily for 7 d. Colony colours were determined after 7 d on MEA at 25 °C in the dark, using the colour charts of Rayner (1970). Descriptions, nomenclature and illustrations were deposited in MycoBank (Crous *et al.* 2004a).

RESULTS

DNA sequencing and analysis

Identification of unknown *Calonectria* isolates

Amplicons of approx. 500 bp were generated for the BT and TEF-1α gene regions and those for the HIS3 region were approx. 450 bp in length. Based on preliminary BT sequence comparisons and morphological characteristics, the sequence data sets for the unknown *Calonectria* spp. were divided into four separate data sets representing the *Ca. colhounii*, *Ca. brassicae*, *Ca. scoparia* and *Ca. morganii* complexes and other closely related species in each data set. These data sets were analysed separately with *Ca. colombiensis* (CBS 112221) and *Ca. chinensis* (CBS 112744)

Table 1. Isolates of *Calonectria* spp. studied.

Species	Isolate number[1]	Other collections[1]	GenBank accession nr.[2]							Reference[3]
			ACT	BT	CAL	HIS3	ITS	LSU	TEF-1α	
Ca. acicola	CBS 114812		GQ280424	DQ190590	GQ267359	DQ190692	GQ280546	GQ280668	GQ267291	Gadgil & Dick (2004)
	CBS 114813^T	CMW 30996	GQ280425	DQ190591	GQ267360	DQ190693	GQ280547	GQ280669	GQ267292	
Ca. angustata	CBS 109065^T	CMW 30990 = CPC 2347 = P99-0454	GQ280426	AF207543	GQ267361	DQ190696	GQ280548	GQ280671	FJ918551	Crous (2002)
	CBS 109169	CMW 30983 = CPC 3152 = P99-1321	GQ280427	DQ190593	GQ267362	DQ190695	GQ280549	GQ280670	FJ918552	
Ca. asiatica	CBS 112711	CPC 3898 = SFE 744	GQ280429	AY725613	AY725738	AY725655	GQ280551	GQ280673	AY725702	Crous *et al.* (2004b)
	CBS 114073^T	CMW 23782 = CPC 3900 = SFE 726	GQ280428	AY725616	AY725741	AY725658	GQ280550	GQ280672	AY725705	
Ca. australiensis	CBS 112954^T	CMW 23669 = CPC 4714	GQ280430	DQ190596	GQ267363	DQ190699	GQ280552	GQ280674	GQ267293	Crous *et al.* (2006)
Ca. avesiculata	CBS 313.92^T	CMW 23670 = CPC 2373 = ATCC 38226	GQ280431	AF333392	GQ267364	DQ190620	GQ280553	GQ280675	GQ267294	Crous (2002)
Ca. brachiatica	CBS 123700^T	CMW 25298	GQ280433	FJ696388	GQ267366	FJ696396	GQ280555	GQ280677	GQ267296	Lombard *et al.* (2009)
	CMW 25302		GQ280432	FJ716708	GQ267365	FJ716712	GQ280554	GQ280676	GQ267295	
Ca. brassicae	CBS 111478	CMW 30981	GQ280455	DQ190611	GQ267383	DQ190719	GQ280577	GQ280699	FJ918567	Crous (2002)
	CBS 111869^T	CMW 30982 = CPC 2409 = PC 551197	GQ280454	AF232857	GQ267382	DQ190720	GQ280576	GQ280698	FJ918566	
Ca. brasiliensis	CBS 230.51^T	CMW 23670 = CPC 2390	GQ280502	GQ267241	GQ267421	GQ267259	GQ280624	GQ280746	GQ267328	Lombard *et al.* (2009c)
	CBS 114257	CMW 32949 = CPC 1944	GQ280503	GQ267242	GQ267422	GQ267260	GQ280625	GQ280747	GQ267329	
Ca. canadensis	CBS 110817^T	CMW 23673 = CPC 499	GQ280434	AF348212	AY725743	AF348228	GQ280556	GQ280678	GQ267297	Crous (2002)
Ca. cerciana	CBS 123693^T	CMW 25309	GQ280437	FJ918510	GQ267369	FJ918528	GQ280559	GQ280681	FJ918559	Lombard *et al.* (2010c)
	CBS 123695	CMW 25290	GQ280438	FJ918511	GQ267370	FJ918529	GQ280560	GQ280682	FJ918560	
Ca. chinensis	CBS 112744	CMW 30986 = CPC 4104	GQ280440	AY725618	AY725746	AY725660	GQ280562	GQ280684	AY725709	Crous *et al.* (2004b)
	CBS 114827^T	CMW 23674 = CPC 4101	GQ280390	AY725619	AY725747	AY725661	GQ280561	GQ280683	AY725710	
Ca. citri	CBS 186.36^T	CMW 23675	GQ280441	AF333393	GQ267371	GQ267247	GQ280563	GQ280685	GQ267299	Crous (2002)
Ca. clavata	CBS 114557^T	CMW 23690 = CPC 2536 = ATCC 66389	GQ280449	AF333396	GQ267377	DQ190623	GQ280571	GQ280693	GQ267305	Crous (2002)
	CBS 114666^T	CMW 30994 = CPC 2537	GQ280450	DQ190549	GQ267378	DQ190624	GQ280572	GQ280694	GQ267306	
Ca. colhounii	CBS 293.79^T	CMW 30999	GQ280443	DQ190564	GQ267373	DQ190639	GQ280565	GQ280687	GQ267301	Crous (2002)
	CBS 114704		GQ280442	DQ190563	GQ267372	DQ190638	GQ280564	GQ280686	GQ267300	
Ca. colombiana	CBS 115127^T	CMW 30871 = CPC 1160	GQ280538	FJ972423	GQ267455	FJ972442	GQ280660	GQ280782	FJ972492	Lombard *et al.* (2010a)
	CBS 115638	CMW 30766 = CPC 1161	GQ280539	FJ972422	GQ267456	FJ972441	GQ280661	GQ280783	FJ972491	
Ca. colombiensis	CBS 112220^T	CMW 23676 = CPC 723	GQ280444	GQ267207	AY725748	AY725662	GQ280566	GQ280688	AY725711	Crous *et al.* (2004b)
	CBS 112221	CMW 30985 = CPC 724	GQ280445	AY725620	AY725749	AY725663	GQ280567	GQ280689	AY725712	Crous (2002)

Table 1. (Continued).

Species	Isolate number[1]	Other collections[1]	GenBank accession nr.[2]							Reference[3]
			ACT	BT	CAL	HIS3	ITS	LSU	TEF-1α	
Ca. curvispora	CBS 116159^T	CMW 23693	GQ280446	AF333394	GQ267374	AY725664	GQ280568	GQ280690	GQ267302	Crous (2002)
Ca. densa	CBS 125249	CMW 31184	GQ280523	GQ267230	GQ267442	GQ267279	GQ280645	GQ280767	GQ267350	This study
	CBS 125250	CMW 31185	GQ280524	GQ267231	GQ267443	GQ267280	GQ280646	GQ280768	GQ267351	
	CBS 125261^T	CMW 31182	GQ280525	GQ267232	GQ267444	GQ267281	GQ280647	GQ280769	GQ267352	
Ca. ecuadoriae	CBS 111394	CMW 30980 = CPC 1628	GQ280448	DQ190599	GQ267376	DQ190704	GQ280570	GQ280692	GQ267304	Crous *et al.* (2006)
	CBS 111406^T	CMW 23677 = CPC 1635	GQ280447	DQ190600	GQ267375	DQ190705	GQ280569	GQ280691	GQ267303	
Ca. eucalypti	CBS 125273	CMW 14890	GQ280510	GQ267217	GQ267429	GQ267266	GQ280632	GQ280754	GQ267337	This study
	CBS 125274	CMW 18443	GQ280509	GQ267216	GQ267428	GQ267265	GQ280631	GQ280753	GQ267336	
	CBS 125275^T	CMW18444	GQ280511	GQ267218	GQ267430	GQ267267	GQ280633	GQ280755	GQ267338	
	CBS 125276	CMW 18445	GQ280512	GQ267219	GQ267431	GQ267268	GQ280634	GQ280756	GQ267339	
Ca. gordoniae	CBS 112142	CMW 23694 = CPC 3136 = ATCC 201837	GQ280453	AF449449	GQ267381	DQ190708	GQ280575	GQ280697	GQ267309	Leahy *et al.* (2000)
Ca. gracilipes	CBS 111141^T		GQ280457	DQ190566	GQ267385	DQ190644	GQ280579	GQ280701	GQ267311	Crous (2002)
	CBS 115674		GQ280456	AF333406	GQ267384	DQ190645	GQ280578	GQ280700	GQ267310	
Ca. gracilis	CBS 111284		GQ280489	DQ190567	GQ267408	DQ190647	GQ280611	GQ280733	GQ267324	Crous (2002)
	CBS 111807		GQ280488	AF232858	GQ267407	DQ190646	GQ280610	GQ280734	GQ267323	
Ca. hawksworthii	CBS 111870^T	CPC 2405 = MUCL 30866	GQ280458	AF333407	GQ267386	DQ190649	GQ280580	GQ280702	FJ918558	Crous (2002)
Ca. hongkongensis	CBS 114711	CMW 30995	GQ280460	AY725621	AY725754	AY725666	GQ280582	GQ280704	AY725716	Crous *et al.* (2004b)
	CBS 114828^T		GQ280459	AY725622	AY725755	AY725667	GQ280581	GQ280703	AY725717	
Ca. humicola	CBS 125251^T	CMW 31183	GQ280526	GQ267233	GQ267445	GQ267282	GQ280648	GQ280770	GQ267353	This study
	CBS 125252	CMW 31186	GQ280527	GQ267234	GQ267446	GQ267283	GQ280649	GQ280771	GQ267354	
	CBS 125269	CMW31187	GQ280528	GQ267235	GQ267447	GQ267284	GQ280650	GQ280772	GQ267355	
Ca. hurae	CBS 114551	CMW 16720 = CPC 2344	GQ280461	AF333408	GQ267387	DQ190728	GQ280583	GQ280705	FJ918548	Crous (2002)
Ca. ilicicola	CBS 190.50^T	CMW 30998 = CPC 2482 = IMI 299389	GQ280483	AY725631	AY725764	AY725676	GQ280605	GQ280727	AY725726	Crous (2002)
	CBS 115897		GQ280484	AY725647	GQ267403	GQ267256	GQ280606	GQ280728	AY725729	
Ca. indonesiae	CBS 112823^T	CMW 23683 = CPC 4508	GQ280463	AY725623	AY725756	AY725668	GQ280585	GQ280707	AY725718	Crous *et al.* (2004b)
	CBS 112840	CPC 4547	GQ280464	AY725625	AY725758	AY725670	GQ280586	GQ280708	AY725720	
Ca. indusiata	CBS 144.36	CMW 23699	GQ280536	GQ267239	GQ267453	GQ267262	GQ280658	GQ280780	GQ267332	Crous (2002)
	CBS 114684	CPC 2446 = UFV 16A	GQ280537	AF232862	GQ267454	DQ190652	GQ280659	GQ280781	GQ267333	
Ca. insularis	CBS 114558^T	CMW 30991	GQ280465	AF210861	GQ267389	FJ918526	GQ280587	GQ280709	FJ918556	Crous (2002)
	CBS 114559	CMW 30992	GQ280466	AF210862	GQ267390	FJ918525	GQ280588	GQ280710	FJ918555	
Ca. kyotensis	CBS 170.77	CMW 23679 = IMI 299388	GQ280452	GQ267209	GQ267380	GQ267249	GQ280574	GQ280696	GQ267308	Crous (2002)
	CBS 413.67	CMW 23678 = CPC 2391	GQ280451	GQ267208	GQ267379	GQ267248	GQ280573	GQ280695	GQ267307	
Ca. leguminum	CBS 728.68^T	CMW 23684 = IMI 299578	GQ280467	AF389837	GQ267391	DQ190654	GQ280589	GQ280711	FJ918547	Crous (2002)
Ca. leucothoës	CBS 109166	CMW 30977 = CPC 3612 = P97-2605	GQ280468	FJ918508	GQ267392	FJ918523	GQ280590	GQ280712	FJ918553	Crous (2002)
Ca. macroconidialis	CBS 114880^T	CPC 307	GQ280469	AF232855	GQ267393	DQ190655	GQ280591	GQ280713	GQ267313	Crous (2002)

Table 1. (Continued).

Species	Isolate number[1]	Other collections[1]	GenBank accession nr.[2]							Reference[3]
			ACT	BT	CAL	HIS3	ITS	LSU	TEF-1α	
Ca. madagascariensis	CBS 114571	CMW 30993 = CPC 2253	GQ280471	DQ190571	GQ267395	DQ190657	GQ280593	GQ280715	GQ267315	Crous (2002)
	CBS 114572^{T}	CMW 23686 = CPC 2252	GQ280470	DQ190572	GQ267394	DQ190658	GQ280592	GQ280714	GQ267314	
Ca. malesiana	CBS 112710	CPC 3899	GQ280473	AY725626	AY725759	AY725671	GQ280595	GQ280717	AY725721	Crous *et al.* (2004b)
	CBS 112752^{T}	CMW 23687 = CPC 4223	GQ280472	AY725627	AY725760	AY725672	GQ280594	GQ280716	AY725722	
Ca. mexicana	CBS 110918^{T}	CMW 9055	GQ280474	AF210863	GQ267396	FJ972460	GQ280596	GQ280718	FJ972526	Crous (2002)
Ca. morganii	CBS 110666	CMW 30978 = P90.1479	GQ280504	FJ918509	GQ267423	FJ918527	GQ280626	GQ280748	FJ9188557	Crous (2002)
Ca. multiphialidica	CBS 112678	CMW 23688	GQ280475	AY725628	AY725761	AY725673	GQ280597	GQ280719	AY725723	Crous *et al.* (2004b)
Ca. multiseptata	CBS 112682	CMW 23692 = CPC 1589	GQ280476	DQ190573	GQ267397	DQ190659	GQ280598	GQ280720	FJ918535	Crous (2002)
Ca. naviculata	CBS 101121^{T}	CMW 30974	GQ280478	GQ267211	GQ267399	GQ267252	GQ280600	GQ280722	GQ267317	Crous (2002)
	CBS 116080	CMW 16723	GQ280477	AF333409	GQ267398	GQ267251	GQ280599	GQ280721	GQ267316	
Ca. orientalis	CBS 125258	CMW 20272	GQ280531	GQ267238	GQ267450	GQ267287	GQ280653	GQ280775	GQ267358	This study
	CBS 125259	CMW 20273	GQ280530	GQ267237	GQ267449	GQ267286	GQ280652	GQ280774	GQ267357	
	CBS 125260^{T}	CMW 20291	GQ280529	GQ267236	GQ267448	GQ267285	GQ267651	GQ280773	GQ267356	
Ca. ovata	CBS 111299	CMW 16724	GQ280479	GQ267212	GQ267400	GQ267253	GQ280601	GQ280723	GQ267318	Crous (2002)
	CBS111307	CMW 30979	GQ280480	AF210868	GQ267401	GQ267254	GQ280602	GQ280724	GQ267319	
Ca. pacifica	CBS 109063	CMW 16726 = IMI 35428	GQ280481	GQ267213	AY725762	GQ267255	GQ280603	GQ280725	AY725724	Crous (2002)
	CBS 114038	CMW 30988	GQ280482	AY725630	GQ267402	AY725675	GQ280604	GQ280726	GQ267320	
Ca. pauciramosa	CMW 5683^{T}	CPC 971	GQ280486	FJ918514	GQ267405	FJ918531	GQ280608	GQ280730	FJ918565	Crous (2002)
	CMW30823	CPC 416	GQ280485	FJ918515	GQ280404	FJ918532	GQ280607	GQ280729	FJ918566	
Ca. penicilloides	CBS 174.55^{T}	CMW 23696	GQ280487	AF333414	GQ267406	GQ267257	GQ280609	GQ280731	GQ267322	Crous (2002)
Ca. pini	CBS 123698^{T}	CMW 31209	GQ280517	GQ267224	GQ267436	GQ267273	GQ280639	GQ280761	GQ267344	This study
	CBS 125523	CMW 31210	GQ280518	GQ267225	GQ267437	GQ267274	GQ280640	GQ280762	GQ267345	
Ca. polizzii	CBS 125270	CMW 7804	GQ280544	FJ972417	GQ267461	FJ972436	GQ280666	GQ280788	FJ972486	Lombard *et al.* (2010a)
	CBS 125271	CMW 10151	GQ280545	FJ972418	GQ267462	FJ972437	GQ280667	GQ280789	FJ972487	
Ca. pseudonaviculata	CBS 114417^{T}	CMW 23672	GQ280490	GQ267214	GQ267409	GQ267258	GQ280612	GQ280734	GQ267325	Crous *et al.* (2002)
Ca. pseudoreteaudii	CBS 123694^{T}	CMW 25310	GQ280492	FJ918504	GQ267411	FJ918519	GQ280614	GQ280736	FJ918541	Lombard *et al.* (2010c)
	CBS 123696	CMW 25292	GQ280491	FJ918505	GQ267410	FJ918520	GQ280613	GQ280735	FJ918542	
Ca. pseudoscoparia	CBS 125254	CMW 15214	GQ280519	GQ267226	GQ267438	GQ267275	GQ280641	GQ280763	GQ267346	This study
	CBS 125255	CMW 15215	GQ280520	GQ267227	GQ267439	GQ267276	GQ280642	GQ280764	GQ267347	
	CBS 125256	CMW 15216	GQ280521	GQ267228	GQ267440	GQ267277	GQ280643	GQ280765	GQ267348	
	CBS 125257^{T}	CMW 15218	GQ280522	GQ267229	GQ267441	GQ267278	GQ280644	GQ280766	GQ267349	
Ca. pseudospathiphylli	CBS 109162^{T}	CMW 30976 = CPC 1623	GQ280493	FJ918513	GQ267412	AF348241	GQ280615	GQ280737	FJ918562	Crous (2002)
Ca. pteridis	CBS 111793^{T}	CMW 16736 = CPC 2372 = ATCC 34395	GQ280494	DQ190578	GQ267413	DQ190679	GQ280616	GQ280738	FJ918563	Crous (2002)
	CBS 111871	CMW 30982 = CPC 2443	GQ280495	DQ190579	GQ267414	DQ190680	GQ280617	GQ280739	FJ918564	
Ca. pyrochoa	CBS 749.70^{T}	CMW 23682	GQ280462	GQ267210	GQ267388	GQ267250	GQ280584	GQ280706	GQ267312	Crous *et al.* (2006)

Table 1. (Continued).

