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ORIGINAL ARTICLE

Phylogeny and taxonomy of the genus *Cylindrocladiella*

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Abstract The genus *Cylindrocladiella* was established to accommodate *Cylindrocladium*-like fungi that have small, cylindrical conidia and aseptate stipe extensions. Contemporary taxonomic studies of these fungi have relied on morphology and to a lesser extent on DNA sequence comparisons of the internal transcribed spacer regions (ITS 1, 2 and 5.8S gene) of the ribosomal RNA and the β-tubulin gene regions. In the present study, the identity of several *Cylindrocladiella* isolates collected over two decades was determined using morphology and phylogenetic inference. A phylogeny constructed for these isolates employing the β-tubulin, histone H3, ITS, 28S large subunit and translation elongation factor 1-alpha gene regions resulted in the identification of several cryptic species in the genus. In spite of the 18 new *Cylindrocladiella* species described in this study based on morphological and sequence data, several species complexes remain unresolved.

Keywords *Cylindrocladiella* · Cryptic species · Phylogeny · Taxonomy

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Introduction

The genus *Cylindrocladiella* was established by Boesewinkel (1982) to accommodate five *Cylindrocladium*-like species producing small, cylindrical conidia. *Cylindrocladiella*, which is based on *C. parva*, is distinguished from the anamorph state of *Calonectria* (= *Cylindrocladium*) by its symmetrically branched conidiophores that can be penicillate and/or subverticillate, producing an asymmetrical bundle of small, cylindrical, 1-septate conidia (<20 μm in length), aseptate stipe extensions, and having *Nectricladiella* teleomorphs (Boesewinkel 1982, Crous and Wingfield 1993, Schoch et al. 2000). The *Nectricladiella* teleomorphs are characterised by their perithecia having smooth walls that collapse laterally when dry, and brown setae arising from the perithecial wall surface (Schoch et al. 2000).

Initially, the generic status of *Cylindrocladiella* was strongly contested (Peerally 1991, Sharma and Mohanan 1991). Morphological evaluations and comparisons by Crous and Wingfield (1993) and Crous et al. (1994), however, confirmed the generic status of this genus, which was later supported by molecular data (Victor et al. 1998, Schoch et al. 2000). Victor et al. (1998) used RFLPs, AT-DNA data and morphological comparisons, to recognise seven species in the genus. This was later supported by phylogenetic inference of the ITS and partial β-tubulin gene regions, resulting in the addition of another species to the

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genus (Schoch et al. 2000). To date, nine species of *Cylindrocladiella* are recognized, with only two connected to their respective *Nectricladiella* teleomorph states (Crous 2002, van Coller et al. 2005).

Cylindrocladiella spp. are soil-borne fungi, and are generally regarded as pathogens and/or saprobes of various plant hosts and substrates in temperate, sub-tropical and tropical regions worldwide (Crous 2002, van Coller et al. 2005, Scattolin and Montecchio 2007). They have been associated with a variety of disease symptoms that included leaf spots, and rots of roots, stems and cuttings of agricultural, forestry and horticultural crops (Crous et al. 1991, Peerally 1991, Crous and Wingfield 1993, Victor et al. 1998, Crous 2002, van Coller et al. 2005, Scattolin and Montecchio 2007).

The aim of this study was to consider the identity of several *Cylindrocladiella* isolates collected over the past two decades from various substrates and regions of the world. To achieve this goal, morphological and culture characteristics were combined with multigene phylogenetic inference for all isolates studied.

Material and methods

Isolates

Isolates and ex-type strains of *Cylindrocladiella* spp. were obtained from various culture collections, isolated from symptomatic plant material and/or baited from soils as described in Crous (2002) and indicated in Table 1. Representative strains have been maintained in the culture collection of the CBS-KNAW Fungal Biodiversity Centre (CBS) and the working collection of Pedro Crous (CPC).

Phylogeny

Total genomic DNA was extracted from 7–10 day old single-conidial cultures using the technique described by Möller et al. (1992). Partial fragments of the following genes and gene regions were amplified using the PCR conditions and primer sets mentioned in Lombard et al. (2010b): β-tubulin (BTUB), histone H3 (HIS3), internal transcribed spacers and 5.8 s rDNA (ITS), 28 s large subunit (LSU) and translation elongation factor 1-alpha (TEF1-α). The PCR reactions were carried out using a MyCycler™ thermal cycler (Bio-Rad Laboratories, Inc.) consisting of an initial step of 95°C for 5 min followed by 40 cycles of 30 s at 95°C, 30 s at 52°C, 1 min at 72°C and ending with a final extension step of 7 min at 72°C.

Amplicons were sequenced in both directions using the same primer sets used for amplification and the consensus sequences were aligned using MAFFT v6.611 (Katoh and Toh 2008) for each gene region. Ambiguous regions in the alignments were removed manually and both ends of the sequences were truncated. All sequences obtained were deposited in GenBank with accession numbers listed in Table 1.

Analyses of the DNA sequence data were done using PAUP (Phylogenetic Analysis Using Parsimony, v4.0b10, Swofford 2002). Initial neighbour-joining analyses (NJ) with the uncorrected ("p"), Juke-Cantor and HKY85 substitution models were done using the LSU sequence data to determine if the *Cylindrocladiella* isolates used in this study formed a monophyletic group. Congruency of the sequence data for each locus were determined using visual inspection of the tree topologies of 70% reciprocal NJ bootstrap trees (Gueidan et al. 2007) determined as described in Lombard et al. (2010c). Thereafter, the combined DNA sequence dataset was subjected to maximum parsimony (MP) and Bayesian analyses.