Species	Isolate number[1]	Other collections[1]	GenBank accession nr.[2]							Reference[3]
			ACT	BT	CAL	HIS3	ITS	LSU	TEF-1α	
Ca. queenslandica	CBS 112146^{T}	CMW 30604 = CPC 3213	GQ280496	AF389835	GQ267415	FJ918521	GQ280618	GQ280740	FJ918543	Lombard *et al.* (2010c)
	CBS 112155	CMW 30603 = CPC 3210	GQ280497	AF389834	GQ267416	DQ190667	GQ280619	GQ280741	FJ918544	
Ca. reteaudii	CBS 112143	CMW 16738 = CPC 3200	GQ280499	GQ240642	GQ267418	DQ190660	GQ280621	GQ280743	FJ918536	Crous (2002)
	CBS 112144^{T}	CMW 30984 = CPC 3201	GQ280498	AF389833	GQ267417	DQ190661	GQ280620	GQ280742	FJ918537	
Ca. rumohrae	CBS 109062	CMW 30989 = CPC 1603	GQ280501	AF232873	GQ267420	DQ190676	GQ280623	GQ280745	FJ918550	Crous (2002)
	CBS 111431^{T}	CMW 23697 = CPC 1716	GQ280500	AF232871	GQ267419	DQ190675	GQ280622	GQ280744	FJ918549	
Ca. scoparia	CMW 31000	CPC 1675 = UFV 117	GQ280435	FJ972426	GQ267367	FJ972476	GQ280557	GQ280679	FJ972525	Crous (2002)
	CMW 31001	UFV 126	GQ280436	FJ972427	GQ267368	GQ267246	GQ280558	GQ280680	GQ267246	
Ca. spathiphylli	CBS 114540	CMW 16742	GQ280505	AF348214	GQ267424	AF348230	GQ280627	GQ280749	GQ267330	Crous (2002)
	CBS 116168	CMW 30997	GQ280506	FJ918512	GQ267425	FJ918530	GQ280628	GQ280750	FJ918561	
Ca. spathulata	CBS 555.92	CMW 16744	GQ280508	GQ267215	GQ267427	GQ267261	GQ280630	GQ280752	GQ267331	Crous (2002)
	CBS 112689	CMW 16745	GQ280507	AF308463	GQ267426	FJ918524	GQ280629	GQ280751	FJ918554	
Ca. sulawesiensis	CBS 125248	CMW 14857	GQ280516	GQ267223	GQ267435	GQ267272	GQ280638	GQ280760	GQ267343	This study
	CBS 125253	CMW 14879	GQ280513	GQ267220	GQ267432	GQ267269	GQ280635	GQ280757	GQ267340	
	CBS 125277^{T}	CMW 14878	GQ280515	GQ267222	GQ267434	GQ267271	GQ280637	GQ280759	GQ267342	
	CMW 14883		GQ280514	GQ267221	GQ267433	GQ267270	GQ280636	GQ280758	GQ267341	
Ca. sumatrensis	CBS 112829^{T}	CMW 23698 = CPC4518	GQ280532	AY725649	AY725771	AY725696	GQ280654	GQ280776	AY725733	Crous *et al.* (2004b)
	CBS 112934	CMW 30987 = CPC 4516	GQ280533	AY725651	AY725773	AY725798	GQ280655	GQ280777	AY725735	
Ca. terrae-reginae	CBS 112151^{T}	CMW 30601 = CPC 3202	GQ280534	FJ918506	GQ267451	FJ918522	GQ280656	GQ280778	FJ918545	Lombard *et al.* (2010c)
	CBS 112634	CMW 30602 = CPC 4233	GQ280535	FJ918507	GQ267452	DQ190668	GQ280657	GQ280779	FJ918546	
Ca. variabilis	CBS 112691	CMW 2914	GQ280541	GQ267240	GQ267458	GQ267264	GQ280663	GQ280785	GQ267335	Crous (2002)
	CBS 114677	CMW 3187	GQ280540	AF333424	GQ267457	GQ267263	GQ280662	GQ280764	GQ267334	
Ca. zuluensis	CBS 125268	CMW 9188^{T}	GQ280542	FJ972414	GQ267459	FJ972433	GQ280664	GQ280786	FJ972483	Lombard *et al.* (2010a)
	CBS 125272	CMW 9896	GQ280543	FJ972415	GQ267460	FJ972434	GQ280665	GQ280787	FJ972484	

[1] CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Pedro Crous working collection housed at CBS; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; ATCC: American Type Culture Collection, Virginia, U.S.A.; UFV: Univeridade Federal de Viçosa, Brazil. [2] ACT = Actin, BT = β-tubulin, CAL = Calmodulin, HIS3 = Histone H3, ITS = Internal transcribed spacer regions 1 and 2 and the 5.8S gene of the ribosomal RNA, LSU = 28S large subunit RNA, TEF-1α = Translation elongation factor 1-alpha. [3] References used for species descriptions. ^{T} Ex-type cultures.

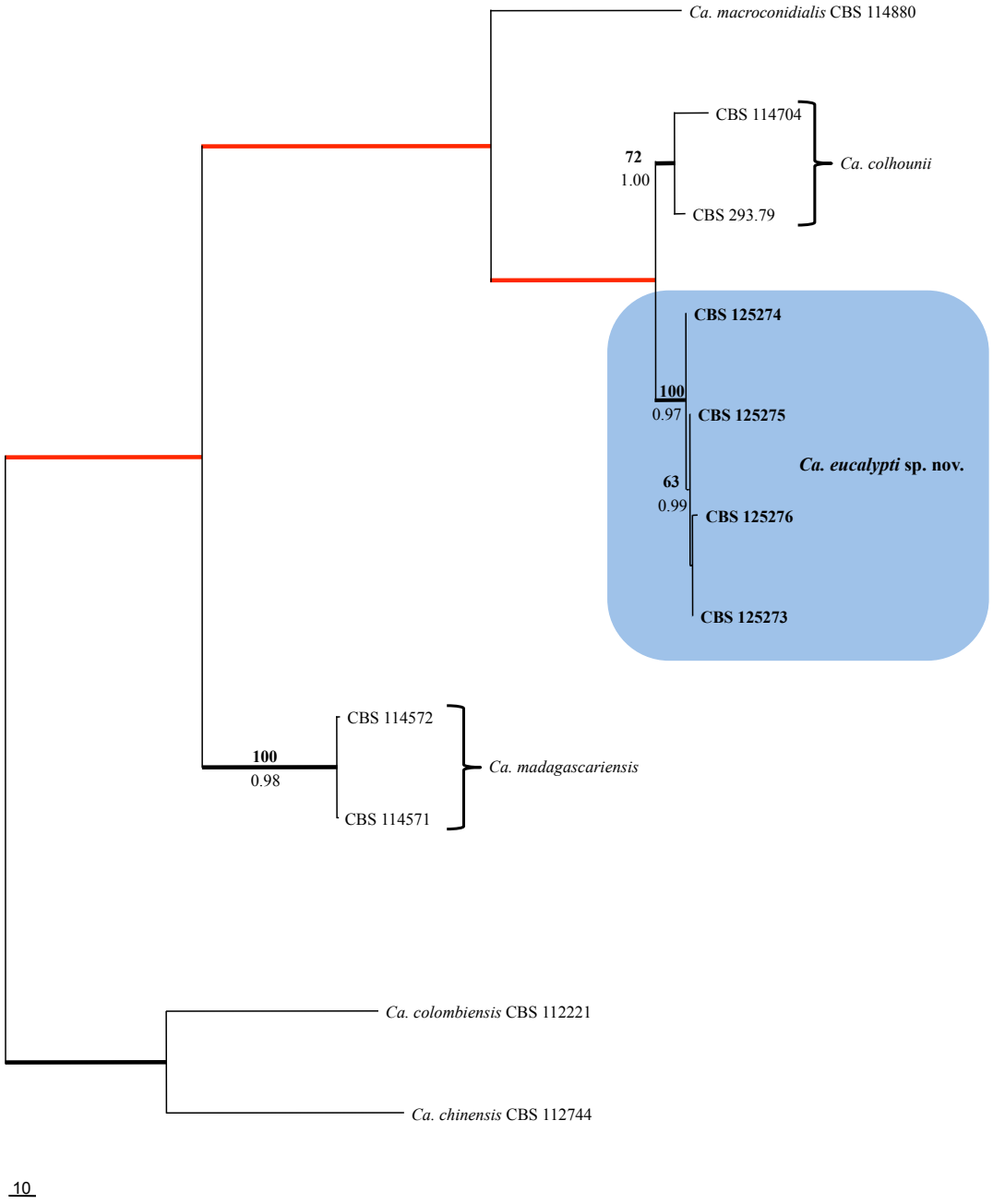

Fig. 1. The most parsimonious trees obtained from a heuristic search with 1 000 random additions sequences of the combined BT, HIS3 and TEF-1α sequence alignments of the *Ca. colhounii* complex. Scale bar shows 10 changes and bootstrap support values (bold) from 1 000 replicates and Bayesian posterior probability values are indicated at the nodes. Red lines indicate bootstrap values of 100 and posterior probabilities of 1.00. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses.

Table 2. Single nucleotide polymorphisms comparisons between *Ca. eucalypti* and *Ca. colhounii*, compared to *Ca. macroconidialis* and *Ca. madagascariensis*.

Species	Isolate no.	β-tubulin				Histone H3					TEF-1α								
		167	207	398	507	58	290	362	454	455	43	105	106	107	108	109	264	457	472
Ca. colhounii	CBS 293.79	A	G	A	C	A	A	C	A	C	C	A	C	A	A	C	G	C	C
	CBS 114704	A	G	A	C	A	A	C	A	C	C	A	C	A	A	C	G	C	C
Ca. eucalypti	CBS 125273	G	T	G	T	-	T	T	C	A	A	-	-	-	-	-	A	T	T
	CBS 125274	G	T	G	T	-	T	T	C	A	A	-	-	-	-	-	A	T	T
	CBS 125275	G	T	G	T	-	T	T	C	A	A	-	-	-	-	-	A	T	T
	CBS 125276	G	T	G	T	-	T	T	C	A	A	-	-	-	-	-	A	T	T
Ca. macroconidialis	CBS 114880	C	G	A	C	A	A	T	A	C	C	C	A	A	C	C	C	T	C
Ca. madagascariensis	CBS 114571	C	G	A	T	T	A	G	A	C	C	C	C	A	C	C	C	C	A
	CBS 114572	C	G	A	T	T	A	G	A	C	C	C	C	A	C	C	C	C	A

Table 3. Single nucleotide polymorphisms from the sequence datasets for *Ca. pini* and *Ca. orientalis* compared to *Ca. brachiatica* and *Ca. brassicae*.

Species	Isolate no.	β-tubulin								Histone H3										
		84	91	121	202	380	382	395	518	12	58	59	61	62	65	71	105	255	268	270
Ca. brachiatica	CBS 123700	A	G	A	A	T	C	A	-	T	-	T	C	A	T	C	C	T	A	A
	CMW 25302	A	G	A	A	T	C	A	-	T	-	T	C	A	T	C	C	T	A	A
Ca. brassicae	CBS 111478	A	G	C	G	G	T	G	-	T	A	T	C	C	C	C	C	C	C	A
	CBS 111869	A	G	C	G	G	T	G	-	T	A	T	C	C	C	C	C	C	C	A
Ca. pini	CBS 123698	A	C	C	G	G	T	G	C	G	-	-	T	C	C	-	A	C	C	A
	CMW 31210	A	C	C	G	G	T	G	C	G	-	-	T	C	C	-	A	C	C	A
Ca. orientalis	CBS 125258	G	G	C	G	G	T	G	-	T	A	T	C	C	C	C	C	C	C	G
	CBS 125259	G	G	C	G	G	T	G	-	T	A	T	C	C	C	C	C	C	C	G
	CBS 125260	G	G	C	G	G	T	G	-	T	A	T	C	C	C	C	C	C	C	G

Species	Isolate no.	TEF-1α																															
		4	12	49	61	62	65	79	93	124	141	142	186	194	195	196	197	198	199	200	201	236	240	246	259	273	428	447	448	449	465	473	493
Ca. brachiatica	CBS 123700	C	T	C	G	C	C	C	A	T	T	T	G	T	-	-	-	-	C	A	T	C	T	G	T	C	G	C	C	A	G	G	-
	CMW 25302	C	T	C	G	C	C	C	A	T	T	T	G	T	-	-	-	-	C	A	T	C	T	G	T	C	G	C	C	A	G	G	-
Ca. brassicae	CBS 111478	T	C	-	G	C	C	C	A	T	-	-	G	T	-	-	-	-	C	A	T	C	T	G	T	C	G	C	C	A	C	G	C
	CBS 111869	T	C	-	G	C	C	C	A	T	-	-	G	T	-	-	-	-	C	A	T	C	T	G	T	C	G	C	C	A	C	G	C
Ca. pini	CBS 123698	G	T	C	C	A	G	C	G	A	T	T	A	A	A	A	A	A	G	C	C	T	A	C	C	C	A	-	-	-	G	A	C
	CMW 31210	G	T	C	C	A	G	C	G	A	T	T	A	A	A	A	A	A	G	C	C	T	A	C	C	C	A	-	-	-	G	A	C
Ca. orientalis	CBS 125258	T	T	C	C	C	C	T	G	T	T	T	G	A	-	-	-	-	G	C	C	T	A	C	C	T	A	-	-	-	G	G	C
	CBS 125259	T	T	C	C	C	C	T	G	T	T	T	G	A	-	-	-	-	G	C	C	T	A	C	C	T	A	-	-	-	G	G	C
	CBS 125260	T	T	C	C	C	C	T	G	T	T	T	G	A	-	-	-	-	G	C	C	T	A	C	C	T	A	-	-	-	G	G	C

as outgroup taxa. For Bayesian analyses, a HKY+I+G model was selected for BT and TEF-1α, and GTR+I+G for HIS3 for all four data sets, which was incorporated in the analyses. The consensus trees obtained from the Bayesian analyses confirmed the tree topologies obtained with maximum-parsimony as well as bootstrap support. Therefore, only maximum-parsimony trees are presented with bootstrap values and posterior probabilities shown for well-supported branches.

The partition homogeneity tests for all possible combinations of the three gene regions used, consistently yielded a P-value of 0.001 for the four separate data sets. The 70 % reciprocal bootstrap trees showed no conflict in tree topologies for the three gene regions in each of the four separate data sets. Based on the tree topologies of the 70 % reciprocal bootstrap trees and a P-value of 0.001 in the PHT (Cunningham 1997, Dettman *et al.* 2003) the DNA sequences for the three gene regions were combined for each of the four separate data sets.

The combined sequence data set representing the *Ca. colhounii* complex, with 10 taxa including outgroups, consisted of 1 497 characters, including gaps. Of these characters, 1 051 were constant, 133 were parsimony-uninformative and 313 characters were parsimony informative. Parsimony analysis of the aligned sequences yielded one most parsimonious tree (Fig. 1; TL = 649 steps; CI = 0.888; RI = 0.891; RC = 0.791). In the tree, isolates CBS 125273–125276, from Indonesia, grouped close to but separate from *Ca. colhounii* (CBS 293.79 and CBS 114704) with 100 % bootstrap support (BP) and a posterior probability (PP) of 0.97. The SNP analyses showed 16 unique alleles for the Indonesian isolates with one shared unique allele with *Ca. madagascariensis* (CBS 114571 and CBS 114572) and two shared alleles with *Ca. macroconidialis* (CBS 114880) for the three gene regions analysed (Table 2). These unique alleles, however, distinguish the Indonesian isolates from *Ca. colhounii*, *Ca. macroconidialis* and *Ca. madagascariensis.*

The data set representing the *Ca. brassicae* complex consisted of 15 taxa including the outgroups, while the combined sequence alignment was made up of 1 509 characters, including gaps. These characters represented 1 092 constant, 127 parsimony-uninformative and 290 parsimony-informative characters. Parsimony analysis yielded one most parsimonious tree (Fig. 2; TL = 569 steps; CI = 0.931; RI = 0.918; RC = 0.855). In the tree, Colombian isolates CBS 123698 and CBS 125523 clustered close to *Ca. brassicae* (CBS 111869 and CBS 111478) and *Ca. brachiatica* (CBS 123700 and CMW 25302) but separately from both these species with high support (BP = 100 and PP = 1.00). Similarly, isolates CBS 125258–125260, from Indonesia, clustered together closely related to *Ca. brassicae* and *Ca. brachiatica*. These Indonesian isolates were also closely related to the Colombian isolates but grouped separately from them in a clade with high support (BP = 97 and PP = 1.00). The SNP analyses showed that isolates CBS 123698 and CBS 125523 have 18 unique alleles and isolates CBS 125258–125260 have four unique alleles distinguishing them from each other for the three gene regions analysed. These isolates also share 14 unique alleles, distinguishing them from *Ca. brassicae* and *Ca. brachiatica* (Table 3).