For the MP analysis, the phylogenetic relationships were determined using a heuristic search with 1 000 random sequence additions with a tree bisection-reconnection algorithm and 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). Branch support was assessed using a 1 000 bootstrap replicates.

For Bayesian analysis, a Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with posterior probabilities using MrBayes v3.1.1 (Ronquist and Heulsenbeck 2003). Nucleotide substitution models were determined for each gene using the Akaike Information Criterion (AIC) in MrModeltest v2.3 (Nylander 2004) and included in the analysis. The DNA sequence data was subjected to two separate analyses of four MCMC chains run from random trees for 1,000,000 generations with sampling at every 100 generations. Both runs converged on the same likelihood score and tree topology, and therefore the first 1,000 trees were discarded as the burn-in phase. Posterior probabilities were determined from the remaining trees. All alignments and trees generated in this study, have been deposited in TreeBASE (http:/www.treebase.org).

Taxonomy

Single-conidial cultures of *Cylindrocladiella* isolates were prepared on synthetic nutrient-poor agar (SNA; Nirenburg 1981) as described in Lombard et al. (2009). In some cases, carnation leaf pieces were added to the SNA to promote sporulation. The gross morphological characteristics were determined with 30 measurements at × 1,000 magnification

Table 1 Isolates of *Cylindrocladiella* studied

Species	Isolates	GenBank Accessions					Substrate	Country	Collector
		BTUB	HIS3	ITS	LSU	TEF-1α			
C. australiensis	CBS 129567=CPC 17507^T	JN098747	JN098932	JN100624	JN099222	JN099060	soil	Australia	P.W. Crous
	CBS 129568=CPC 17562	JN098748	JN098931	JN100623	JN099221	JN099059	soil	Australia	P.W. Crous
C. camelliae	CPC234=PPRI 3990=IMI 346845	AY793471	AY793509	AF220952	JN099249	JN099087	*Eucalyptus grandis*	South Africa	P.W. Crous
	CPC 237	JN098749	JN098839	JN100573	JN099252	JN099090	*Eucalyptus grandis*	South Africa	P.W. Crous
	CPC 239	JN098750	JN098838	JN100571	JN099250	JN099088	*Eucalyptus grandis*	South Africa	P.W. Crous
	CBS 114891=CPC 277	AY793472	AY793510	AF220953	JN099248	JN099086	*Eucalyptus grandis*	South Africa	P.W. Crous
C. clavata	CBS 129563=CPC 17591	JN098751	JN098859	JN099096	JN099136	JN098975	soil	Australia	P.W. Crous
	CBS 129564=CPC 17592^T	JN098752	JN098858	JN099095	JN099135	JN098974	soil	Australia	P.W. Crous
C. cymbiformis	CBS 129553=CPC 17393^T	JN098753	JN098866	JN099103	JN099143	JN098988	soil	Australia	P.W. Crous
	CBS 129554=CPC 17392	JN098754	JN098867	JN099104	JN099144	JN098989	soil	Australia	P.W. Crous
C. elegans	CBS 338.92=PPRI 4050=IMI 346847^T	AY793474	AY793512	AY793444	JN099201	JN099039	leaf litter	South Africa	I. Rong
	CBS 110801=CPC 525	JN098755	JN098916	JN100609	JN099206	JN099044	leaf litter	South Africa	P.W. Crous
C. ellipsoidea	CBS 129572=CPC 17558	JN098756	JN098943	JN100636	JN099235	JN099073	soil	Australia	P.W. Crous
	CBS 129573=CPC 17560^T	JN098757	JN098857	JN099094	JN099134	JN098973	soil	Australia	P.W. Crous
	CPC 17559	JN098758	JN098942	JN100635	JN099234	JN099072	soil	Australia	P.W. Crous
	CPC 17561	JN098759	JN098853	JN099093	JN099133	JN098972	soil	Australia	P.W. Crous
C. hawaiiensis	CBS 118704	JN098760	JN098878	JN099115	JN099158	JN098996	soil	Hawaii	Y. Degawa
	CBS 129569=CPC 12272^T	JN098761	JN098929	JN100621	JN099219	JN099057	soil	Hawaii	Y. Degawa
C. infestans	CBS 111795=ATCC 44816=CPC 2380^T	AF320190	AY793513	AF220955	JN099199	JN099037	*Pinus pinea*	New Zealand	H.J. Boesewinkel
	CBS 191.50=IMI 299376=CPC 2480	AY793475	AY793514	AF220956	JN099198	JN099036	*Arenga pinnata*	Indonesia	K.B. Boedijn & J. Reitsma
	CBS 192.50	JN098762	JN098882	JN099120	JN099163	JN099001	*Theobroma cacoa*	Indonesia	K.B. Boedijn & J. Reitsma
	CBS 114465=CPC 1619	JN098763	JN098887	JN099125	JN099170	JN099008		Ecuador	M.J. Wingfield
C. kurandica	CBS 129576=CPC 17547	JN098764	JN098941	JN100634	JN099233	JN099071	soil	Australia	P.W. Crous
	CBS 129577=CPC 17551^T	JN098765	JN098953	JN100646	JN099245	JN099083	soil	Australia	P.W. Crous
	CPC 17548	JN098766	JN098872	JN099109	JN099149	JN098983	soil	Australia	P.W. Crous
	CPC 17549	JN098767	JN098871	JN099108	JN099148	JN098982	soil	Australia	P.W. Crous
	CPC 17550	JN098768	JN098870	JN099107	JN099147	JN098981	soil	Australia	P.W. Crous
	CPC 17553	JN098769	JN098869	JN099106	JN099146	JN098980	soil	Australia	P.W. Crous
C. lageniformis	CBS 340.92=PPRI 4449=UFV 115^T	AY793481	AY793520	AF220959	JN099165	JN099003	*Eucalyptus* sp.	Brazil	A.C. Alfenas
	CBS 111060=CPC 1240	JN098770	JN098918	JN100611	JN099208	JN099046	*Eucalyptus* sp.	South Africa	P.W. Crous
	CBS 111061=CPC 1241	JN098771	JN098913	JN100606	JN099202	JN099040	*Eucalyptus* sp.	South Africa	P.W. Crous
	CBS 112898=CPC 5607	AY725652	AY725699	AY793445	JN099151	JN098990	*Vitis vinifera*	South Africa	L. Mostert
C. lageniformis	CBS 112899=CPC 5608	AY793476	AY793515	AY793446	JN099183	JN099021	*Vitis vinifera*	South Africa	L. Mostert
	CBS 113011=CPC 4283	JN098772	JN098903	JN100596	JN099188	JN099026	*Eucalyptus* sp.	South Africa	P.W. Crous
	CBS 113017=CPC 4287	JN098773	JN098884	JN099122	JN099167	JN099005	*Eucalyptus* sp.	South Africa	P.W. Crous