The third data set, represented by 16 ingroup taxa residing in the *Ca. scoparia* complex and closely related species, consisted of 1 530 characters including gaps for the three gene regions analysed. Of these characters, 1 114 were constant, 138 were parsimony-uninformative and 278 characters were parsimony informative. Parsimony analysis of the aligned sequences yielded two most parsimonious trees (TL = 551 steps; CI = 0.902; RI = 0.925; RC = 0.834), one of which is presented in Fig. 3. In the tree,

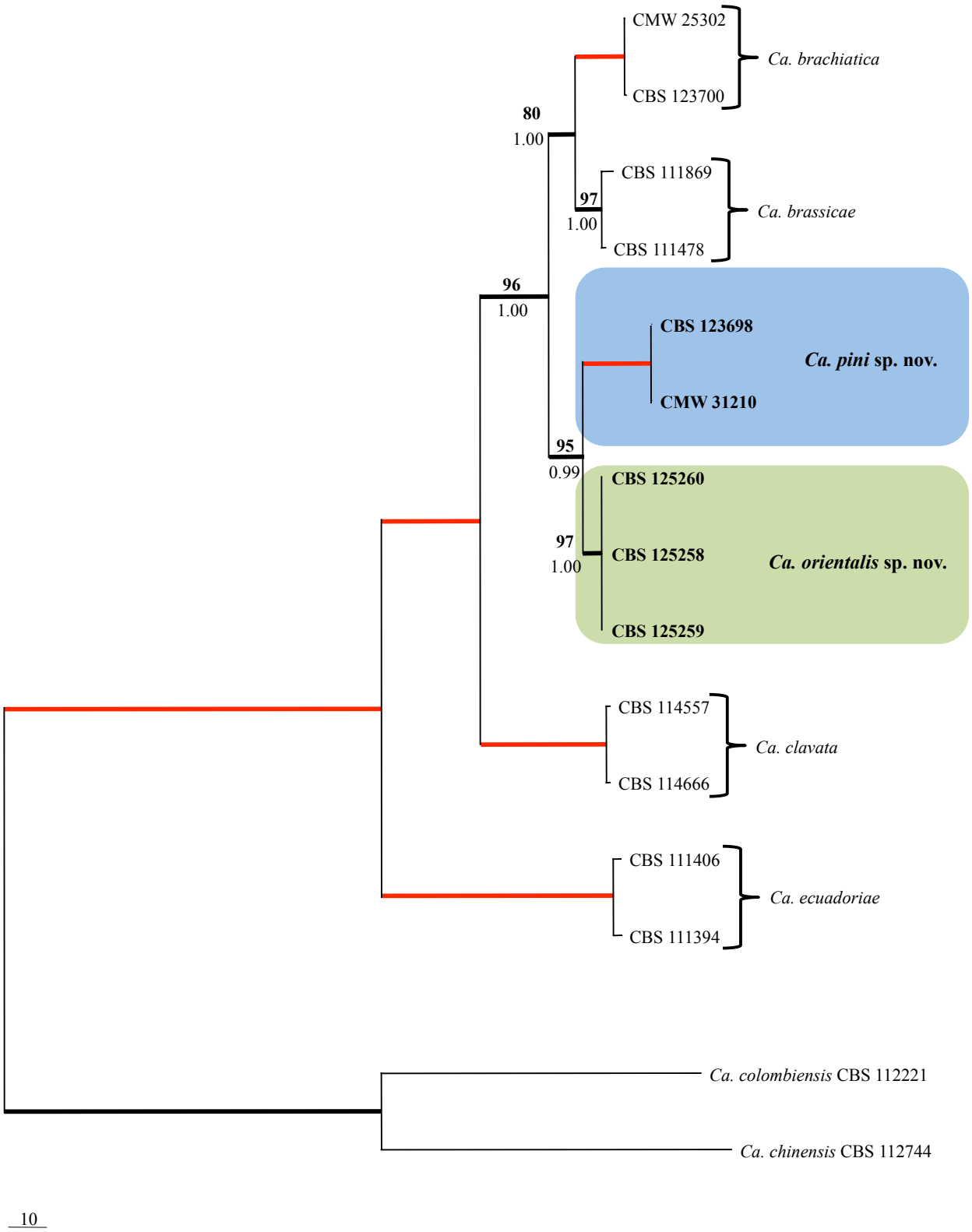

Fig. 2. The most parsimonious trees obtained from a heuristic search with 1 000 random additions sequences of the combined BT, HIS3 and TEF-1α sequence alignments of the *Ca. brassicae* complex. Scale bar shows 10 changes and bootstrap support values (bold) from 1 000 replicates and Bayesian posterior probability values are indicated at the nodes. Red lines indicate bootstrap values of 100 and posterior probabilities of 1.00. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses.

isolates CBS 125254–125257 from Ecuador, clustered closely but separately from *Ca. scoparia* (CMW 31000 and CMW 31001) and other species in the *Ca. pauciramosa* complex with low support (BP = 63 and PP = 1.00). The Ecuadorian isolates also had three unique alleles separating them from *Ca. scoparia* and *Ca. pauciramosa* (CMW 5683 and CMW 30823) for the BT and TEF-1α regions, but there were no unique alleles for these isolates in the HIS3 region (Table 4).

The aligned sequence data set for the *Ca. morganii* complex included 25 ingroup taxa consisting of 1 535 characters. Of these characters, 975 were constant, 211 were parsimony-uninformative and 349 characters were parsimony-informative. Parsimony analysis

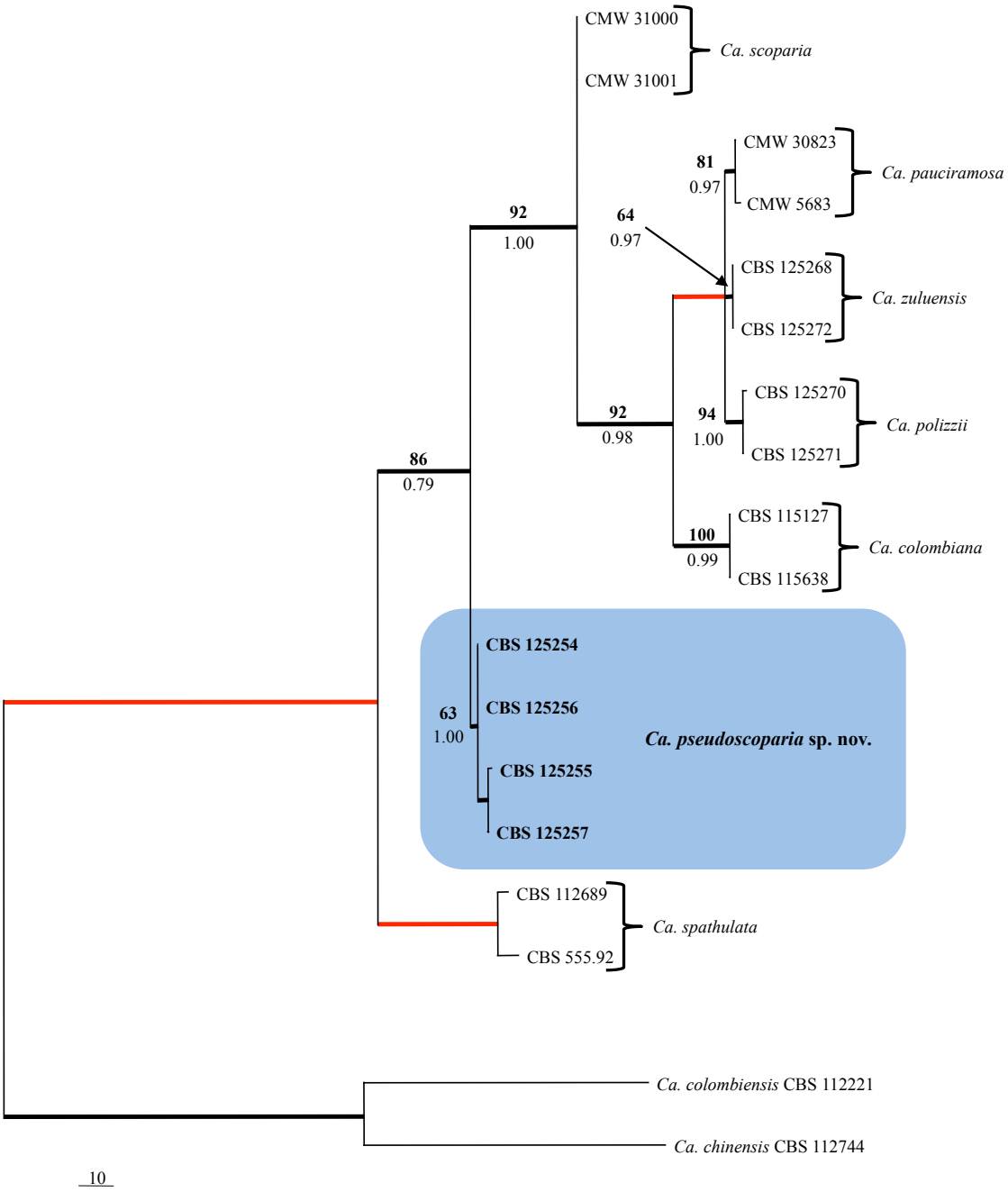

Fig. 3. One of two most parsimonious trees obtained from a heuristic search with 1 000 random additions sequences of the combined BT, HIS3 and TEF-1α sequence alignments of the *Ca. scoparia* complex. Scale bar shows 10 changes and bootstrap support values (bold) from 1 000 replicates and Bayesian posterior probability values are indicated at the nodes. Red lines indicate bootstrap values of 100 and posterior probabilities of 1.00. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses.

Table 4. Single nucleotide polymorphisms comparisons between *Ca. scoparia* and *Ca. pseudoscoparia*, compared to *Ca. pauciramosa*.

Species	Isolate no.	β-tubulin	TEF-1α	
		193	288	490
Ca. scoparia	CMW 31000	T	-	-
	CMW 31001	T	-	-
Ca. pauciramosa	CMW 5683	T	-	-
	CMW 30823	T	-	-
Ca. pseudoscoparia	CBS 125254	C	C	C
	CBS 125255	C	C	C
	CBS 125256	C	C	C
	CBS 125257	C	C	C

of the aligned sequences yielded three most parsimonious trees (TL = 977 steps; CI = 0.784; RI = 0.825; RC = 0.647), one of which is presented in Fig. 4. In the tree, isolates CBS 125249–125252, CBS 125261 and CBS 125269 from Ecuador clustered in a clade (BP = 99 and PP = 1.00) with *Ca. spathiphylli* (CBS 114540 and CBS 116168) and *Ca. pseudospathiphylli* (CBS 109165), whereas isolates CBS 125248, CBS 125253, CBS 125277 and CMW 14883 from Indonesia clustered close to *Ca. brasiliensis* (CBS 230.51 and CBS 114257) but with low support (BP = 52; PP = 0.90) in a separate, well-supported clade (BP = 100; PP = 1.00). Isolates CBS 125249, CBS 125250 and CBS 125261 clustered together in a well-supported clade (BP = 93; PP = 1.00) separate from CBS 125251, CBS 125252 and CBS 125269, that also clustered together in a well-supported clade (BP = 81; PP = 1.00). Both clades were separate from *Ca. spathiphylli* and *Ca. pseudospathiphylli* but closely related to these species. The SNP analyses showed that isolates CBS 125249, CBS 125250 and CBS 125261 shared four unique alleles and CBS 125251, CBS 125252 and CBS 125269 shared seven unique alleles for the three gene regions. These isolates also shared an additional 33 alleles, distinguishing them from *Ca. spathiphylli* (Table 5). Isolates CBS 125248, CBS 125253, CBS 125277 and CMW 14883 shared eight unique alleles, distinguishing them from *Ca. brasiliensis* (CBS 230.51 and CBS 114257), *Ca. cerciana* (CBS 123693 and CBS 123695) and *Ca. insularis* (CBS 114558 and CBS 114559) (Table 6).

Phylogenetic relationships amongst *Calonectria* spp.

Approximately 250 bases were determined for ACT, 450 bases for HIS3, 500 for BT, CAL and TEF-1α, 700 for ITS and 880 for LSU. The adjusted sequence alignments for each gene region consisted of 122 ingroup taxa with *Cylindrocladiella lageniformis* (CBS 112898) and *C. peruviana* (CPC 5614) as outgroup taxa for each gene region. For Bayesian analyses, a K80+G model was selected for ACT, HKY+I+G for BT, CAL and TEF-1α, GTR+I+G for HIS3 and LSU, and SYM+I+G for ITS and incorporated in the analyses. The consensus trees obtained from the Bayesian analyses confirmed the tree topologies obtained with maximum-parsimony as well as bootstrap support.

Individual analyses of the gene regions showed similar tree topologies for the protein coding regions (ACT, BT, CAL, HIS3 and TEF-1α) with well-supported clades for *Calonectria* spp. with similar morphological characteristics. In contrast, the non-coding gene regions (ITS and LSU) provided little or no support for the clades that emerged from the protein coding regions, with several *Calonectria* spp. clustering together with no significant similarities. The trees for the ITS and LSU regions showed a single monophyletic clade for all *Calonectria* spp. and did not reveal the two clades observed for the coding gene regions. The phylogeny constructed based on CAL sequences showed the best resolution of the species and it had the highest support for the individual clades, followed by TEF-1α gene region. Statistical data for the individual trees (not shown) are presented in Table 7.

The partition homogeneity tests for all possible combinations of the seven gene regions used, consistently yielded a P-value of 0.001. The 70 % reciprocal bootstrap trees showed no conflict in tree topologies for the five coding gene regions (ACT, BT, CAL, HIS3 and TEF-1α), however conflicts were observed between the non-coding gene regions (ITS and LSU) and the coding gene regions. Based on the tree topologies and a P-value of 0.001 (Cunningham 1997, Dettman *et al.* 2003) the sequence data for coding gene regions were combined. The data for the ITS and LSU datasets were treated separately, but these are not presented

Table 5. Single nucleotide polymorphisms from the sequence datasets for *Ca. densa* and *Ca. humicola* compared to *Ca. spathiphylli*.

Species	Isolate no.	β-tubulin 8	74	103	151	193	220	225	234	235	241	388	393	515	524	527
Ca. spathiphylli	CBS 114540	A	T	G	C	C	G	C	T	T	C	T	G	C	T	A
	CBS 116168	A	T	G	C	C	G	C	T	T	C	T	G	C	T	A
Ca. densa	CBS 125249	A	-	A	G	A	A	T	C	C	T	T	C	T	C	T
	CBS 125250	A	-	A	G	A	A	T	C	C	T	T	C	T	C	T
	CBS 125261	A	-	A	G	A	A	T	C	C	T	T	C	T	C	T
Ca. humicola	CBS 125251	T	-	A	G	A	A	T	T	T	T	C	C	T	C	T
	CBS 125252	T	-	A	G	A	A	T	T	T	T	C	C	T	C	T
	CBS 125269	T	-	A	G	A	A	T	T	T	T	C	C	T	C	T

Species	Isolate no.	Histone H3 71	83	101	103	105	127	209	253	256	261	262	266	279	459	460
Ca. spathiphylli	CBS 114540	C	C	A	C	T	T	T	T	A	C	T	T	T	A	C
	CBS 116168	C	C	A	C	T	T	T	T	A	C	T	T	T	A	C
Ca. densa	CBS 125249	T	G	G	T	C	C	C	C	G	T	C	C	C	A	C
	CBS 125250	T	G	G	T	C	C	C	C	G	T	C	C	C	A	C
	CBS 125261	T	G	G	T	C	C	C	C	G	T	C	C	C	A	C
Ca. humicola	CBS 125251	T	C	G	T	C	C	C	C	G	T	C	C	C	C	A
	CBS 125252	T	C	G	T	C	C	C	C	G	T	C	C	C	C	A
	CBS 125269	T	C	G	T	C	C	C	C	G	T	C	C	C	C	A

Species	Isolate no.	TEF-1α 49	72	84	100	102	104	113	114	115	116	207	262	454	469
Ca. spathiphylli	CBS 114540	T	T	-	G	A	G	-	-	-	-	T	A	C	T
	CBS 116168	T	T	-	G	A	G	-	-	-	-	T	A	C	T
Ca. densa	CBS 125249	C	C	-	A	G	A	C	A	-	-	C	T	T	G
	CBS 125250	C	C	-	A	G	A	C	A	-	-	C	T	T	G
	CBS 125261	C	C	-	A	G	A	C	A	-	-	C	T	T	G
Ca. humicola	CBS 125251	C	C	C	A	G	A	C	A	C	A	T	A	T	G
	CBS 125252	C	C	C	A	G	A	C	A	C	A	T	A	T	G
	CBS 125269	C	C	C	A	G	A	C	A	C	A	T	A	T	G

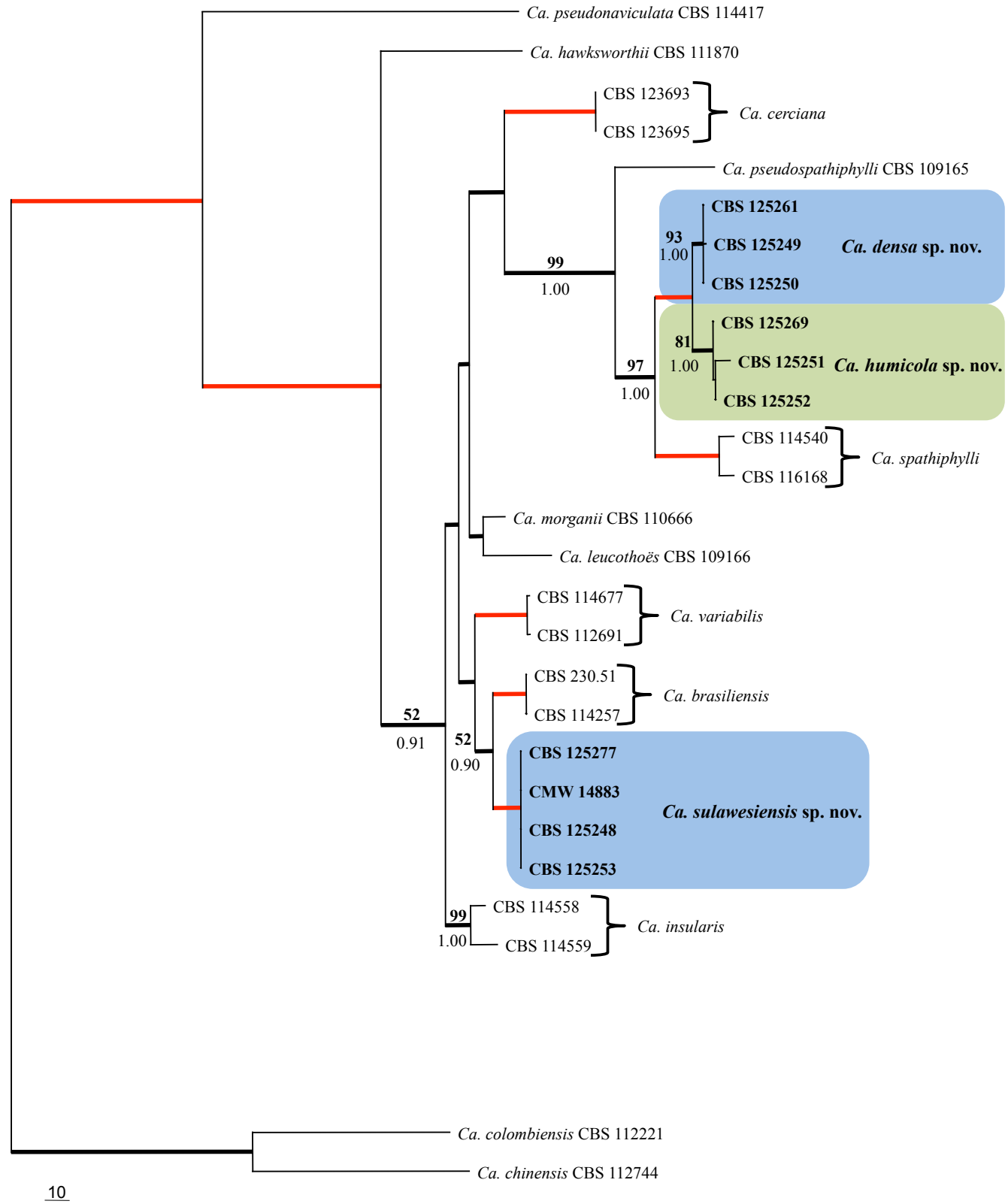

Fig. 4. One of three most parsimonious trees obtained from a heuristic search with 1 000 random additions sequences of the combined BT, HIS3 and TEF-1α sequence alignments of the *Ca. morganii* complex. Scale bar shows 10 changes and bootstrap support values (bold) from 1 000 replicates and Bayesian posterior probability values are indicated at the nodes. Red lines indicate bootstrap values of 100 and posterior probabilities of 1.00. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses.

because they add little taxonomic value. However, all ITS and LSU sequences generated in this study have been deposited in GenBank and TreeBase (Table 1).

The combined sequence alignment of the five coding gene regions consisted of 2 472 characters, including gaps. Of these characters, 925 were constant, 267 were parsimony-uninformative and 1 280 characters were parsimony-informative. Parsimony analysis of the aligned sequences yielded 24 most parsimonious trees (TL = 7319 steps; CI = 0.397; RI = 0.820; RC = 0.326), one of which is presented in Fig. 5. The tree topology obtained with

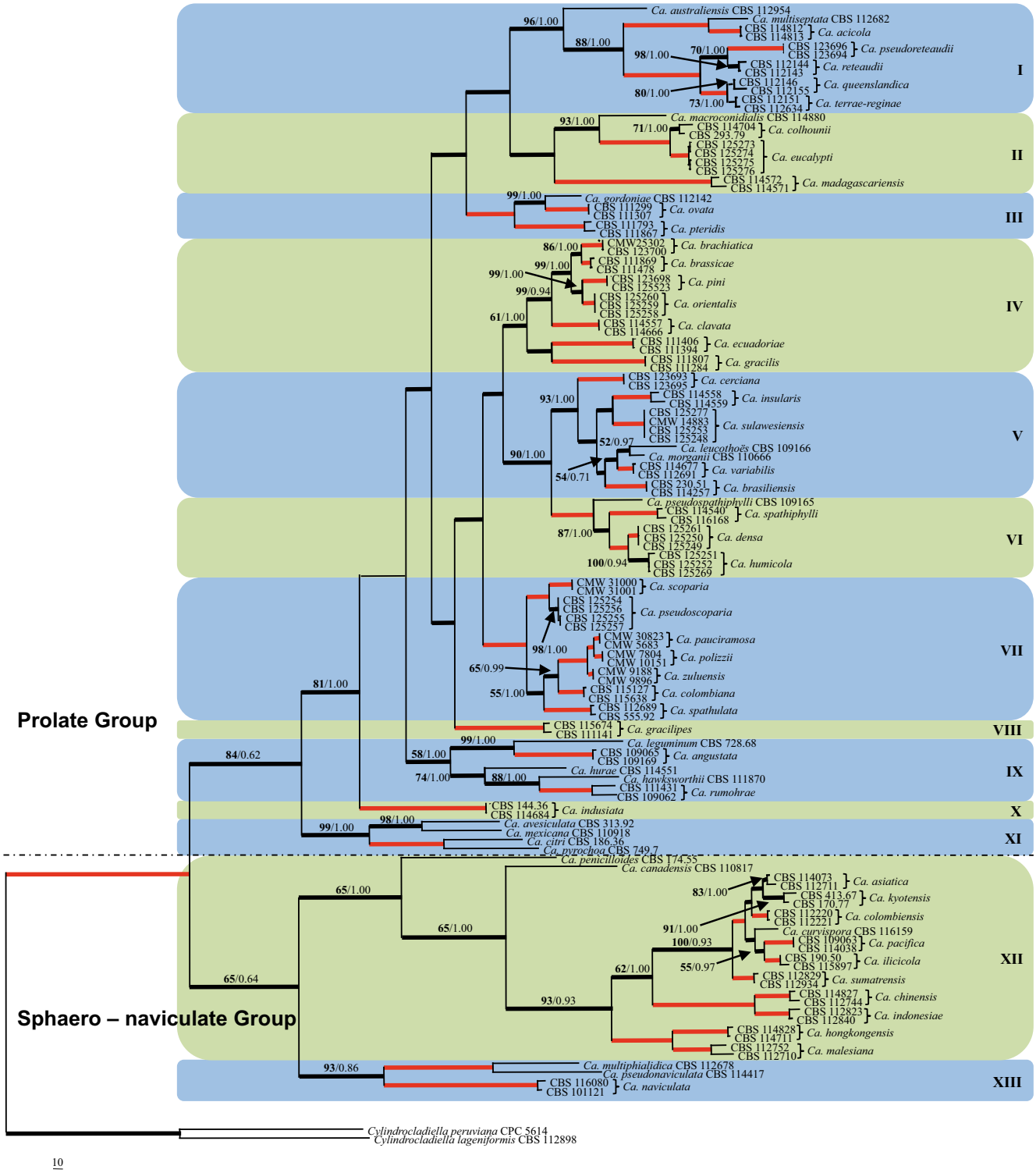

Fig. 5. One of 24 most parsimonious trees obtained from a heuristic search with 1 000 random additions sequences of the combined actin, β-tubulin, calmodulin, histone H3 and translation elongation factor 1-alpha sequence alignments of the *Calonectria*. Scale bar shows 10 changes and bootstrap support values (bold) from 1 000 replicates and Bayesian posterior probability values are indicated at the nodes. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses. Red lines indicate bootstrap values of 100 and posterior probabilities of 1.00. The tree was rooted to *Cylindrocladiella lageniformis* (CBS 112898) and *C. peruviana* (CPC 5614). Phylogenetic groups are indicated on the right.

Table 6. Single nucleotide polymorphisms comparisons between *Ca. brasiliensis*, *Ca. insularis* and *Ca. sulawesiensis* compared to *Ca. cerciana*.

Species	Isolate no.	β-tubulin					Histone H3								TEF-1α									
		117	360	395	472	509	95	100	253	259	260	390	417	452	98	100	103	104	105	109	143	263	439
Ca. brasiliensis	CBS 230.51	C	A	A	T	C	G	C	G	A	C	T	T	A	G	-	-	-	-	G	T	C	G
	CBS 114257	C	A	A	T	C	G	C	G	A	C	T	T	A	G	-	-	-	-	G	T	C	G
Ca. cerciana	CBS 123693	T	A	A	T	T	A	C	C	A	C	C	T	C	G	-	-	C	G	A	-	C	G
	CBS 123695	T	A	A	T	T	A	C	C	A	C	C	T	C	G	-	-	C	G	A	-	C	G
Ca. insularis	CBS 114558	T	G	A	C	C	A	C	G	A	C	C	C	A	G	C	A	C	A	A	-	C	A
	CBS 114559	T	G	A	C	C	A	C	G	A	C	C	C	A	G	C	A	C	A	A	-	C	A
Ca. sulawesiensis	CBS 125248	T	A	G	T	T	A	T	T	G	T	T	T	C	C	G	A	C	G	A	-	T	A
	CBS 125253	T	A	G	T	T	A	T	T	G	T	T	T	C	C	G	A	C	G	A	-	T	A
	CBS 125277	T	A	G	T	T	A	T	T	G	T	T	T	C	C	G	A	C	G	A	-	T	A
	CMW 14883	T	A	G	T	T	A	T	T	G	T	T	T	C	C	G	A	C	G	A	-	T	A

Table 7. Statistical information on the sequence dataset and maximum parsimony trees for each locus.

	Actin	β-tubulin	Calmodulin	Histone H3	ITS	LSU	TEF-1α
Aligned characters	290	532	531	499	706	887	596
Variable characters	15	42	39	62	32	10	57
Informative characters	151	268	323	223	112	37	337
Most parsimonious trees	2622	91	1000	372	1000	100	9970
Tree length	573	1454	1282	1843	296	91	1641
CI	0.490	0.431	0.467	0.352	0.618	0.538	0.477
RI	0.867	0.840	0.849	0.793	0.882	0.913	0.871
RC	0.425	0.569	0.397	0.648	0.545	0.492	0.416

the combined sequence dataset was similar to that obtained for the individual gene regions analysed and therefore the only tree presented is that of the combined dataset.

In the tree (Fig. 5), the *Calonectria* spp. were found to clearly reside in two main clades which was consistent for the analyses for these gene regions separately. One of these clades (BP = 82, PP = 0.62) which we refer to as representing the Prolate Group, includes *Calonectria* spp. with clavate to pyriform to ellipsoidal vesicles. This clade (Fig. 5) is made up of two sub-clades, one (BP = 81, PP = 1.00) of which includes 10 minor clades representing *Calonectria* spp. that have vesicles and conidia that have similar morphology. The second sub-clade (BP = 99, PP = 1.00) representing the Prolate Group includes taxa represented by single isolates and for which there were no obvious unifying morphological characters.

The second main clade (BP = 65, PP = 0.64) which is referred to as the Sphaero-Naviculate Group of species included *Calonectria* spp. characterised by sphaeropedunculate and naviculate vesicles and these were also seen in the analyses based on the individual gene regions. This clade is further sub-divided into two clades. The first of these sub-clades (BP = 65, PP = 1.00) includes *Calonectria* spp. characterised by sphaeropedunculate vesicles. The second sub-clade (BP = 93, PP = 0.86) accommodates *Calonectria* spp. with naviculate vesicles.

Sexual compatibility

The only isolates in the mating tests that yielded perithecia were CBS 125273–125276 (Fig. 6). These isolates all produced perithecia containing viable ascospores within 6 wk when mated with themselves, indicating that they are self-fertile (homothallic). All other control inoculations with the selected isolates failed to yield perithecia, indicating that they were either self-sterile (heterothallic) and non-compatible, or that they had lost the ability to undergo sexual recombination.

Taxonomy

Based on morphological observations, phylogenetic inference and mating, numerous isolates of *Calonectria* spp. included in this study represent undescribed species. Species of *Cylindrocladium* (1892) represent anamorph states of *Calonectria* (1867) (Rossman *et al.* 1999). In an attempt to move to a single nomenclature for pleomorphic fungi, the teleomorph name takes precedence over the anamorph name when both types belong to the same holomorph. The species below are described as new species in *Calonectria,* which represents the older generic name for these holomorphs and follows Lombard *et al.* (2009, 2010a, c). All *Cylindrocladium* species without a *Calonectria* state, are also transferred to *Calonectria*.

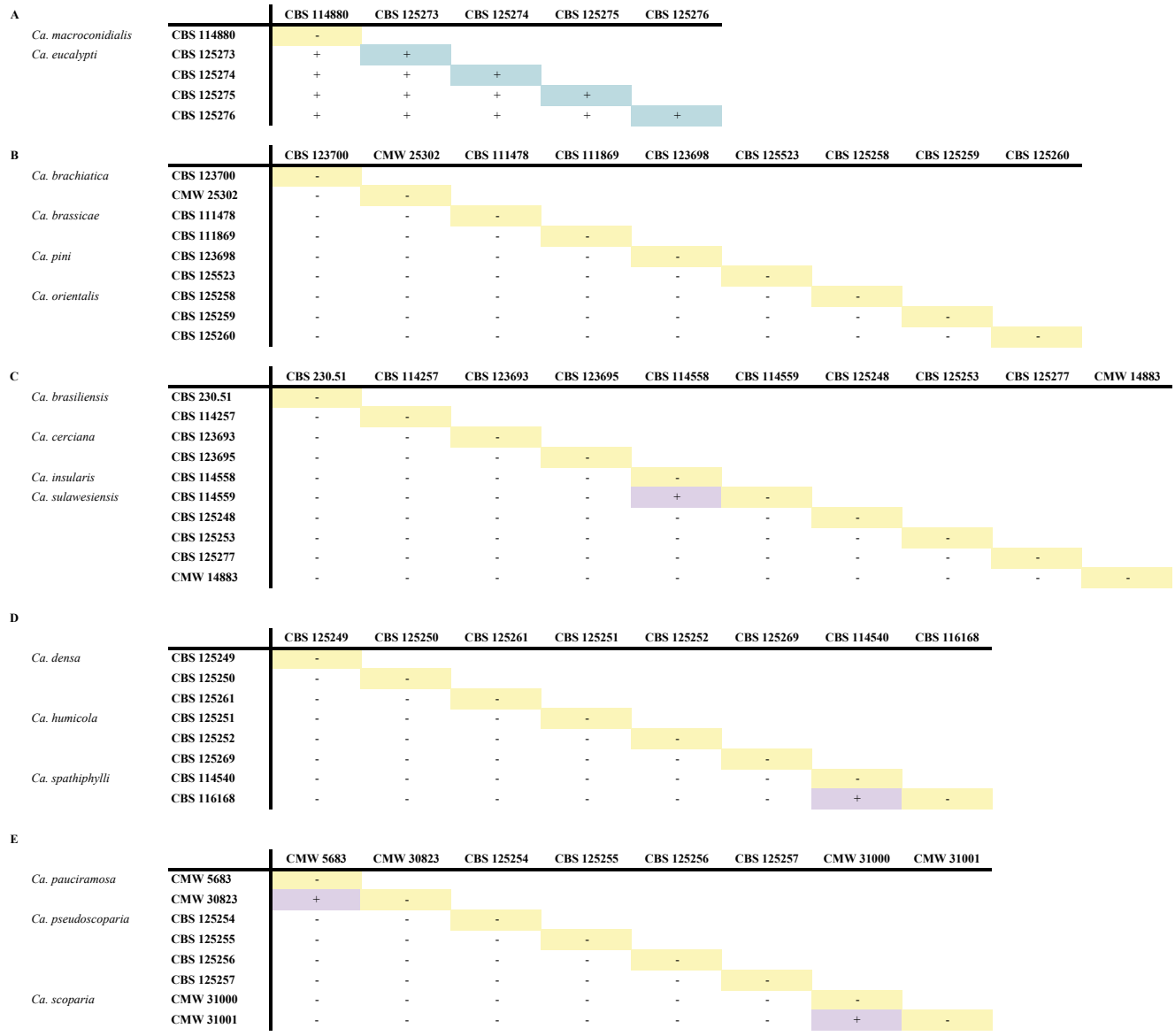

Fig 6. Results of sexual compatibility tests. Successful matings are indicated by (+) and unsuccessful matings is indicated with (-). Blue highlighted blocks indicate homothallic matings. Yellow blocks highlight unsuccessful self-self matings. Purple blocks indicate mating tester strain matings. A. Matings between isolates of *Ca. macroconidialis* and *Ca. eucalypti*. B. Matings between isolates of *Ca. brachiatica*, *Ca. brassicae*, *Ca. pini* and *Ca. orientalis*. C. Matings between isolates of *Ca. brasiliensis*, *Ca. cerciana*, *Ca. insularis* and *Ca. sulawesiensis*. D. Matings between isolates of *Ca. densa*, *Ca. humicola* and *Ca. spathiphylli*. E. Matings between isolates of *Ca. pauciramosa*, *Ca. pseudoscoparia* and *Ca. scoparia*.

Calonectria densa L. Lombard, M.J. Wingf. & Crous, **sp. nov.** MycoBank MB515529, Fig. 7.

Etymology: Name refers to the fact that lateral stipe extensions are readily formed in this species, giving it a bushy appearance.

Teleomorpha ignota. Anamorpha *Cy. spathiphylli* similis sed extensiones laterales stiparum facit, macroconidiis cylindricis utrinque rotundatis rectis (47–)50–58(–62) × 5–6 µm mediocriter 54 × 6 µm, semel septatis, sine cicatrice abscissionis manifesta, cum muco hyalino in fasciculis parallelis cylindricis differ.

Teleomorph unknown. *Conidiophores* with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 54–90 × 6–10 µm; stipe extensions septate, straight to flexuous, 149–192 µm long, 5–6 µm wide at the apical septum, terminating in ovoid to ellipsoid to sphaeropedunculate vesicles, 10–12 µm diam; lateral stipe extensions (90˚ to the axis) also present. *Conidiogenous apparatus* 49–78 µm long, and 63–123 µm wide; primary branches aseptate, 20–29 × 5–6 µm; secondary branches aseptate, 16–20 × 4–6 µm; tertiary and additional branches (–4) aseptate, 9–16 × 3–5 µm, each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, 11–16 × 2–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (47–)50–58(–62) × (5–)6 µm (av. = 54 × 6 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not seen.

Specimens examined: **Ecuador**, Pichincha Province, Las Golondrinas, from soil, Dec. 2004, M.J. Wingfield, Herb. PREM 60302, **holotype** of *Ca. densa*, culture ex-type CMW 31182 = CBS 125261; Pichincha Province, Las Golondrinas, from soil, Dec. 2004, M.J. Wingfield, cultures CMW 31184 = CBS 125249; Las Golondrinas, from soil, Dec. 2004, M.J. Wingfield, culture CMW 31185 = CBS 125250.

Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–35 ºC) on MEA, reverse umber

Fig. 7. *Calonectria densa*. A–D. Macroconidiophores. E–I. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. J–L. Ovoid to ellipsoid vesicles. M–N. One-septate macroconidia. Scale bars = 10 µm.

to verona-brown after 7 d; moderate white aerial mycelium with moderate sporulation; chlamydospores extensive throughout the medium forming microsclerotia.

Substrate: Soil.

Distribution: Ecuador.

Notes: Morphologically, *Ca. densa* is very similar to *Ca. spathiphylli* and *Ca. pseudospathiphylli*. However, macroconidia of *Ca. densa* (av. 54 × 6 µm) are smaller than those of *Ca. spathiphylli* (av. 70 × 6 µm), but slightly larger and broader than those of *Ca. pseudospathiphylli* (av. 52 × 4 µm). *Calonectria densa* also readily forms lateral stipe extensions, not reported for the other two species.

Calonectria eucalypti L. Lombard, M.J. Wingf. & Crous, **sp. nov.** MycoBank MB515530, Fig. 8.

Etymology: Name refers to *Eucalyptus* from which the fungus was isolated.

Teleomorpha *Ca. colhounii* similis sed ascocarpo flavo vel aurantiaco differt. Anamorpha *Cy. colhounii* similis sed macroconidiis cylindricis utrinque rotundatis rectis (66–)69–75(–80) × 5–6 µm mediocriter 72 × 6 µm, ter septatis, sine cicatrice abscissionis manifesta, cum muco hyalino in fasciculis parallelis cylindricis, differt.