Table 1 (continued)

Species	Isolates	GenBank Accessions					Substrate	Country	Collector
		BTUB	HIS3	ITS	LSU	TEF-1α			
	CBS 113018=CPC 4286	JN098774	JN098904	JN100597	JN099189	JN099027	*Eucalyptus* sp.	South Africa	P.W. Crous
	CBS 113019=CPC 4285	JN098775	JN098905	JN100598	JN099190	JN099028	*Eucalyptus* sp.	South Africa	P.W. Crous
	CPC 17508	JN098776	JN098837	JN100570	JN099247	JN099085	soil	Australia	P.W. Crous
	CPC 17509	JN098777	JN098935	JN100628	JN099227	JN099065	soil	Australia	P.W. Crous
	CPC 17522	JN098778	JN098863	JN099100	JN099140	JN098985	soil	Australia	P.W. Crous
	CPC 17523	JN098779	JN098948	JN100641	JN099240	JN099078	soil	Australia	P.W. Crous
	CPC 17526	JN098780	JN098856	JN099092	JN099132	JN098971	soil	Australia	P.W. Crous
	CPC 17527	JN098781	JN098947	JN100640	JN099239	JN099077	soil	Australia	P.W. Crous
	CPC 17537	JN098782	JN098946	JN100639	JN099238	JN099076	soil	Australia	P.W. Crous
	CPC 17540	JN098783	JN098926	JN100619	JN099217	JN099055	soil	Australia	P.W. Crous
	CPC 17599	JN098784	JN098937	JN100630	JN099229	JN099067	soil	Australia	P.W. Crous
	CPC 17600	JN098785	JN098938	JN100631	JN099230	JN099068	soil	Australia	P.W. Crous
	CPC 18712	JN098786	JN098842	JN100576	JN099255	JN098957	*Rosa* sp.	USA	M. Munster
C. lanceolata	CBS 114950=CPC 396	JN098787	JN098898	JN100591	JN099181	JN099019	*Eucalyptus* sp.	South Africa	P.W. Crous
	CBS 129565=CPC 17566	JN098788	JN098939	JN100632	JN099231	JN099069	soil	Australia	P.W. Crous
	CBS 129566=CPC 17567^{T}	JN098789	JN098862	JN099099	JN099139	JN098978	soil	Australia	P.W. Crous
C. longiphialidica	CBS 129557=CPC 18839^{T}	JN098790	JN098851	JN100585	JN099264	JN098966	soil	Thailand	P.W. Crous
	CBS 129558=CPC 18841	JN098791	JN098852	JN100586	JN099265	JN098967	soil	Thailand	P.W. Crous
C. longistipitata	CBS 112953=CPC 4720	JN098792	JN098902	JN100595	JN099187	JN099025	*Opisthiolepsis heterophylla*	Australia	C. Pearce & B. Paulus
	CBS 116075=CPC 708^{T}	AY793506	AY793546	AF220958	JN099155	JN098993	soil	China	M.J. Wingfield
C. microcylindrica	CBS 111794=ATCC 38571=CPC 2375^{T}	AY793483	AY793523	AY793452	JN099203	JN099041	*Echeveria elegans*	Indonesia	C.F. Hill
C. natalensis	CBS 110800=CPC 529	JN098793	JN098915	JN100608	JN099205	JN099043	soil	South Africa	P.W. Crous
	CBS 114943=CPC 456^{T}	JN098794	JN098895	JN100588	JN099178	JN099016	*Arachis hypogaea*	South Africa	M.J. Wingfield
	CBS 114944=CPC 457	JN098795	JN098896	JN100589	JN099179	JN099017	*Arachis hypogaea*	South Africa	M.J. Wingfield
	CBS 114945=CPC 459	JN098796	JN098897	JN100590	JN099180	JN099018	*Arachis hypogaea*	South Africa	M.J. Wingfield
	CPC 17395	JN098797	JN098936	JN100629	JN099228	JN099066	soil	Australia	P.W. Crous
C. nederlandica	CBS 143.95=PD94/1353	JN098798	JN098891	JN099129	JN099175	JN099013	*Kalanchoë* sp.	Netherlands	J.W. Veenbaas-Rijks
	CBS 146.94=PD39/1776	JN098799	JN098889	JN099127	JN099173	JN099011	*Rhododendron* sp.	Netherlands	
	CBS 152.91=PD90/2015^{T}	JN098800	JN098910	JN100603	JN099195	JN099033	*Pelargonium* sp.	Netherlands	J.W. Veenbaas-Rijks
C. novaezelandica	CBS 486.77=ATCC 44815=CPC 2397^{T}	AY793485	AY793525	AF220963	JN099212	JN099050	*Rhododendron indicum*	New Zealand	H.J. Boesewinkel
C. parva	CBS 114524=ATCC 28272=CPC 2370^{T}	AY793486	AY793526	AF220964	JN099171	JN099009	*Telopea speciosissima*	New Zealand	H.J. Boesewinkel
C. peruviana	CBS 113022=CPC 4291	JN098801	JN098906	JN100599	JN099191	JN099029	*Eucalyptus* sp.	South Africa	P.W. Crous
	CBS 114697=CPC 2573	JN098802	JN098886	JN099124	JN099169	JN099007	*Vitis vinifera*	South Africa	S. Lambrecht
	CBS 114942=CPC 267	JN098803	JN098893	JN100587	JN099177	JN099015	*Acacia mearnsii*	South Africa	P.W. Crous
	CBS 114952=CPC 398	JN098804	JN098854	JN100572	JN099251	JN099089	*Eucalyptus* sp.	South Africa	P.W. Crous