Perithecia solitary or in groups, yellow to orange, becoming brown with age; in section apex and body yellow to orange, base red-brown, sub-globose to ovoid, 325–510 µm high, 285–360 µm diam, body turning dark red, and base dark red-brown (KOH+). Perithecial walls rough consisting of 2 thick-walled layers: outside

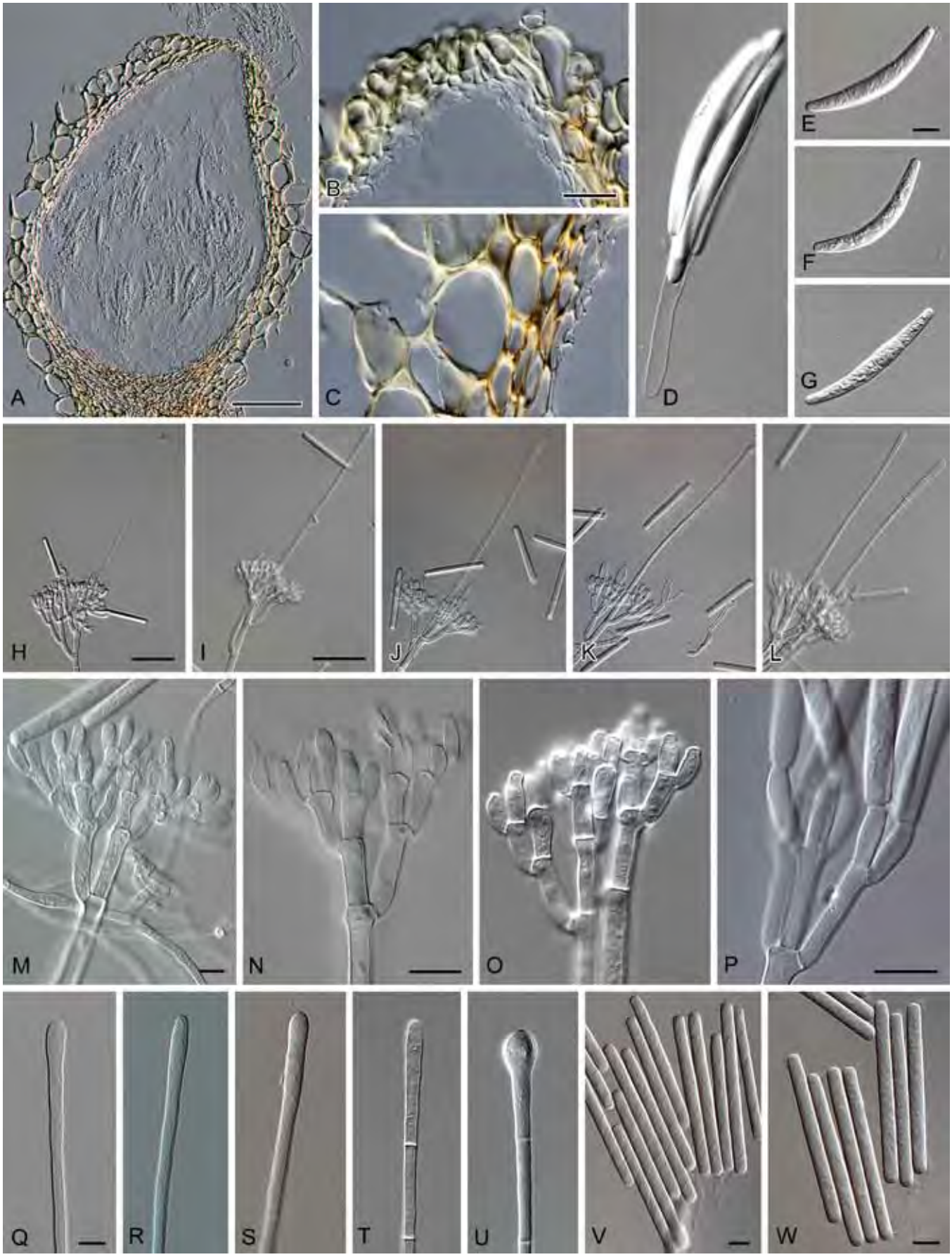

Fig. 8. *Calonectria eucalypti*. A. Perithecium. B. Section through ostiolar region of a perithecium. C. A vertical section through a perithecium, showing wall layers. D. Ascus. E–G. Ascospores. H–L. Macroconidiophores. M–P. Conidiogenous apparatus with conidiophore branches and doliiform to reniform or allantoid phialides. Q–U. Clavate to broadly clavate vesicles. V–W. Three-septate macroconidia. Scale bars: A = 90 µm, H–I = 70 µm, Other bars = 10 µm.

layer of *textura globulosa*, 45–90 µm wide; becoming more compressed towards inner layer of *textura angularis,* 12–18 µm wide; becoming thin-walled and hyaline towards the centre, outer cells 24–50 × 10–40 µm; inner cells 6–19 × 3–6 µm: perithecial base up to 125 µm wide; consisting of dark red, angular cells; merging with an erumpent stroma, cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. *Asci* 4-spored, clavate, 92–188 × 10–27 µm, tapering to a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, (1–)3-septate, not or slightly constricted at the septum, (25–)30–36(–56) × (3–)5–6(–8) µm (av. = 33 × 6 µm). Cultures were homothallic. *Conidiophores* with a stipe bearing a suit of penicillate, fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, 45–91 × 7–10 µm; stipe extensions septate, straight to flexuous, 110–235 µm long, 5–6 µm wide at the apical septum, terminating in broadly clavate vesicles, 4–6 µm diam. *Conidiogenous apparatus* 52–82 µm long, and 40–95 µm wide; primary branches aseptate or 1-septate, 21–29 × 5–6 µm; secondary branches aseptate, 14–21 × 3–5 µm; tertiary branches and additional branches (–5), aseptate, 11–16 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 10–14 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (66–)69–75(–80) × (5–)6 µm (av. = 72 × 6 µm), 3-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Megaconidia* and *microconidia* not seen.

Specimens examined: **Indonesia**, Sumatra Utara, Aek Nauli, on leaf of *Eucalyptus grandis*, May 2005, M.J. Wingfield, Herb. PREM 60298, **holotype** of *Ca. eucalypti*, culture ex-type CMW 18444 = CBS 125275; Aek Nauli, on leaf of *Eucalyptus grandis*, May 2005, M.J. Wingfield, PREM 60299, culture CMW 14890 = CBS 125273; Aek Nauli, on leaf of *Eucalyptus grandis*, May 2005, M.J. Wingfield, culture CMW 18443 = CBS 125274, Aek Nauli, on leaf of *Eucalyptus grandis*, May 2005, M.J. Wingfield, culture CMW 18445 = CBS 125276.

Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–30 ºC) on MEA, reverse colour tawny-brown after 7 d; abundant white aerial mycelium and sporulation; chlamydospores abundant throughout the medium, forming microsclerotia.

Substrate: *Eucalyptus grandis.*

Distribution: Indonesia.

Notes: The perithecia of *Ca. eucalypti* can be distinguished from *Ca. colhounii* and *Ca. macroconidialis* based on their yellow to orange colour in KOH. Macroconidia of *Ca. eucalypti* (av. 72 × 6 µm) are also larger than those of *Ca. colhounii* (av. 55 × 6 µm) and *Ca. madagascariensis* (av. 55 × 4.5 µm), but smaller than those of *Ca. macroconidialis* (av. 90 × 6.5 µm). Mating tests (Fig. 6) also showed that *Ca. eucalypti* is homothallic, a characteristic shared by *Ca. colhounii* and *Ca. madagascariensis* but not with *Ca. macroconidialis*, which is heterothallic (Crous 2002).

Calonectria humicola L. Lombard, M.J. Wingf. & Crous, **sp. nov.** MycoBank MB515531, Fig. 9.

Etymology: Name refers to the fact that this fungus was isolated from soil.

Teleomorpha ignota. Anamorpha Cy. spathiphylli similis sed macroconidiis cylindricis utrinque rotundatis rectis (45–)48–54(–56) × 4–5 µm mediocriter 51 × 5 µm, semel septatis, sine cicatrice abscissionis manifesta, cum muco hyalino in fasciculis parallelis cylindricis differt.

Teleomorph unknown. *Conidiophores* with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 44–90 × 6–8 µm; stipe extensions septate, straight to flexuous, 126–157 µm long, 4–5 µm wide at the apical septum, terminating in globose to ovoid to sphaeropedunculate vesicles, 10–12 µm diam. *Conidiogenous apparatus* 43–71 µm long, and 42–49 µm wide; primary branches aseptate, 20–29 × 4–6 µm; secondary branches aseptate, 12–19 × 3–5 µm; tertiary branches aseptate, 9–16 × 3–5 µm, each terminal branch producing 2–4 phialides; phialides elongated doliiform to reniform, hyaline, aseptate, 10–15 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (45–)48–54(–56) × (4–)5 µm (av. = 51 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not seen.

Specimens examined: **Ecuador**, Pichincha Province, Las Golondrinas, from soil, Dec. 2004, M.J. Wingfield, Herb. PREM 60369 **holotype** of *Ca. humicola*, culture ex-type CMW 31183 = CBS 125251; Las Golondrinas, from soil, Jan. 2006, L. Lombard, culture CMW 31186 = CBS 125252; Las Golondrinas, from soil, Jan. 2006, L. Lombard, (Herb. PREM 60368) culture CMW 31187 = CBS 125269.

Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–35 ºC) on MEA, reverse umber to verona-brown after 7 d; moderate white aerial mycelium with moderate sporulation; chlamydospores extensive throughout the medium, forming microsclerotia.

Substrate: Soil.

Distribution: Ecuador.

Notes: *Calonectria humicola* is morphologically very similar to *Ca. densa*, *Ca. pseudospathiphylli* and *Ca. spathiphylli*. However, no lateral stipe extensions occur in this species, whereas these are common in *Ca. densa*. Macroconidia of *Ca. humicola* (av. 51 × 5 µm) are slightly smaller than those of *Ca. densa* (av. 54 × 6 µm) and *Ca. spathiphylli* (av. 70 × 6 µm), but slightly broader than those of *Ca. pseudospathiphylli* (av. 52 × 4 µm).

Calonectria orientalis L. Lombard, M.J. Wingf. & Crous, **sp. nov.** MycoBank MB515532, Fig. 10.

Etymology: Name refers to the East Asian region, where the fungus was isolated.

Teleomorpha ignota. Anamorpha *Ca. brachiaticae* similis sed ramis conidiophorae tres vel minus sine extensionibus lateralibus stipae, macroconidiis cylindricis utrinque rotundatis rectis (43–)46–50(–53) × 4–5 µm mediocriter 48 × 4 µm, semel septatis, sine cicatrice abscissionis manifesta, cum muco hyalino in fasciculis parallelis cylindricis differt.

Teleomorph unknown. *Conidiophores* with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 60–169 × 6–12 µm; stipe extensions septate, straight to flexuous, 90–218 µm long, 5–10 µm wide at the apical septum, terminating in clavate to broadly clavate vesicles, 5–10 µm diam. *Conidiogenous apparatus* 54–174 µm long, and 67–92 µm wide; primary branches aseptate, 19–30 × 4–7 µm; secondary branches aseptate, 16–29 × 4–6 µm; tertiary and additional branches (–5) aseptate, 10–20 × 5–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 10–19 × 2–5 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (43–)46–50(–53) × 4(–5) µm (av. = 48 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not seen.

Specimens examined: **Indonesia**, Langam, from soil, June 2005, M.J. Wingfield, Herb. PREM 60303, **holotype** of *Ca. orientalis*, culture ex-type CMW 20291 = CBS 125260; Teso East, from soil, June 2005, M.J. Wingfield culture CMW 20273 = CBS 125259; Teso East, from soil, June 2005, M.J. Wingfield, culture CMW 20272 = CBS 125258.

Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–35 ºC) on MEA, reverse sepia-brown after 7 d; abundant white aerial mycelium with moderate to extensive sporulation; chlamydospores extensive throughout the medium, forming microsclerotia.

Substrate: Soil.

Distribution: Indonesia.

Notes: *Calonectria orientalis* is closely related to *Calonectria* spp. in the *Ca. brassicae* complex, based on phylogenetic inference and SNP analyses. Morphological comparisons showed that the macroconidia of *Ca. orientalis* (av. 48 × 4 µm) are shorter than those of *Ca. brassicae* (av. 53 × 4.5 µm), *Ca. clavata* (av. 65 × 5 µm) and *Ca. gracilis* (av. 56 × 4.5 µm) but larger than those of *Ca. brachiatica* (av. 44 × 5 µm) and *Ca. gracilipes* (av. 45 × 4.5 µm). As with *Ca. pini*, perithecia could not be induced when this species was mated with *Ca. brachiatica* and *Ca. brassicae*, highlighting the rarity of teleomorph structures for this group of fungi.

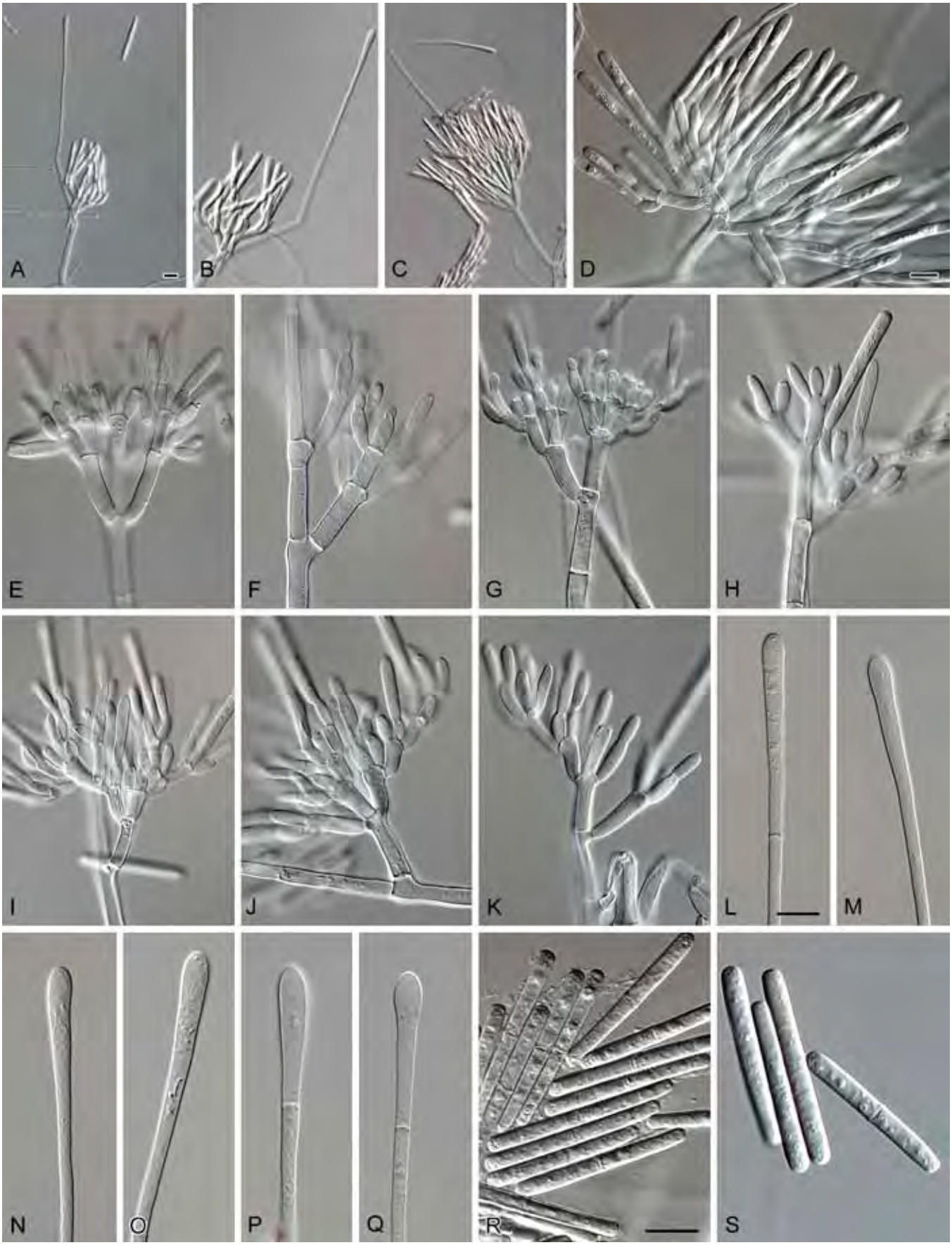

Fig. 10. *Calonectria orientalis*. A–C. Macroconidiophores. D–K. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. L–Q. Clavate vesicles. R–S. One-septate macroconidia. Scale bars = 10 µm.

Fig. 9. (p. 50) *Calonectria humicola*. A–F. Macroconidiophores. G–I. Conidiogenous apparatus with conidiophore branches and somewhat elongated, doliiform to reniform phialides. J–N. Globose to ovoid to sphaeropedunculate vesicles. O–P. One-septate macroconidia. Scale bars = 10 µm.

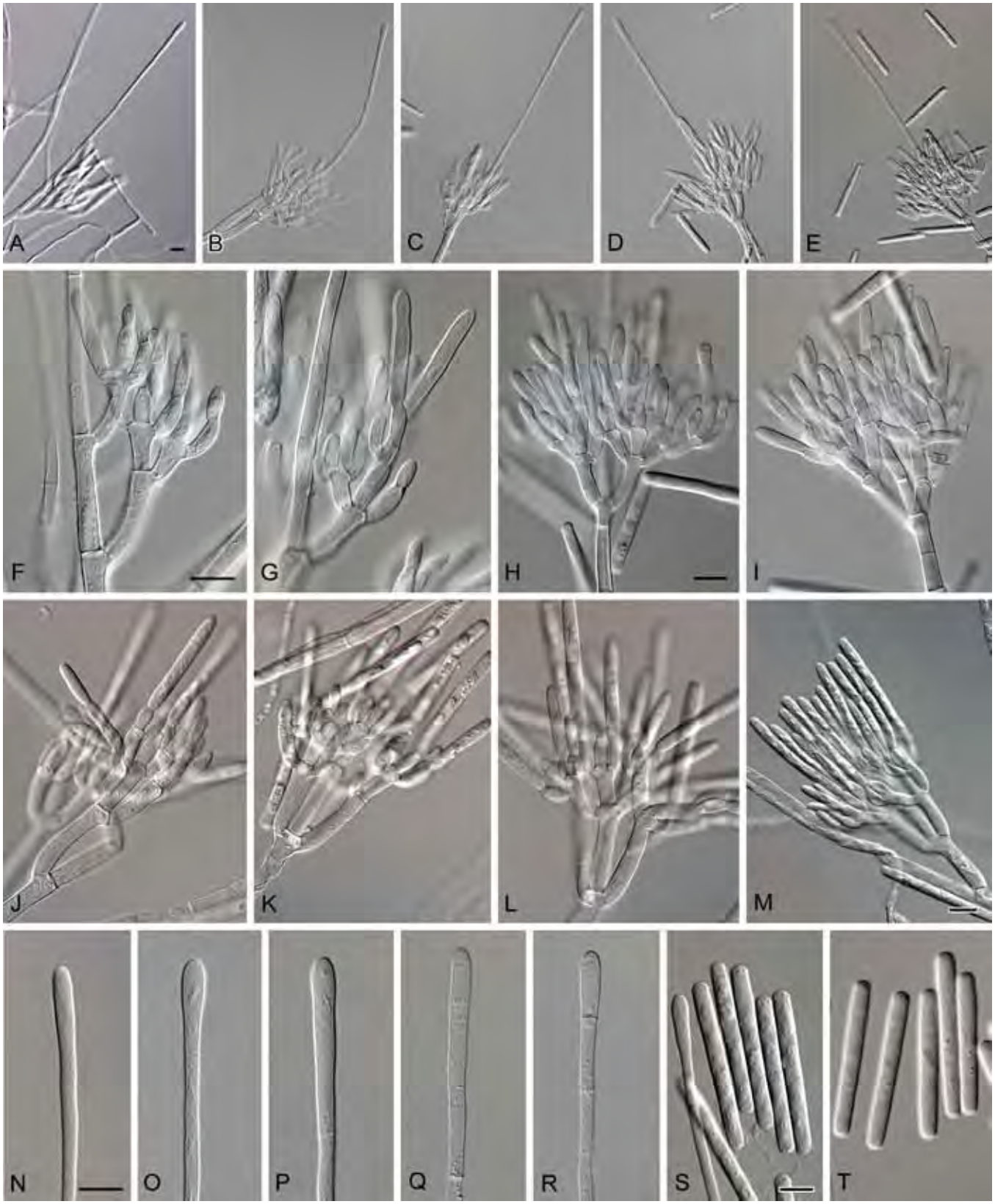

Fig. 11. *Calonectria pini*. A–E. Macroconidiophores. F–M. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. N–R. Clavate vesicles. S–T. One-septate macroconidia. Scale bars = 10 µm.

Calonectria pini L. Lombard, M.J. Wingf. & Crous, **sp. nov.** MycoBank MB515533, Fig. 11.

Etymology: Name refers to *Pinus,* the host from which the fungus was isolated.

Teleomorpha ignota. Anamorpha *Ca. brachiaticae* similis sed ramis conidiophorae tres vel minus sine extensionibus lateralibus stipae, macroconidiis cylindricis utrinque rotundatis rectis (37–)40–48(–50) × 4–6 µm mediocriter 44 × 5 µm, semel septatis, sine cicatrice abscissionis manifesta, cum muco hyalino in fasciculis parallelis cylindricis differ.

Teleomorph unknown. *Conidiophores* with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 40–99 × 6–7 µm; stipe extensions septate, straight to flexuous, 121–266 µm long, 5–7

µm wide at the apical septum, terminating in clavate vesicles, 4–6 µm diam. *Conidiogenous apparatus* 49–81 µm long, and 35–84 µm wide; primary branches aseptate, 20–30 × 4–6 µm; secondary branches aseptate, 13–22 × 3–5 µm; tertiary branches aseptate, 11–15 × 3–4 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 10–15 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (37–)40–48(–50) × 4–6 µm (av. = 44 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not seen.

Specimens examined: **Colombia**, Valle del Cauca, Buga, from *Pinus patula*, Sept. 2007, C.A. Rodas, Herb. PREM 60304, **holotype** of *Ca. pini*, culture ex-type CMW 31209 = CBS 123698; Buga, from *Pinus patula*, Sept. 2007, *C.A. Rodas*; Buga, from *Pinus patula*, Sept. 2007, C.A. Rodas, culture CMW 31210 = CBS 125523.

Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–30 ºC) on MEA, reverse amber to sepia-brown after 7 d; abundant white aerial mycelium with moderate to extensive sporulation; chlamydospores extensive throughout the medium forming microsclerotia.

Substrate: *Pinus patula.*

Distribution: Colombia.

Notes: *Calonectria pini* is very similar to *Ca. brachiatica*, but can be distinguished morphologically by the fact that it has three or fewer conidiophore branches and no lateral stipe extensions (Lombard *et al.* 2009). Macroconidia of *Ca. pini* (av. 44 × 5 µm) are shorter than those of *Ca. brassicae* (av. 53 × 4.5 µm), *Ca. gracilis* (56 × 4.5 µm) and *Ca. orientalis* (av. 48 × 4 µm). This species also has fewer conidiophore branches than those mentioned above. *Calonectria pini* failed to produce perithecia when crossed with *Ca. brachiatica* and *Ca. brassicae*. This supports the findings of Crous *et al.* (2004b) and Lombard *et al.* (2009), that teleomorph structures are rarely observed in members of the *Ca. brassicae* complex.

Calonectria pseudoscoparia L. Lombard, M.J. Wingf. & Crous, **sp. nov.** MycoBank MB515534, Fig. 12.

Etymology: Name reflects the fact that the species resembles the anamorph state of *Ca. scoparia*.

Teleomorpha ignota. Anamorpha *Ca. scopario* similis sed phialidibus elongato-doliiformibus vel reniformibus hyalinis non septatis 7–11 × 2–4 µm apice minute periclinale incrassatis colliculo inconspicuo, macroconidiis cylindricis utrinque rotundatis rectis (41–)45–51(–52) × 3–5 µm mediocriter 48 × 4 µm, semel septatis, sine cicatrice abscissionis manifesta, cum muco hyalino in fasciculis parallelis cylindricis differ.

Teleomorph unknown. *Conidiophores* with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 56–107 × 6–10 µm; stipe extensions septate, straight to flexuous, 124–201 µm long, 4–6 µm wide at the apical septum, terminating in obpyriform to ellipsoidal vesicles, 6–10 µm diam. *Conidiogenous apparatus* 34–87 µm long, and 52–74 µm wide; primary branches aseptate, 26–38 × 4–7 µm; secondary branches aseptate, 17–28 × 4–6 µm; tertiary branches and additional branches (–4) aseptate, 14–19 × 3–4 µm, each terminal branch producing 2–6 phialides; phialides elongate-doliiform to reniform, hyaline, aseptate, 7–11 × 2–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (41–)45–51(–52) × 3–5 µm (av. = 48 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not seen.

Specimens examined: **Ecuador**, Pichincha Province, Las Golondrinas, Buenos Aires Nursery, from *Eucalyptus grandis* cutting, Dec. 2004, M.J. Wingfield, Herb. PREM 60305, **holotype** of *Ca. pseudoscoparia*, culture ex-type CMW 15218 = CBS 125257; Buenos Aires Nursery, from *Eucalyptus grandis* cutting, Dec. 2004, M.J. Wingfield, Herb. PREM 60306, cultures from different cuttings, CMW 15214 = CBS 125254, CMW 15215 = CBS 125255, CMW 15216 = CBS 125256.

Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 10–30 ºC) on MEA, reverse amber to sepia-brown after 7 d; colony margins irregular with sparse to moderate white aerial mycelium with moderate sporulation; chlamydospores extensive throughout the medium forming microsclerotia.

Substrate: *Eucalyptus grandis.*

Distribution: Ecuador.

Notes: *Calonectria pseudoscoparia* (conidia av. 48 × 4 µm) can be distinguished from *Ca. scoparia* (conidia av. 60 × 4.5 µm) based on smaller macroconidia and the fact that it has elongated-doliiform to reniform phialides unlike those of *Ca. pauciramosa* and *Ca. scoparia*. Mating tests between this fungus and *Ca. scoparia* and *Ca. pauciramosa* failed to produce perithecia. Control crosses with both *Ca. pauciramosa* (CMW 5683 and CMW 30823) and *Ca. scoparia* tester isolates (CMW 31000 and CMW 31001) produced perithecia with viable ascospores showing that culture conditions were appropriate for mating.

Calonectria sulawesiensis L. Lombard, M.J. Wingf. & Crous, **sp. nov.** MycoBank MB515535, Fig. 13.

Etymology: Name refers to the Indonesian island of Sulawesi, where the fungus was collected.

Teleomorpha ignota. Anamorpha *Ca. morganii* similis sed vesiculo terminali late clavato vel ellipsoideo 5–7 µm diametro, macroconidiis cylindricis utrinque rotundatis rectis (41–)45–51(–54) × (3–)4–6 µm mediocriter 48 × 4 µm, semel septatis, sine cicatrice abscissionis manifesta, cum muco hyalino in fasciculis parallelis cylindricis differt.

Teleomorph unknown. *Conidiophores* with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 37–139 × 5–11 µm; stipe extensions septate, straight to flexuous, 113–262 µm long, 5–7 µm wide at the apical septum, terminating in broadly clavate to ellipsoidal vesicles, 5–7 µm diam. *Conidiogenous apparatus* 41–79 µm long, and 43–81 µm wide; primary branches aseptate, 17–41 × 3–6 µm; secondary branches aseptate, 10–27 × 3–6 µm; tertiary branches and additional branches (–5), aseptate, 9–15 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 9–15 × 2–5 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (41–)45–51(–54) × (3–)4(–6) µm (av. = 48 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not seen.

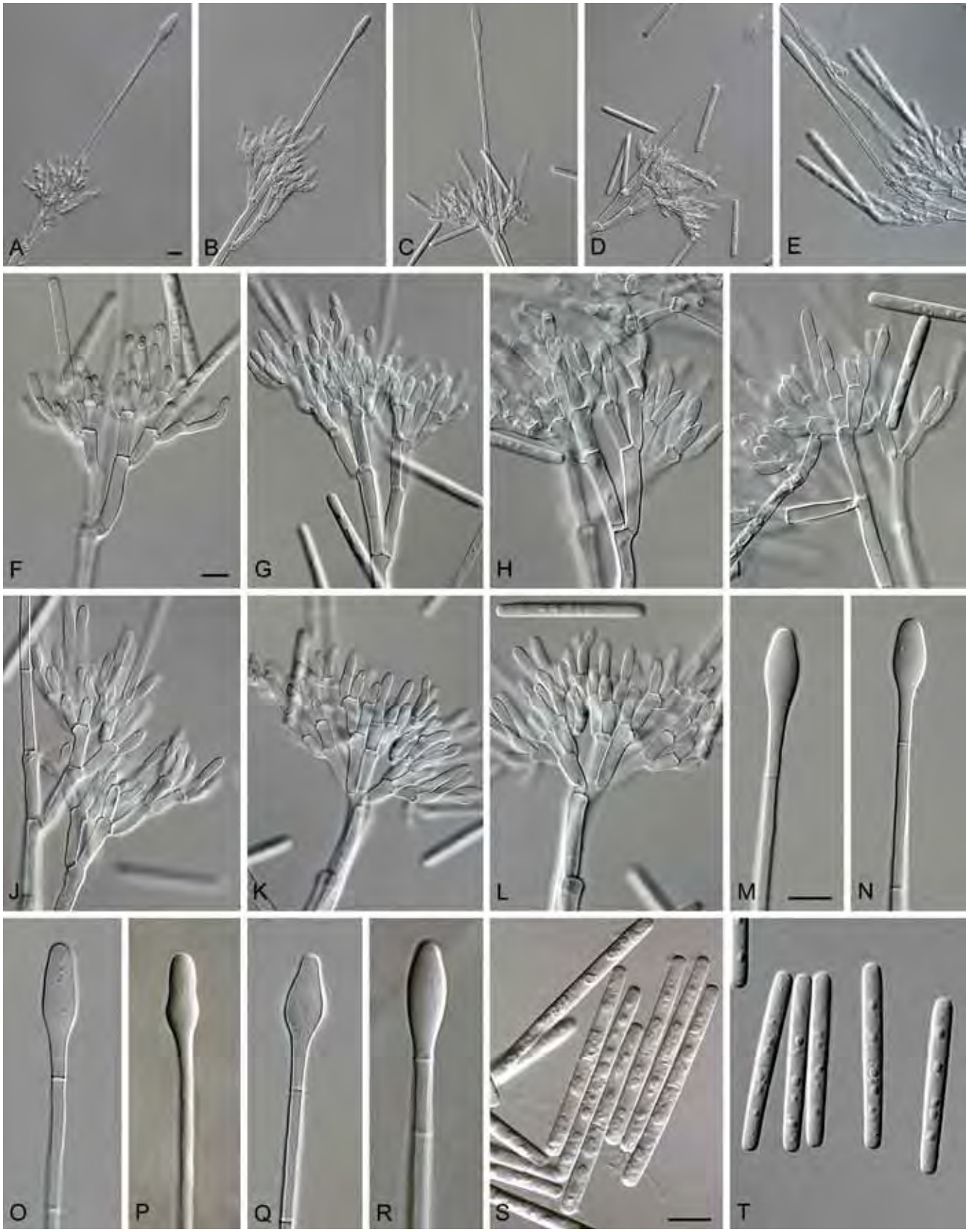

Fig. 12. *Calonectria pseudoscoparia*. A–E. Macroconidiophores. F–L. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. M–R. Obpyriform to ellipsoidal vesicles. S–T. One-septate macroconidia. Scale bars = 10 µm.

Specimens examined: **Indonesia**, Sulawesi, from leaf of *Eucalyptus* sp., July 2003, M.J. Wingfield, Herb. PREM 60300, **holotype** of *Ca. sulawesiensis*, culture ex-type CMW 14878 = CBS 125277; Sulawesi, from leaf of *Eucalyptus* sp., July 2003, M.J. Wingfield, PREM 60301 culture CMW 14883; from different leaves, culture CMW 14859 = CBS 125248, CMW 14879 = CBS 125253.

Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–30 ºC) on MEA, reverse amber to sepia-brown after 7 d; abundant white aerial mycelium with moderate to extensive sporulation; chlamydospores extensive throughout the medium, forming microsclerotia.

Fig. 13. *Calonectria sulawesiensis*. A–E. Macroconidiophores. F–K. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. L–Q. Clavate to ellipsoidal vesicles. R–S. One-septate macroconidia. Scale bars = 10 µm.

Substrate: *Eucalyptus* sp.

Distribution: Indonesia.

Notes: There are a few morphological differences distinguishing *Ca. sulawesiensis* from other species in the *Ca. morganii* complex. Macroconidia of *Ca. sulawesiensis* (av. 48 × 4 µm) are slightly larger than those of *Ca. brasiliensis* (av. 30 × 4 µm), *Ca. cerciana* (av. 44 × 5 µm), *Ca. insularis* (av. 45 × 4 µm) and *Ca. morganii* (av. 45 × 4 µm), but smaller than those of *Ca. hawksworthii* (av. 56 × 4 µm), *Ca. leucothoës* (av. 73 × 5 µm) and *Ca. variabilis* (av. 73 × 5 µm). Mating tests where *Ca. sulawesiensis* was crossed with *Ca. brasiliensis*, *Ca. cerciana* and *Ca. insularis* failed to produce perithecia, or produced perithecia without viable ascospores.

Calonectria angustata (Crous & El-Gholl) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515536.
Basionym: *Cylindrocladium angustatum* Crous & El-Gholl, Mycoscience 41: 522. 2000.

Calonectria australiensis (Crous & K.D. Hyde) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515537.
Basionym: *Cylindrocladium australiense* Crous & K.D. Hyde, Stud. Mycol. 55: 221. 2006.

Calonectria canadensis (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515538.
Basionym: *Cylindrocladium canadense* J.C. Kang, Crous & C.L. Schoch, Syst. Appl. Microbiol. 24: 210. 2001.

Calonectria chinensis (Crous) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515539.
Basionym: *Cylindrocladium chinense* Crous, Stud. Mycol. 50: 420. 2004.

Calonectria citri (H.S. Fawc. & Klotz) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515540.
Basionym: *Candelospora citri* H.S. Fawc. & Klotz, Mycologia 29: 213. 1937.
≡ *Cylindrocladium citri* (H.S. Fawc. & Klotz) Boedijn & Reitsma, Reinwardtia 1: 57. 1950.

Calonectria curvata (Boedijn & Reitsma) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515541.
Basionym: *Cylindrocladium curvatum* Boedijn & Reitsma, Reinwardtia 1: 54. 1950.

Calonectria curvispora (Crous & D. Victor) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515542.
Basionym: *Cylindrocladium curvisporum* Crous & D. Victor, Syst. Appl. Microbiol. 20: 283. 1997.

Calonectria ecuadoriae (Crous & M.J. Wingf.) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515543.
Basionym: *Cylindrocladium ecuadoriae* Crous & M.J. Wingf., Stud. Mycol. 55: 222. 2006.

Calonectria gordoniae (Leahy, T.S. Schub. & El-Gholl) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515544.
Basionym: *Cylindrocladium gordoniae* Leahy, T.S. Schub. & El-Gholl, Mycotaxon 76: 80. 2000.

Calonectria hawksworthii (Peerally) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515545.
Basionym: *Cylindrocladium hawksworthii* Peerally, Mycotaxon 40: 375. 1991.

Calonectria hurae (Linder & Whetzel) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515546.
Basionym: *Cercosporella hurae* Linder & Whetzel, Mycologia 29: 656. 1937.
≡ *Cylindrocladiopsis hurae* (Linder & Whetzel) U. Braun, Mycotaxon 51: 40. 1994.
≡ *Cylindrocladium hurae* (Linder & Whetzel) Crous, In: *Taxonomy and pathology of Cylindrocladium (Calonectria) and allied genera:* 185. 2002.
= *Cylindrocladium heptaseptatum* Sober, Alfieri & Knauss, Phytopathology 65: 333. 1975.
= *Cylindrocladiopsis lagerstroemiae* J.M. Yen, Mycotaxon 8: 236. 1979.

Calonectria indonesiae (Crous) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515547.
Basionym: *Cylindrocladium indonesiae* Crous, Stud. Mycol. 50: 424. 2004.

Calonectria leucothoës (El-Gholl, Leahy & T.S. Schub.) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515548.
Basionym: *Cylindrocladium leucothoës* El-Gholl, Leahy & T.S. Schub., Canad. J. Bot. 67: 2530. 1989.
= *Cylindrocladium perseae* T.S. Schub., Leahy & El-Gholl, Mycotaxon 73: 474. 1999.

Calonectria malesiana (Crous) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515549.
Basionym: *Cylindrocladium malesianum* Crous, Stud. Mycol. 50: 425. 2004.

Calonectria multiphialidica (Crous, Simoneau & Risède) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515550.
Basionym: *Cylindrocladium multiphialidicum* Crous, Simoneau & Risède, Stud. Mycol. 50: 425. 2004.

Calonectria pacifica (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515551.
Basionym: *Cylindrocladium pacificum* J.C. Kang, Crous & C.L. Schoch, Syst. Appl. Microbiol. 24: 213. 2001.

Calonectria penicilloides (Tubaki) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515552.
Basionym: *Candelospora penicilloides* Tubaki, Nogaoa 2: 58. 1952.
≡ *Cylindrocladium penicilloides* (Tubaki) Tubaki, J. Hattori Bot. Lab. 20: 154. 1958.