Table 1 (continued)

Species	Isolates	GenBank Accessions					Substrate	Country	Collector
		BTUB	HIS3	ITS	LSU	TEF-1α			
	CBS 114953=CPC 399	JN098805	JN098885	JN099123	JN099168	JN099006	*Eucalyptus* sp.	South Africa	P.W. Crous
	CBS 116089=CPC 640=UFO 200	JN098806	JN098875	JN099112	JN099154	JN098969	*Piptadenia* sp.	Brazil	A.O. Carvalho
	CBS 116103=CPC 637=UFO 197	JN098807	JN098908	JN100601	JN099193	JN099031	*Psidium guajava*	Brazil	A.O. Carvalho
	CPC 17517	JN098808	JN098944	JN100637	JN099236	JN099074	soil	Australia	P.W. Crous
	CPC 17532	JN098809	JN098855	JN099091	JN099131	JN098970	soil	Australia	P.W. Crous
	CPC 17533	JN098810	JN098940	JN100633	JN099232	JN099070	soil	Australia	P.W. Crous
	CPC 17534	JN098811	JN098873	JN099110	JN099150	JN098984	soil	Australia	P.W. Crous
	CPC 17535	JN098812	JN098945	JN100638	JN099237	JN099075	soil	Australia	P.W. Crous
	CPC 17556	JN098813	JN098954	JN100569	JN099246	JN099084	soil	Australia	P.W. Crous
	IMUR 1843=CPC 2404^{T}	AY793500	AY793540	AF220966	JN099266	JN098968	ants	Peru	M.P. Herrera
C. pseudocamelliae	CBS 129555=CPC 18825^{T}	JN098814	JN098843	JN100577	JN099256	JN098958	soil	Thailand	P.W. Crous
	CBS 129556=CPC 18832	JN098815	JN098846	JN100580	JN099259	JN098961	soil	Thailand	P.W. Crous
	CPC 18826	JN098816	JN098844	JN100578	JN099257	JN098959	soil	Thailand	P.W. Crous
	CPC 18836	JN098817	JN098849	JN100583	JN099262	JN098964	soil	Thailand	P.W. Crous
	CPC 18838	JN098818	JN098850	JN100584	JN099263	JN098965	soil	Thailand	P.W. Crous
C. pseudohawaiiensis	CBS 210.94=PPRI 4450=UFV 125^{T}	JN098819	JN098890	JN099128	JN099174	JN099012	*Eucalyptus* sp.	Brazil	A.C. Alfenas
	CBS 115610=CPC 909	JN098820	JN098901	JN100594	JN099186	JN099024		Madagascar	P.W. Crous
C. pseudoinfestans	CBS 114530=CPC 2320	JN098821	JN098888	JN099126	JN099172	JN099010	soil	Madagascar	J.E. Taylor
	CBS 114531=CPC 2319^{T}	AY793508	AY793548	AF220957	JN099166	JN099004	soil	Madagascar	J.E. Taylor
C. pseudoparva	CBS 113624=CPC 752	JN098822	JN098883	JN099121	JN099164	JN099002	*Quercus* sp	Switzerland	L. Petrini
	CBS 122594	JN098823	JN098907	JN100600	JN099192	JN099030	*Vitis riparia*	New Zealand	K. Paice
	CBS 129560=CPC 18149^{T}	JN098824	JN098927	JN100620	JN099218	JN099056	soil	Netherlands	P.W. Crous
	CPC 18150	JN098825	JN098864	JN099101	JN099141	JN098986	soil	Netherlands	P.W. Crous
C. queenslandica	CBS 129574=CPC 17568^{T}	JN098826	JN098861	JN099098	JN099138	JN098977	soil	Australia	P.W. Crous
	CBS 129575=CPC 17569	JN098827	JN098860	JN099097	JN099137	JN098976	soil	Australia	P.W. Crous
C. stellenboschensis	CBS 386.67	JN098828	JN098920	JN100613	JN099210	JN099048	*Fragaria* sp.	Netherlands	G.H. Boerema
	CBS 110668=CPC 517^{T}	JN098829	JN098922	JN100615	JN099213	JN099051	soil	South Africa	P.W. Crous
	CBS 115611=CPC 4074	JN098830	JN098900	JN100593	JN099185	JN099023	*Geum* sp.	New Zealand	P.W. Crous
	CBS 116170=CPC 753	JN098831	JN098894	JN099117	JN099160	JN098998	*Quercus* sp	Switzerland	L. Petrini
	CBS 129559=CPC 15200	JN098832	JN098868	JN099105	JN099145	JN098979	leaf litter	Canada	P.W. Crous
C. thailandica	CBS 129570=CPC 18834	JN098833	JN098847	JN100581	JN099260	JN098962	soil	Thailand	P.W. Crous
	CBS 129571=CPC 18835^{T}	JN098834	JN098848	JN100582	JN099261	JN098963	soil	Thailand	P.W. Crous
	CPC 18831	JN098835	JN098845	JN100579	JN099258	JN098960	soil	Thailand	P.W. Crous
C. variabilis	CBS 375.93=IMI 317057	JN098836	JN098881	JN099119	JN099162	JN099000	*Mangifera indica*	India	P.N. Chowdhry
	CBS 129561=CPC 17505^{T}	JN098719	JN098950	JN100643	JN099242	JN099080	soil	Australia	P.W. Crous

Table 1 (continued)

Species	Isolates	GenBank Accessions					Substrate	Country	Collector
		BTUB	HIS3	ITS	LSU	TEF-1α			
	CBS 129562=CPC17506	JN098720	JN098951	JN100644	JN099243	JN099081	soil	Australia	P.W. Crous
	CPC 17504	JN098721	JN098949	JN100642	JN099241	JN099079	soil	Australia	P.W. Crous
	CPC 17563	JN098722	JN098933	JN100625	JN099223	JN099061	soil	Australia	P.W. Crous
C. viticola	CBS 112897=CPC 5606^{T}	AY793504	AY793544	AY793468	JN099226	JN099064	*Vitis vinifera*	South Africa	G.J. van Coller
	CBS 114682=IMI 297470=CPC 2509	JN098723	JN098919	JN100612	JN099209	JN099047	*Amorphophallus* sp.	Thailand	R. Stevenson
Cylindrocladiella sp.	CBS 139.26	JN098724	JN098912	JN100605	JN099197	JN099035		Netherlands	C.J. Buisman
	CBS 114960=CPC 375	JN098725	JN098874	JN099111	JN099152	JN098991	*Pinus radiata*	South Africa	P.W. Crous
	CBS 114961=CPC 377	JN098726	JN098934	JN100626	JN099224	JN099062	*Pinus radiata*	South Africa	P.W. Crous
	CBS 115687=CPC 530	JN098727	JN098909	JN100602	JN099194	JN099032	leaf litter	South Africa	P.W. Crous
	CBS 115895=CPC 502	JN098728	JN098876	JN099113	JN099156	JN098994		South Africa	S. Lambrecht
	CPC 374	JN098729	JN098841	JN100575	JN099254	JN098956	*Pinus radiata*	South Africa	P.W. Crous
Cylindrocladiella sp.	CBS 199.62	JN098730	JN098911	JN100604	JN099196	JN099034	*Viburnum* sp.	Netherlands	G.H. Boerema
	CBS 110669=CPC 509	JN098731	JN098914	JN100607	JN099204	JN099042	soil	South Africa	P.W. Crous
Cylindrocladiella sp.	CBS 874.68=ATCC 16315=IMI 299377	JN098732	JN098921	JN100614	JN099211	JN099049	soil	Germany	W. Gams
Cylindrocladiella sp.	CBS 100283	JN098733	JN098892	JN099130	JN099176	JN099014	twig on ground	Japan	H.-J. Schroers
Cylindrocladiella sp.	CBS 110946=CPC 970	JN098734	JN098917	JN100610	JN099207	JN099045			P.W. Crous
	CBS 115673=CPC 917	AY793502	AY793542	AY793466	JN099153	JN098992	soil	South America	P.W. Crous
	CBS 115675=CPC 968	AY793503	AY793543	AY793467	JN099184	JN099022	soil	South America	P.W. Crous
Cylindrocladiella sp.	CBS 112364	AY793507	AY793547	AY793470	JN099200	JN099038	*Archontophoenix purpurea*	Australia	F. Hill
Cylindrocladiella sp.	CBS 114780=CPC 278	JN098735	JN098925	JN100618	JN099216	JN099054	*Eucalyptus grandis*	South Africa	P.W. Crous
	CBS 114884=CPC 279	JN098736	JN098924	JN100617	JN099215	JN099053	*Eucalyptus grandis*	South Africa	P.W. Crous
Cylindrocladiella sp.	CBS 114881=CPC 238	JN098737	JN098880	JN099118	JN099161	JN098999	*Eucalyptus grandis*	South Africa	P.W. Crous
Cylindrocladiella sp.	CBS 114885=CPC 262	JN098738	JN098923	JN100616	JN099214	JN099052	*Eucalyptus* sp.	South Africa	P.W. Crous
Cylindrocladiella sp.	CBS 114890=CPC 259	JN098739	JN098928	JN100627	JN099225	JN099063	*Eucalyptus* sp.	South Africa	P.W. Crous
Cylindrocladiella sp.	CBS 114957=CPC 426	JN098740	JN098899	JN100592	JN099182	JN099020	*Eucalyptus* sp.	South Africa	P.W. Crous
	CPC 260	JN098741	JN098840	JN100574	JN099253	JN098955	*Eucalyptus* sp.	South Africa	P.W. Crous
Cylindrocladiella sp.	CBS 116095=CPC 678	JN098742	JN098879	JN099116	JN099159	JN098997	soil	South Africa	M.J. Wingfield
Cylindrocladiella sp.	CBS 122595	JN098743	JN098877	JN099114	JN099157	JN098995	*Vitis riparia*	New Zealand	K. Paice
Cylindrocladiella sp.	CPC 15198	JN098744	JN098930	JN100622	JN099220	JN099058	soil	Canada	P.W. Crous
Cylindrocladiella sp.	CPC 15199	JN098745	JN098952	JN100645	JN099244	JN099082	soil	Canada	P.W. Crous
Cylindrocladiella sp.	CPC 17603	JN098746	JN098865	JN0990102	JN099142	JN098987	soil	Australia	P.W. Crous

CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: working collection of Pedro Crous housed at CBS; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; ATCC: American Type Culture Collection, Virginia, U.S.A.; PPRI: Plant Protection Research Institute, Agricultural Research Council, Pretoria, South Africa.; UFV: Univeridade Federal de Vicosa, Brazil., BTUB=β-tubulin, 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. ^{T} Ex-type cultures.

of the fungal structures mounted in 85% lactic acid. The conidial measurements are presented as the 95% confidence level with extremes in parentheses. Only the extremes are presented for other structures. The colony colours were determined on 2% w/v malt extract agar (MEA) after 7 day incubation at 24°C in the dark using the colour charts of Rayner (1970). Descriptions, nomenclature and illustrations were deposited in MycoBank (Crous et al. 2004).

Results

Phylogeny

Amplicons of approximately 530 bases were determined for BTUB, HIS3 and TEF-1α, 500 for ITS, and 850 for LSU. The phylogenetic analysis included 136 ingroup taxa, with *Ca. pauciramosa* (CBS 114861) and *Ca. brachiatica* (CBS 123700) as outgroup taxa. The initial NJ analysis of the LSU sequence data revealed that all the isolates included in the study formed a monophyletic clade (results not shown). Comparisons of the 70% reciprocal bootstrap NJ tree topologies of the individual gene regions showed no conflict and therefore the sequence datasets were combined. The resulting dataset of 2,956 characters, including alignment gaps, consisted of 2,070 constant and 131 parsimony-uninformative characters. Analysis of the 755 parsimony-informative characters yielded 1,224 trees (TL=,4486; CI=0.308; RI=0.843; RC=0.260), of which the first tree is presented (Fig. 1). For the Bayesian analysis, a HKY + I + G model was selected for BTUB and TEF-1α, GTR + I + G for HIS3 and LSU, and SYM + I + G for ITS which was incorporated into the analysis. The Bayesian consensus tree confirmed both the tree topology and bootstrap support of the strict consensus tree obtained with maximum-parsimony.

In the phylogenetic tree (Fig. 1) the *Cylindrocladiella* isolates are divided into two main clades. The first main clade [bootstrap support (BS)=98; posterior probability (PP)=0.70] is further divided into two subclades. The first subclade (BS=98; PP=0.70) represents *C. novaezelandiae* (CBS 486.77), *C. elegans* (CBS 338.92) and other closely related isolates that could represent novel phylogenetic species. The second subclade (BS=100; PP=0.75) representing *C. camelliae* (CPC 234; Crous 2002) and *C. peruviana* (IMUR 1843) also consists of closely related isolates clustering together in smaller well-supported terminal clades, each representing possible novel species.

The second main clade (BS=59; PP=0.53) is also divided into two subclades. In the first subclade (BS=82; PP=0.90) representing *C. parva* (CBS 114524) several isolates form well-supported terminal clades, also indicating potentially new species. The second subclade (BS=91; PP=0.93) further divides into a clade (BS=89; PP=0.65) representing *C. viticola* (CBS 112897), and a clade (BS=100; PP=1.00) containing *C. lageniformis* (CBS 340.92) and *C. infestans*. This clade also consists of several well-supported terminal clades that could represent novel species. The ex-type strain of the anamorph state of *C. infestans* (CBS 111795) clustered (BS=83; PP=0.97) separately from the ex-type strain of the purported teleomorph state of this species (CBS 114531, BS=100; PP=0.98), indicating that each ex-type strain represents a distinct species.