Calonectria pseudonaviculata (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515554.
Basionym: *Cylindrocladium pseudonaviculatum* Crous, J.Z. Groenew. & C.F. Hill, Sydowia 54: 26. 2002.
= *Cylindrocladium buxicola* Henricot, Mycologia 94: 993. 2002.

Calonectria sumatrensis (Crous) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515555.
Basionym: *Cylindrocladium sumatrense* Crous, Stud. Mycol. 50: 426. 2004.

DISCUSSION

In this study, a collection of isolates of unknown identity were shown to represent seven new species of *Calonectria*. These species, provided with the names *Ca. eucalypti*, *Ca. orientalis* and *Ca. sulawesiensis* from Indonesia, *Ca. densa*, *Ca. humicola* and *Ca. pseudoscoparia* from Ecuador and *Ca. pini* from Colombia were recognised based on morphological characteristics and phylogenetic inference. Recognition of a relatively large number of new species, mainly from soil samples collected in areas not previously intensively sampled, suggests that many more species of *Calonectria* remain to be discovered, particularly from the tropics and Southern Hemisphere.

Calonectria eucalypti, isolated from the leaves of *Eucalyptus grandis*, adds a new species to the *Ca. colhounii* complex (Crous 2002, Crous *et al.* 2006), which includes *Ca. colhounii*, *Ca. macroconidialis* and *Ca. madagascariensis*. Members of this complex are characterised by their unique yellow perithecia (Crous 2002). Although *Ca. eucalypti* was isolated from lesions typical of Cylindrocladium leaf blight, its importance as a pathogen is unknown. *Calonectria eucalypti* was shown to be homothallic, which is a characteristic that this species shares with *Ca. colhounii* and *Ca. madagascariensis.*

The descriptions of *Ca. pini* and *Ca. orientalis* add two species to the *Ca. brassicae* complex (Crous *et al.* 2006, Lombard *et al.* 2009). *Calonectria pini* was isolated from *Pinus patula* rooted cuttings with symptoms similar to those associated with root and collar infections caused by *Ca. brassicae* and *Ca. brachiatica* on other *Pinus* spp. (Lombard *et al.* 2009). In contrast, *Ca. orientalis* was isolated from soils collected in Indonesia and nothing is known regarding its pathogenicity. Phylogenetic inference and SNP allele analyses showed that these are closely related sibling species (Taylor *et al.* 2000) with genetic isolation having apparently occurred recently. Crosses between isolates of *Ca. pini* and *Ca. orientalis* as well as those with themselves and other *Calonectria* spp. in the group failed to produce perithecia. This is consistent with the observations of Crous *et al.* (2006) and Lombard *et al.* (2009), that *Calonectria* spp. in this complex rarely produce teleomorph structures in culture. *Calonectria sulawesiensis* resides in the *Ca. morganii* complex, closely related to *Ca. brasiliensis* and *Ca. insularis*. Morphologically, *Ca. sulawesiensis* can be distinguished from other species in the complex based only on macroconidial dimensions. Therefore phylogenetic inference based on DNA sequence data is necessary to distinguish it from other members of the *Ca. morganii* complex. Members of this complex are well-known pathogens of various hosts worldwide (Crous 2002), but nothing is known regarding the pathogenicity of *Ca. sulawesiensis.*

Calonectria pseudoscoparia is a new species in the *Ca. scoparia* complex (Schoch *et al.* 1999), isolated from *E. grandis* cuttings collected in Ecuador that displayed basal rot symptoms. *Calonectria* spp. in this group are well known causal agents of cutting rot in commercial forestry nurseries worldwide (Crous *et al.* 1991, Crous 2002, Lombard *et al.* 2010a). However, the pathogenicity of *Ca. pseudoscoparia* is only assumed based on the symptoms with which the fungus was associated.

The two newly described species, *Ca. densa* and *Ca. humicola*, isolated from Ecuadorian soils reside in the *Ca. spathiphylli* complex as defined by Kang *et al.* (2001b). *Calonectria pseudospathiphylli* and *Ca. spathiphylli*, that define this complex, are not easily distinguished based on morphology and DNA sequence comparisons are required for their identification. They can, however, be distinguished based on their mating strategies, with *Ca. pseudospathiphylli* being homothallic and *Ca. spathiphylli* being heterothallic (Kang *et al.* 2001b, Crous 2002). The mating strategies of *Ca. densa* and *Ca. humicola* could not be determined in this study. This complex of species appears to originate from Central and South America (Chase & Poole 1987, Kang *et al.* 2001b, Crous 2002).

DNA sequence data for the ITS, BT and HIS3 have been used more extensively to explore phylogenetic relationships amongst *Calonectria* spp. (Schoch *et al*. 1999, Kang *et al*. 2001a, 2001b, Henricot & Culham 2002, Crous *et al*. 2004b, 2006). In this regard, BT is the gene region that provides the most valuable insights into relationships between all species of *Calonectria* (Schoch *et al.* 2000b, 2001b, Crous 2002, Henricot & Culham 2002). Application of the CAL and TEF-1α partial gene sequences has only recently been introduced for *Calonectria* spp. (Crous *et al.* 2004b, 2006, Lombard *et al.* 2009, 2010a, c) and data for these gene regions have been available for only a small sub-set of species. The present study has attempted to address this problem and also introduce the ACT and LSU gene sequences that have not been employed previously for *Calonectria* spp. It has also provided sequence data for all seven gene regions for all accepted species in the genus.

The ITS and LSU sequences provided little valuable information to separate *Calonectria* spp. In contrast, sequence data for the protein-coding gene regions ACT, BT, CAL, HIS3 and TEF-1α provided good resolution of *Calonectria* spp., confirming the results of previous studies (Schoch *et al.* 1999, 2001a, Crous 2002, Henricot & Culham 2002, Crous *et al.* 2004b, 2006). This study also introduced sequence data for the ACT gene region, although it had few informative sites, consistent with the results of previous studies on other groups of fungi (Helgason *et al.* 2003, Hunter *et al.* 2006). Phylogenetic analyses of the individual coding gene regions and single nucleotide polymorphisms showed that CAL sequence data provide the best resolution distinguishing *Calonectria* spp. from each other followed by sequence data for the TEF-1α, HIS3, BT and ACT gene regions.

In addition to identifying the most useful gene regions to accurately identify species of *Calonectria*, an important goal of this study was to re-consider the phylogenetic relationships between all the species in this genus. Having determined that the ACT, BT, CAL, HIS3 and TEF-1α gene regions give the best resolution when identifying species of *Calonectria*, a phylogenetic tree for the genus was generated. This showed that the group includes two major clades and that these define morphologically similar groups of *Calonectria* spp. These two major clades have substantial sub-structure with all of the 66 species of *Calonectria* residing in one of 13 sub-clades. Eleven of these sub-clades, that include 50 species, represent the Prolate Group of isolates and two sub-clades that include 16 species representing the Sphaero-Naviculate Group of isolates.

The Prolate group of isolates incorporates the majority of the plant pathogenic *Calonectria* spp. and includes the type species for *Calonectria* (*Ca. pyrochoa*) and *Cylindrocladium* (*Cy. scoparium*). Most of these pathogenic species have been reported from forestry crops (Peerally 1991, Crous & Wingfield 1994, Crous 2002, Crous *et al.* 2006) but a few have also been found to infect horticultural and agronomic crops (Boedijn & Reitsma 1950, Kim *et al.* 1998, Crous 2002, Polizzi *et al.* 2007, Vitale *et al.* 2008). None of the sub-clades in this group could, however, be correlated with any specific host type.

The geographic distribution of the *Calonectria* spp. representing the various sub-clades of the unifying Prolate Group of isolates

shows some correlation in their distribution. *Calonectria* spp. in the sub-clade representing the *Ca. reteaudii* complex (Sub-clade I) have been reported only from Australia, China, Indonesia and New Zealand (Crous 2002, Gadgil & Dick 2004, Crous *et al.* 2006, Lombard *et al.* 2010c). Another sub-clade of isolates that appears to have geographical structure resides in the *Ca. brassicae* complex (Sub-clade IV). Species in this sub-clade, with the exception of *Ca. orientalis*, have all been reported from South and Central America (Crous 2002, Crous *et al.* 2004b, Lombard *et al.* 2009). Isolates in other sub-clades appeared to have broad geographic distribution and not to occur in any defined part of the world.

Species residing in the Sphaero-Naviculate Group had no obvious patterns of pathogenicity, or distribution. This group consisted of two sub-clades in which only vesicle morphology was a consistent character. The majority of the species in the *Ca. kyotensis* complex (sub-clade XII) have been isolated from debris and soil (Crous *et al.* 2004b) but a few such as *Ca. kyotensis*, *Ca. ilicicola* and *Ca. pacifica* are important pathogens of agronomic and forestry crops (Crous 2002, Crous *et al.* 2004b). Members of this sub-clade also had a broad distribution with the majority reported from Asia (Crous *et al.* 2004b) and they included both heterothallic and homothallic species (Crous 2002, Crous *et al.* 2004b).

The second sub-clade in the Sphaero-Naviculate Group of isolates (sub-clade XIII) included three *Calonectria* spp., only two of which have morphological similarities. *Calonectria multiphialidica* is morphologically similar to the *Calonectria* spp. in sub-clade XII but there were no obvious patterns of distribution and pathogenicity for this group.

The intention of this phylogenetic study was to include all *Calonectria* spp. recognised to date. *Calonectria curvata* and *Ca. hederae* were, however, not included because there are no cultures for them as has previously been mentioned by Crous (2002). Furthermore, *Ca. rajasthanensis*, *Cy. avesiculatum* var. *microsporum*, *Cy. bambusae*, *Cy. couratarii*, *Cy. crataegi*, *Cy. intermedium* and *Cy. musae* were not included due either to the fact that they have not been validly described or not recognised as true species of *Calonectria* (Crous 2002). Based on the results of this study, 68 *Calonectria* spp. are recognised as valid and cultures are available for 66 of them.

The teleomorph state has not been seen for several species of *Calonectria*. Nonetheless C*ylindrocladium* spp., irrespective of whether their perithecial states are known or not, have been provided names in *Calonectria.* This is consistent with the view that for all newly described pleomorphic fungal species, the teleomorph name or the oldest typified name takes precedence over the anamorph or more recent name when both types belong to the same holomorph taxon (Hawksworth 2005, McNeill *et al.* 2005). It has already been established that *Calonectria* spp. have only *Cylindrocladium* anamorphs (Rossman *et al.* 1999, Schoch *et al.* 2001b), with micro- and megaconidial states that have thus far not been named. The name *Calonectria* was typified in 1867 (Rossman 1979) whereas that of *Cylindrocladium* was typified in 1892 (Morgan 1892). Therefore *Calonectria* has preference above *Cylindrocladium* and should henceforth be used for all species irrespective of whether the perithecial state has been found.

KEYS

Both synoptic and dichotomous keys to species of *Calonectria* are presented. In the synoptic key, numbers grouped with each character refer to the species that are alphabetically arranged below:

1. *Ca. acicola* P.D. Gadgill & M.A. Dick
2. *Ca. angustata* (Crous & El-Gholl) L. Lombard, M.J. Wingf. & Crous
3. *Ca. asiatica* Crous & N.L. Hywel-Jones
4. *Ca. australiensis* (Crous & K.D. Hyde) L. Lombard, M.J. Wingf. & Crous
5. *Ca. avesiculata* T.S. Schub., El-Gholl, Alfieri & Schoult.
6. *Ca. brachiatica* L. Lombard, M.J. Wingf. & Crous
7. *Ca. brassicae* (Panwar & Borha) L. Lombard, M.J. Wingf. & Crous
8. *Ca. brasiliensis* (Peerally) L. Lombard, M.J. Wingf. & Crous
9. *Ca. canadensis* (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous
10. *Ca. cerciana* L. Lombard, M.J. Wingf. & Crous
11. *Ca. chinensis* (Crous) L. Lombard, M.J. Wingf. & Crous
12. *Ca. citri* (H.S. Fawc. & Klotz) L. Lombard, M.J. Wingf. & Crous
13. *Ca. clavata* Alfieri, El-Gholl & E.L. Barnard
14. *Ca. colhounii* Peerally
15. *Ca. colombiana* L. Lombard, M.J. Wingf. & Crous
16. *Ca. colombiensis* Crous
17. *Ca. curvata* (Boedijn & Reitsma) L. Lombard, M.J. Wingf. & Crous
18. *Ca. curvispora* (Crous & D. Victor) L. Lombard, M.J. Wingf. & Crous
19. *Ca. densa* L. Lombard, M.J. Wingf. & Crous
20. *Ca. ecuadoriae* (Crous & M.J. Wingf.) L. Lombard, M.J. Wingf. & Crous
21. *Ca. eucalypti* L. Lombard, M.J. Wingf. & Crous
22. *Ca. gracilipes* Crous & G.R.A. Mchau
23. *Ca. gracilis* Crous, M.J. Wingf. & Alfenas
24. *Ca. gordoniae* (Leahy, T.S. Schub. & El-Gholl) L. Lombard, M.J. Wingf. & Crous
25. *Ca. hawksworthii* (Peerally) L. Lombard, M.J. Wingf. & Crous
26. *Ca. hederae* C. Booth & J.S. Murray
27. *Ca. hongkongensis* Crous

28. *Ca. humicola* L. Lombard, M.J. Wingf. & Crous
29. *Ca. hurae* (Linder & Whetzel) L. Lombard, M.J. Wingf. & Crous
30. *Ca. ilicicola* Boedijn & Reitsma
31. *Ca. indonesiae* (Crous) L. Lombard, M.J. Wingf. & Crous
32. *Ca. indusiata* (Seaver) Crous
33. *Ca. insularis* C.L. Schoch & Crous
34. *Ca. kyotensis* Tersh.
35. *Ca. leguminum* (Rehm) Crous
36. *Ca. leucothoës* (El-Gholl, Leahy & T.S. Schub.) L. Lombard, M.J. Wingf. & Crous
37. *Ca. macroconidialis* (Crous, M.J. Wingf. & Alfenas) Crous
38. *Ca. madagascariensis* Crous
39. *Ca. malesiana* (Crous) L. Lombard, M.J. Wingf. & Crous
40. *Ca. mexicana* C.L. Schoch & Crous
41. *Ca. morganii* Crous, Alfenas & M.J. Wingf.
42. *Ca. multiphialidica* (Crous, Simoneau & Risède) L. Lombard, M.J. Wingf. & Crous
43. *Ca. multiseptata* Crous & M.J. Wingf.
44. *Ca. naviculata* Crous & M.J. Wingf.
45. *Ca. orientalis* L. Lombard, M.J. Wingf. & Crous
46. *Ca. ovata* D. Victor & Crous
47. *Ca. pacifica* (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous
48. *Ca. pauciramosa* C.L. Schoch & Crous
49. *Ca. penicilliodes* (Tubaki) L. Lombard, M.J. Wingf. & Crous
50. *Ca. pini* L. Lombard, M.J. Wingf. & Crous
51. *Ca. polizzii* L. Lombard, M.J. Wingf. & Crous
52. *Ca. pseudonaviculata* (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M.J. Wingf. & Crous
53. *Ca. pseudoreteaudii* L. Lombard, M.J. Wingf. & Crous
54. *Ca pseudoscoparia* L. Lombard, M.J. Wingf. & Crous
55. *Ca. pseudospathiphylli* J.C. Kang, Crous & C.L. Schoch
56. *Ca. pteridis* Crous, M.J. Wingf. & Alfenas
57. *Ca. pyrochoa* (Desm.) Sacc.
58. *Ca. queenslandica* L. Lombard, M.J. Wingf. & Crous
59. *Ca. reteaudii* (Bugn.) C. Booth
60. *Ca. rumohrae* El-Gholl & Alfenas
61. *Ca. scoparia* Peerally
62. *Ca. spathiphylli* El-Gholl, J.Y. Uchida, Alfenas, T.S. Schub., Alfieri & A.R. Chase
63. *Ca. spathulata* El-Gholl, Kimbr., E.L. Barnard, Alfieri & Schoult.
64. *Ca. sulawesiensis* L. Lombard, M.J. Wingf. & Crous
65. *Ca. sumatrensis* (Crous) L. Lombard, M.J. Wingf. & Crous
66. *Ca. terrae-reginae* L. Lombard, M.J. Wingf. & Crous
67. *Ca. variabilis* Crous, B.J.H. Janse, D. Victor, G.F. Marias & Alfenas
68. *Ca. zuluensis* L. Lombard, M.J. Wingf. & Crous

Synoptic key to *Calonectria* species

1. Teleomorph:
    a. Teleomorph state known
        1, 3, 5, 13, 14, 15, 16, 21, 22, 23, 26, 27, 29, 30, 31, 32, 33, 34, 35, 37, 38, 40, 41, 43, 44, 46, 48, 55, 56, 57, 59, 60, 61, 62, 63, 67, 68
    b. Teleomorph state unknown
        2, 4, 6, 7, 8, 9, 10, 11, 12, 17, 18, 19, 20, 24, 25, 28, 36, 39, 42, 45, 47, 49, 50, 51, 52, 53, 54, 58, 64, 65, 66

2. Ascocarps:
    a. Red-brown to red in colour, changing to dark-red in 3 % KOH
        1, 23, 44, 56, 61, 67
    b. Orange to red in colour, changing to dark-red in 3 % KOH
        3, 5, 15, 16, 22, 26, 30, 32, 33, 34, 40, 43, 55, 62, 68
    c. Orange to red-brown in colour, changing to dark-red in 3 % KOH
        13, 27, 35, 46, 48, 57, 59, 60, 63
    d. Yellow to orange in colour, only base and stroma changing to dark-red in 3 % KOH
        14, 21, 37, 38, 41

3. Asci:
    a. 8-spored and clavate
        1, 3, 5, 13, 15, 16, 22, 23, 26, 27, 30, 32, 33, 34, 35, 38, 40, 41, 43, 44, 46, 48, 55, 56, 57, 59, 60, 61, 62, 63, 67, 68
    b. 4-spored and clavate
        14, 21, 37

4. Ascospore septation:
    a. 1-septate
        3, 15, 16, 22, 23, 27, 33, 34, 40, 41, 48, 61, 68
    b. (1–)3-septate
        5, 13, 14, 21, 26, 30, 32, 35, 37, 38, 44, 46, 55, 56, 57, 59, 62, 63, 67
    c. (3–)4-septate
        1
    d. (1–)3–6(–9) septate
        43, 60

5. Ascospore width (av. in µm)
    a. 4–5
        15, 16, 22, 34, 44, 62, 67, 68
    b. 5.5–6
        1, 3, 5, 13, 14, 21, 26, 27, 30, 33, 37, 38, 40, 41, 46, 55, 56, 57, 59, 61, 63
    c. 6.5–7
        22, 32, 35, 43, 48, 60