Taxonomy

Based on the phylogenetic inference and morphological observations, numerous *Cylindrocladiella* isolates included in this study represent novel species. Following the approach of Lombard et al. (2009, 2010a–c) and Crous et al. (2006, 2008, 2009) for other fungal groups, all new species are described in *Cylindrocladiella,* as this represents the older generic (Boesewinkel 1982), and best established name for this group of fungi.

Cylindrocladiella australiensis L. Lombard & Crous, sp. nov. – MycoBank MB561676, Fig. 2.

Etymology – Named after the country from where it was collected, Australia.

Cylindrocladiellae infestantis morphologice valde similis, sed conidiis minoribus, (9–)11–13(–15)×2–4 μm, distinguitur.

Teleomorph unknown. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 2a–e) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 41–96×6–9 μm; stipe extension aseptate, straight, 101–152 μm long, thick-walled with one basal septum, terminating in thin-walled, ellipsoidal to fusoid vesicles (Fig. 2j–l), 6–8 μm wide. *Penicillate conidiogenous apparatus* (Fig. 2f–i) with primary branches aseptate, 13–21×3–5 μm, secondary branches aseptate, 11–15×3–6 μm, each terminal branch producing 2–4 phialides; phialides cymbiform to cylindrical, hyaline, aseptate, 8–17×2–4 μm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* (Fig. 2m–n) abundant, comprising of a septate stipe, primary and secondary branches terminating in 2–3 phialides; primary branches straight, hyaline, 0–1-septate, 22–54×2–5 μm, secondary branches straight, hyaline, aseptate, 21–36×4–5 μm; phialides cymbiform to cylindrical, hyaline, aseptate, 19–40×2–4 μm, apex with minute periclinal thickening and collarette. *Conidia* (Fig. 2o) cylindrical, rounded at both ends, straight, 1-septate, (9–)11–13(–15)×2–4 μm (av. = 12×3 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