6. Ascospore length (av. in µm)
    a. 30–39
        3, 15, 16, 21, 22, 23, 27, 33, 34, 41, 48, 68
    b. 40–49
        5, 13, 30, 44, 55, 57, 61, 62, 67
    c. 50–59
        14, 26, 32, 37, 38, 40, 56, 63
    d. 60–69
        46
    e. 70 and above
        1, 35, 43, 59, 60

7. Stipe length (av. in µm)
    a. 40–100
        1, 5, 6, 9, 10, 16, 18, 20, 21, 27, 30, 31, 33, 34, 36, 38, 40, 44, 47, 48, 49, 50, 57, 58, 61, 63, 65, 66, 68
    b. 101–150
        4, 7, 11, 13, 15, 24, 32, 41, 42, 51, 53, 54, 60, 62, 64,
    c. 151–200
        2, 3, 12, 14, 19, 22, 23, 28, 29, 35, 39, 45, 46, 52, 56, 67
    d. above 200
        25, 26, 37, 55, 59

8 Stipe extension length (av. in µm)
    a. Less than 100
        1
    b. 100–200
        9, 11, 12, 15, 16, 18, 19, 25, 27, 28, 31, 34, 39, 41, 44, 51, 52, 57, 58, 68
    c. 201–300
        2, 3, 10, 13, 14, 21, 22, 24, 26, 30, 33, 35, 36, 40, 45, 46, 47, 48, 50, 54, 55, 56, 61, 62, 63, 64, 65, 66, 67
    d. Above 300
        4, 5, 6, 7, 20, 23, 29, 32, 37, 38, 42, 53, 59, 60

9. Vesicle shape
    a. Avesiculate to clavate
        5
    b. Clavate
        1, 2, 4, 6, 7, 13, 14, 20, 21, 22, 23, 24, 29, 32, 35, 37, 38, 43, 45, 50, 53, 56, 58, 59, 60, 64, 66
    c. Ellipsoidal to pyriform to obovoid
        8, 12, 25, 26, 41, 55, 61, 63

d. Ellipsoidal to ovoid
   19, 46
e. Ellipsoidal to obpyriform
   10, 15, 33, 36, 40, 48, 51, 54, 57, 68
f. Sphaeropedunculate
   3, 9, 11, 16, 17, 18, 19, 27, 30, 31, 34, 39, 42, 47, (49), 64, 67
g. Globose
   19, 28, 62
h. Naviculate
   44, 52

10. Shape of phialides on macroconidiophore
    a. Reniform to doliiform
       3, 6, 7, 8, 9, 10, 12, 15, 17, 19, 20, 21, 22, 23, 24, 25, 26, 33, 34, 36, 40, 41, 44, 45, 46, 48, 49, 50, 51, 52, 54, 57, 61, 63, 64, 68
    b. Elongate reniform to doliiform
       5, 11, 13, 14, 16, 18, 27, 28, 30, 31, 39, 42, 47, 55, 56, 62, 65, 67
    c. Cylindrical to allantoid
       1, 2, 4, 29, 32, 35, 37, 38, 53, 58, 59, 60, 66

11. Number of fertile branches on macroconidiophore
    a. 1–3
       1, 5, 8, 9, 11, 12, 17, 18, 28, 30, 46, 48, 49, 50, 51, 52, 53, 57, 58, 60, 63, 66, 67, 68
    b. 4–6
       2, 3, 4, 6, 7, 14, 16, 19, 21, 24, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 44, 45, 46, 47, 54, 55, 56, 59, 61, 62, 64, 65
    c. More than 6
       20, 27, 42

12. Microconidia
    a. Microconidia absent
       2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 28, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 44, 45, 47, 48, 49, 50, 51, 52, 54, 55, 57, 58, 61, 63, 64, 65, 66, 68
    b. Microconidia present
       1, 13, 24, 29, 30, 43, 46, 53, 56, 59, 60, 62, 67

13. Microconidial septation
    a. 1-septate
       13, 29, 30, 46, 56, 62, 67
    b. 1(–3)-septate
       24, 59, 60
    c. 1–3-septate
       1, 43, 53

14. Microconidial width (mean in µm)
    a. Up to 3
       13, 29, 43, 46, 56, 59
    b. Up to 4
       24, 53, 62, 67
    c. Up to 5
       1, 30, 60

15. Microconidial length (mean in µm)
    a. Below 20
       29
    b. 20–30
       1, 30, 46, 56, 59, 60, 67
    c. 31–40
       13, 24, 62
    d. above 40
       43, 53

16. Macroconidial septation
    a. 1-septate
       3, 6, 7, 8, 9, 10, 11, 12, 15, 17, 19, 22, 25, 27, 28, 31, 33, 34, 39, 40, 41, 42, 44, 45, 47, 48, 50, 51, 52, 54, 61, 64, 65, 68
    b. 1(–3)-septate
       5, 13, 16, 18, 20, 23, 24, 36, 46, 53, 55, 56, 62
    c. (1–)3-septate
       4, 14, 21, 30, 32, 38, 49, 57,
    d. (1–)3(–6)-septate
       26, 37, 58, 66
    e. (1–)5(–6)-septate
       1, 26, 35, 59, 60
    f. (1–)7(–8)-septate
       29
    g. More than 8-septate
       2
17. Macroconidial width (av. in µm)
    a. 3–4
       8, 9, 11, 12, 15, 17, 25, 27, 31, 33, 34, 39, 40, 41, 44, 45, 51, 54, 55, 63, 64, 68
    b. 4.5–5
       3, 5, 6, 7, 10, 13, 14, 16, 18, 20, 22, 23, 24, 28, 35, 36, 38, 42, 46, 47, 48, 49, 50, 52, 61, 65, 67
    c. 5.5–6
       19, 21, 26, 30, 32, 56, 57, 58, 62, 66
    d. 6.5–7
       1, 4, 37, 59
    e. above 7
       2, 29, 53, 60

18. Macroconidial length (av. in µm)
    a. Less than 40
       8, 15, 51, 68
    b. 40–46
       6, 10, 11, 17, 22, 30, 33, 34, 40, 41, 44, 50
    c. 47–55
       3, 7, 9, 14, 16, 19, 20, 27, 28, 31, 38, 39, 42, 45, 47, 48, 49, 52, 54, 55, 63, 64
    d. 56–66
       4, 5, 12, 13, 18, 23, 24, 25, 26, 35, 57, 61, 65
    e. 67–75
       1, 21, 36, 46, 58, 62, 67
    f. 76–95
       32, 37, 56, 59, 66
    g. above 95
       29, 53, 60

Dichotomous key to *Calonectria* species

The following key is an adaptation of the key provided by Crous (2002) to include all *Calonectria* spp. described subsequent to 2002. Measurements and observations are those of Crous (2002) and other authors who have described species subsequent to 2002 (Table 1). Only average conidial dimensions, where available, and a few distinguishing characters are presented in the key. Complete descriptions should be consulted to determine species variations. *Calonectria penicilloides* has been omitted from the keys, due to the fact that there is little morphological information available for this species.

1. Stipe extensions thick-walled; acicular to clavate vesicles 2
1. Stipe extensions and vesicles not as above 28

2. Stipe extensions thick-walled, terminating in acicular to clavate vesicles; fertile branches –3; phialides elongate-doliiform to reniform; macroconidia 1(–3)-septate, 64 × 5 µm; perithecia orange to red; ascospores 1(–3)-septate, 40 × 6 µm ..... ***Ca. avesiculata***
2. Stipe extensions not thick-walled and vesicles clavate 3

3. Teleomorph state unknown 4
3. Teleomorph state known 15

4. Macroconidia 1-septate only 5
4. Macroconidia more than 1-septate 8

5. Fertile branches –3; phialides doliiform to reniform; macroconidia 1-septate, 44 × 5 µm; stipe extensions terminating in clavate vesicles ..... ***Ca. pini***
5. Fertile branches –5 6

6. Lateral stipe extensions present; macroconidia 1(–2)-septate, 44 × 5 µm; stipe extensions terminating in clavate vesicles; fertile branches –5; phialides doliiform to reniform ..... ***Ca. brachiatica***
6. Lateral stipe extensions absent 7

7. Stipe extensions terminating in clavate vesicles; fertile branches –5; phialides doliiform to reniform; macroconidia 1-septate, 53 × 4.5 µm ..... ***Ca. brassicae***
7. Stipe extensions terminating in clavate vesicles; fertile branches –5; phialides doliiform to reniform; macroconidia 1-septate, 48 × 4 µm ..... ***Ca. orientalis***

8. Macroconidia longer than 100 µm 9
8. Macroconidia shorter than 100 µm 10

9. Macroconidia 5–8-septate, 104 × 8 µm; stipe extension terminate in clavate vesicles; fertile branches –3; phialides cylindrical to allantoid; microconidiophores lacking stipe extension; microconidia 1–3-septate, 44 × 4 µm ..... ***Ca. pseudoreteaudii***
9. Macroconidia 1–3-septate 12

10. Macroconidia (1–)3-septate, 63 × 6.5 µm; stipe extensions terminating in clavate vesicles; fertile branches –6; phialides cylindrical to allantoid ..... ***Ca. australiensis***
10. Macroconidia 1(–3)-septate 11

11. Fertile branches –7; phialides doliiform to reniform; macroconidia 51 × 4.5 µm; stipe extensions terminating in clavate vesicles ..... ***Ca. ecuadoriae***
11. Fertile branches –4; phialides doliiform to reniform; macroconidia 62 × 5 µm; stipe extensions terminating in clavate vesicles ..... ***Ca. gordoniae***

12. Macroconidia longer than 100 µm with more than 6 septa 13
12. Macroconidia shorter than 100 µm with 6 or less septa 14

13. Stipe extensions terminating in narrowly clavate vesicles; fertile branches –4; phialides cylindrical; macroconidia (1–)7–10(–12)-septate with slight swelling in the middle, 110 × 10 µm; Mega- and microconidia absent ..... ***Ca. angustata***
13. Stipe extensions terminating in narrowly clavate vesicles; fertile branches –3; phialides cylindrical; microconidia present, 1-septate, 18 × 3 µm; macroconidia (1–)7(–8)-septate, 120 × 7.5 µm; megaconidia present, 9–16-septate, bent or curved, (150–)200–250(–270) × 6–7(–8) µm ..... ***Ca. hurae***

14. Stipe extensions terminating in narrowly clavate vesicles; fertile branches –3; phialides cylindrical to allantoid, obpyriform when carried singly; macroconidia 4–6-septate, 69 × 6 µm ..... ***Ca. queenslandica***
14. Stipe extensions terminating in a narrowly clavate vesicles; fertile branches –3; phialides cylindrical to allantoid, obpyriform when carried singly; macroconidia 4–6-septate, 76 × 6 µm ..... ***Ca. terrae-reginae***

15. Macroconidial state unknown; megaconidiophores with stipe extensions terminating in clavate vesicles when present; megaconidia 6–10-septate, boomerang-shaped or curved, (120–)150–170(–220) × 8–9 µm; microconidia 1–3-septate, straight or curved, 20–65 × 2.5–3.5 µm ..... ***Ca. multiseptata***
15. Macroconidial state known 16

16. Teleomorph state known and macroconidia 1-septate to 1(–3)-septate 17
16. Teleomorph state known and macroconidia multi-septate 20

17. Teleomorph homothallic 18
17. Teleomorph heterothallic 19

18. Perithecia orange with a red apex; ascospores 1-septate, 35 × 6.5 µm; stipe extensions terminating in clavate vesicles; fertile branches –4; phialides doliiform to reniform; macroconidia 1-septate, 45 × 4.5 µm ..... ***Ca. gracilipes***

18. Perithecia red; ascospores 1-septate, 37 × 5 µm; stipe extensions terminating in narrowly clavate vesicles; fertile branches –4; phialides doliiform to reniform; macroconidia 1(–3)-septate, 56 × 4.5 µm ..... ***Ca. gracilis***

19. Perithecia orange; ascospores 1(–3)-septate, 44 × 5.5 µm; stipe extensions terminating in narrowly clavate vesicles; fertile branches –4; phialides elongate-doliiform to reniform; macroconidia 1(–3)-septate, 65 × 5 µm; microconidia 1-septate, 32 × 3 µm ..... ***Ca. clavata***

19. Perithecia red-brown; ascospores 1(–3)-septate, 52 × 6 µm; stipe extensions terminating in clavate to narrowly ellipsoidal vesicles; fertile branches –5; phialides elongate-doliiform to reniform; macroconidia 1(–3)-septate, 82 × 5.5 µm; microconidia 1-septate, 30 × 3.5 µm ..... ***Ca. pteridis***

20. Macroconidia 3-septate 21

20. Macroconidia 3- to multi-septate 25

21. Perithecia yellow to orange 22

21. Perithecia yellow 23

22. Teleomorph state homothallic; perithecia yellow to orange; ascospores (1–)3-septate, 33 × 6 µm; stipe extensions terminating in broadly clavate vesicles; fertile branches –5; phialides doliiform to reniform; macroconidia 3-septate, 72 × 6 µm ..... ***Ca. eucalypti***

22. Teleomorph state homothallic; perithecia orange to red; ascospores (1–)3-septate, 53 × 7 µm; stipe extensions terminating in narrowly clavate vesicles; fertile branches –5; phialides allantoid to reniform; macroconidia (1–)3-septate, 81 × 6 µm; megaconidia 7–9(–14)-septate, boomerang-shaped to curved, 130–200 × 5–6 µm ..... ***Ca. indusiata***

23. Macroconidia and ascospores shorter than 65 µm; teleomorph state homothallic; perithecia bright yellow; ascospores (1–)3-septate, 50 × 5.5 µm; stipe extensions terminating in clavate vesicles; fertile branches –5; phialides allantoid to cylindrical; macroconidia (1–)3-septate, 55 × 4.5 µm ..... ***Ca. madagascariensis***

23. Macroconidia and ascospores longer than 65 µm 24

24. Teleomorph state homothallic; perithecia bright yellow; ascospores (1–)3-septate, 55 × 6 µm; stipe extensions terminating in clavate vesicles; fertile branches –5; phialides elongate-doliiform to reniform; macroconidia (1–)3-septate, 65 × 5 µm ..... ***Ca. colhounii***

24. Teleomorph state heterothallic; perithecia dirty yellow, ascospores (1–)3-septate, 55 × 6 µm; stipe extensions terminating in clavate vesicles; fertile branches –5; phialides allantoid to cylindrical; macroconidia (1–)3(–4)-septate, 90 × 6.5 µm ..... ***Ca. macroconidialis***

25. Macroconidiophore branches –2 or less 26

25. Macroconidiophore with more than 2 series of branches 27

26. Teleomorph state homothallic; perithecia orange-brown; ascospores 3–6(–9)-septate, 90 × 6.5 µm; stipe extensions terminating in narrowly clavate vesicles; fertile branches –2; phialides cylindrical; microconidia 1(–3)-septate, (8–)15–30(–50) × 3–5 µm; macroconidia 5(–7)-septate, 110 × 9 µm; megaconidia 7–13-septate, bent or curved, (120–)180–230 × (8–)10–11(–13) µm ..... ***Ca. rumohrae***

26. Teleomorph state homothallic; perithecia red to red-brown; ascospores 3–4-septate, 70 × 6 µm; stipe extensions, when present, terminating in narrowly clavate vesicles; fertile branches –1; macroconidia 5–7-septate, 75 × 7 µm; microconidia 1–3-septate, 10–30 × 3–5 µm ..... ***Ca. acicola***

27. Teleomorph state homothallic; perithecia orange to red-brown; ascospores (1–)3-septate, 70 × 6.5 µm; stipe extensions terminating in narrowly clavate vesicles; fertile branches –6; phialides cylindrical to allantoid; macroconidia (1–)3–5(–6)-septate, 60 × 5 µm ..... ***Ca. leguminum***

27. Teleomorph state heterothallic; perithecia orange to red-brown; ascospores (1–)5(–6)-septate, 70 × 5.5 µm; stipe extensions terminating in clavate vesicles; fertile branches –6; phialides cylindrical to allantoid; macroconidia (1–)5(–6)-septate, 84 × 6.5 µm; microconidia 1(–3)-septate, 30 × 3 µm ..... ***Ca. reteaudii***

28. Vesicles sphaeropedunculate, globose or ovoid 29

28. Vesicles not as above 48

29. Vesicles consistently ovate; teleomorph state heterothallic; perithecia orange; ascocpores 1–3(–7)-septate, 60 × 5.5 µm; fertile branches –3; phialides doliiform to reniform; macroconidia straight or curved, 1(–3)-septate, 70 × 5 µm; microconidia 1-septate, 21 × 3 µm ..... ***Ca. ovata***

29. Vesicles not consistently ovate 30

30. Macroconidia 1(–3)-septate 31
30. Macroconidia only 1-septate 36

31. Teleomorph state unknown; stipe extensions terminating in sphaeropedunculate vesicles; fertile branches –3; phialides elongate-doliiform to reniform; macroconidia 1(–3)-septate, 60 × 5 µm ..... ***Ca. curvispora***
31. Teleomorph state known 32

32. Perithecia red-brown; teleomorph state homothallic; ascospores 1(–3)-septate, 42 × 5 µm; stipe extensions terminating in sphaeropedunculate to ovoid or ellipsoidal to clavate vesicles; fertile branches –3; phialides elongate-doliiform to reniform; macroconidia (1–)3(–4)-septate, 73 × 5 µm; microconidia 1-septate, 27 × 4 µm ..... ***Ca. variabilis***
32. Perithecia orange to red 33

33. Teleomorph state heterothallic; perithecia orange to red; ascospores 1(–3)-septate, 45 × 5 µm; stipe extensions terminating in globoid or ellipsoid to obpyriform vesicles; fertile branches –5; phialides elongate-doliiform to reniform; macroconidia 1(–3)-septate, 70 × 6 µm; microconidia 1-septate, 39 × 4 µm ..... ***Ca. spathiphylli***
33. Teleomorph state homothallic 34

34. Lateral stipe extensions abundant; perithecia orange; ascospores 1-septate, 33 × 5 µm; stipe extensions terminating in sphaeropedunculate vesicles; fertile branches –5; phialides elongate-doliiform to reniform; macroconidia 1(–3)-septate, 53 × 4.5 µm ..... ***Ca. colombiensis***
34. Lateral stipe extensions absent 35

35. Ascospores 1(–3)-septate, 42 × 5.5 µm; stipe extensions terminating in sphaeropedunculate to ellipsoidal vesicles; fertile branches –4; phialides elongate-doliiform to reniform; macroconidia 1(–3)-septate, 52 × 4 µm ..... ***Ca. pseudospathiphylli***
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