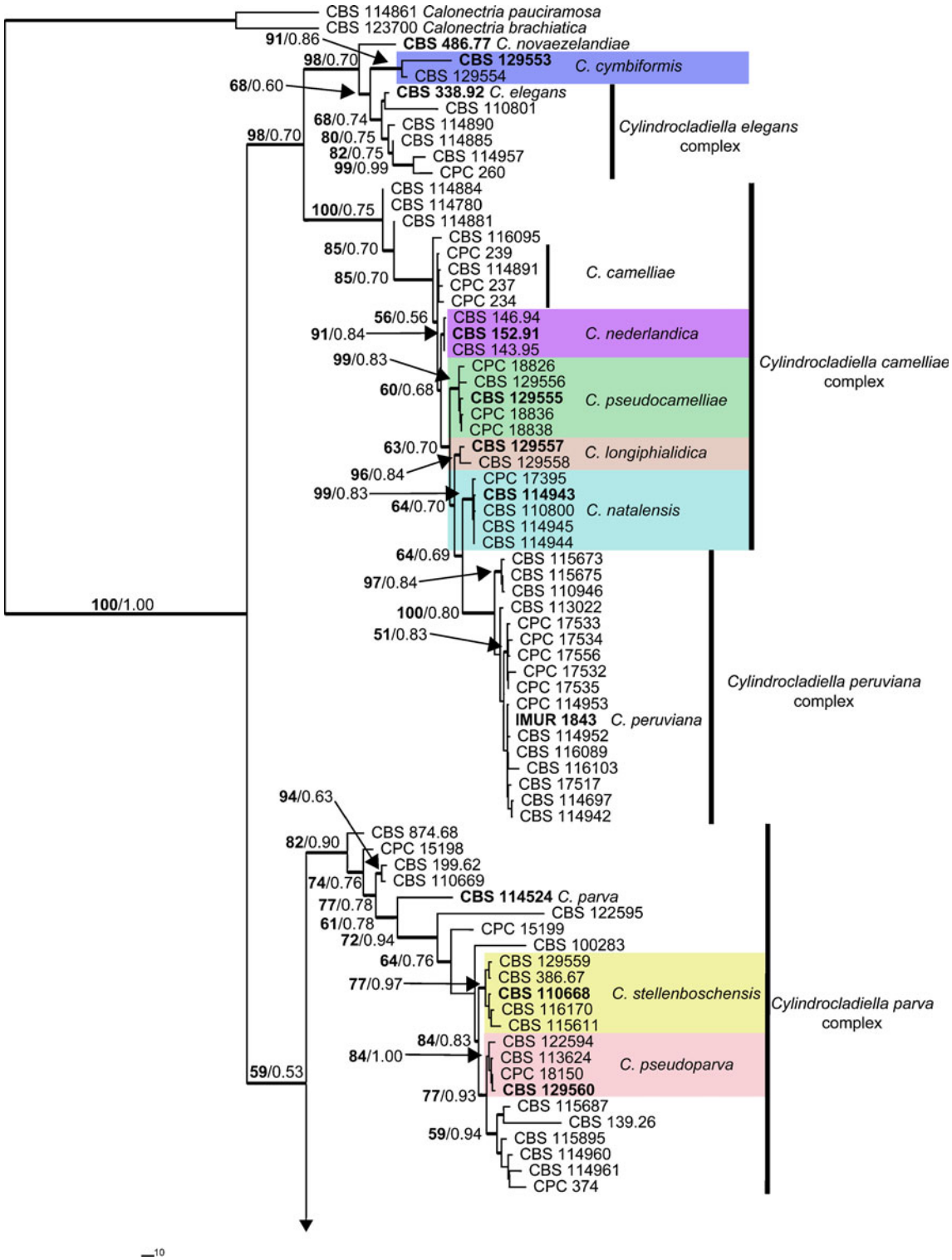

Fig. 1 One of 1,224 most parsimonious trees obtained from a heuristic search with 1,000 random additions sequences of the combined β-tubulin, histone H3, internal transcribed spacer regions 1 and 2 and the 5.8S gene of the ribosomal RNA, 28S large subunit and translation elongation factor-1alpha sequence alignments of the *Cylindrocladiella* isolates. 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. Ex-type strains are indicated in bold and coloured block indicate the novel species described. The tree was rooted to *Calonectria brachiatica* (CBS123700) and *Ca. pauciramosa* (CBS 114861). Species complexes are indicated on the right

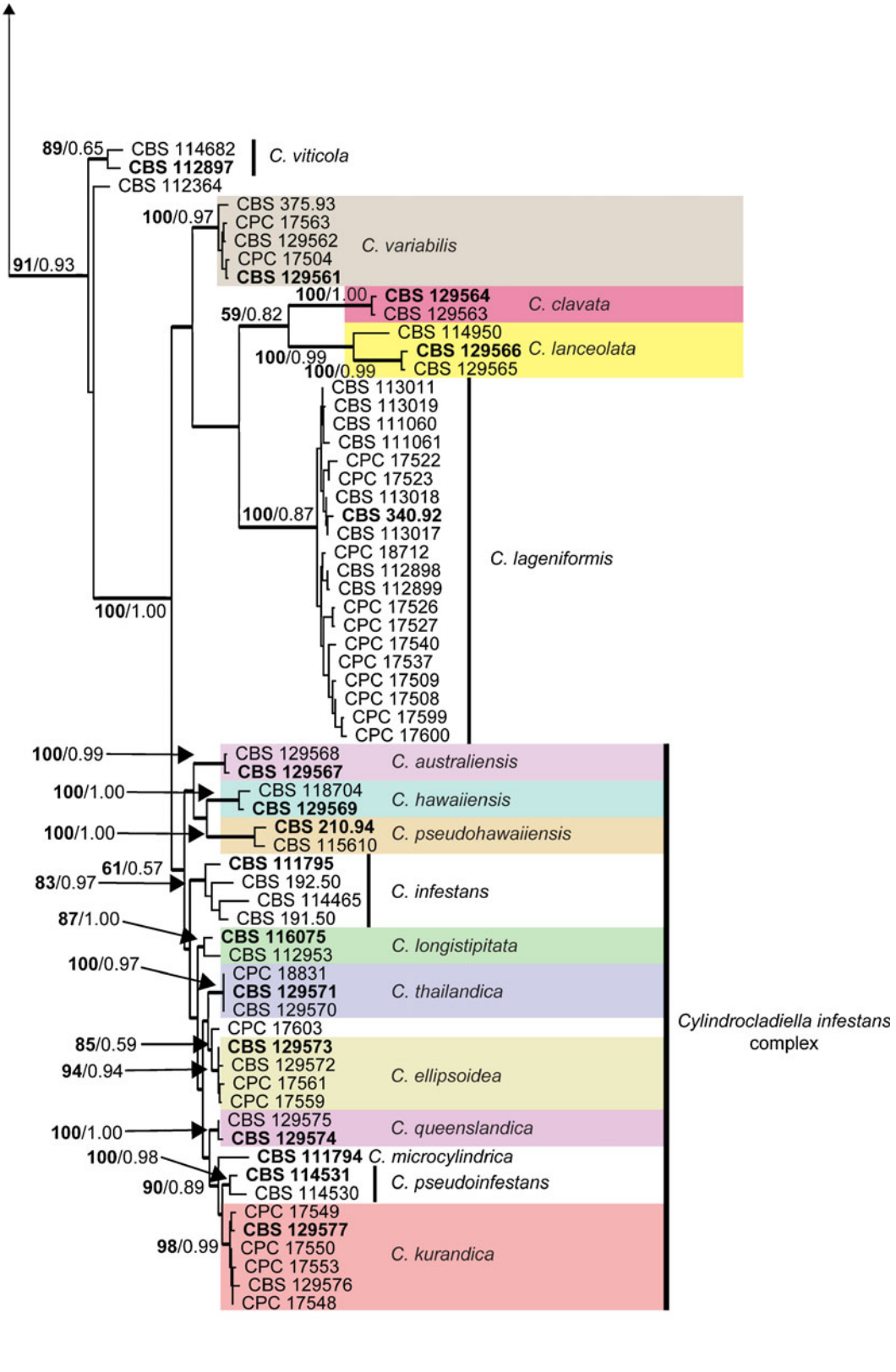

Fig. 1 (continued)

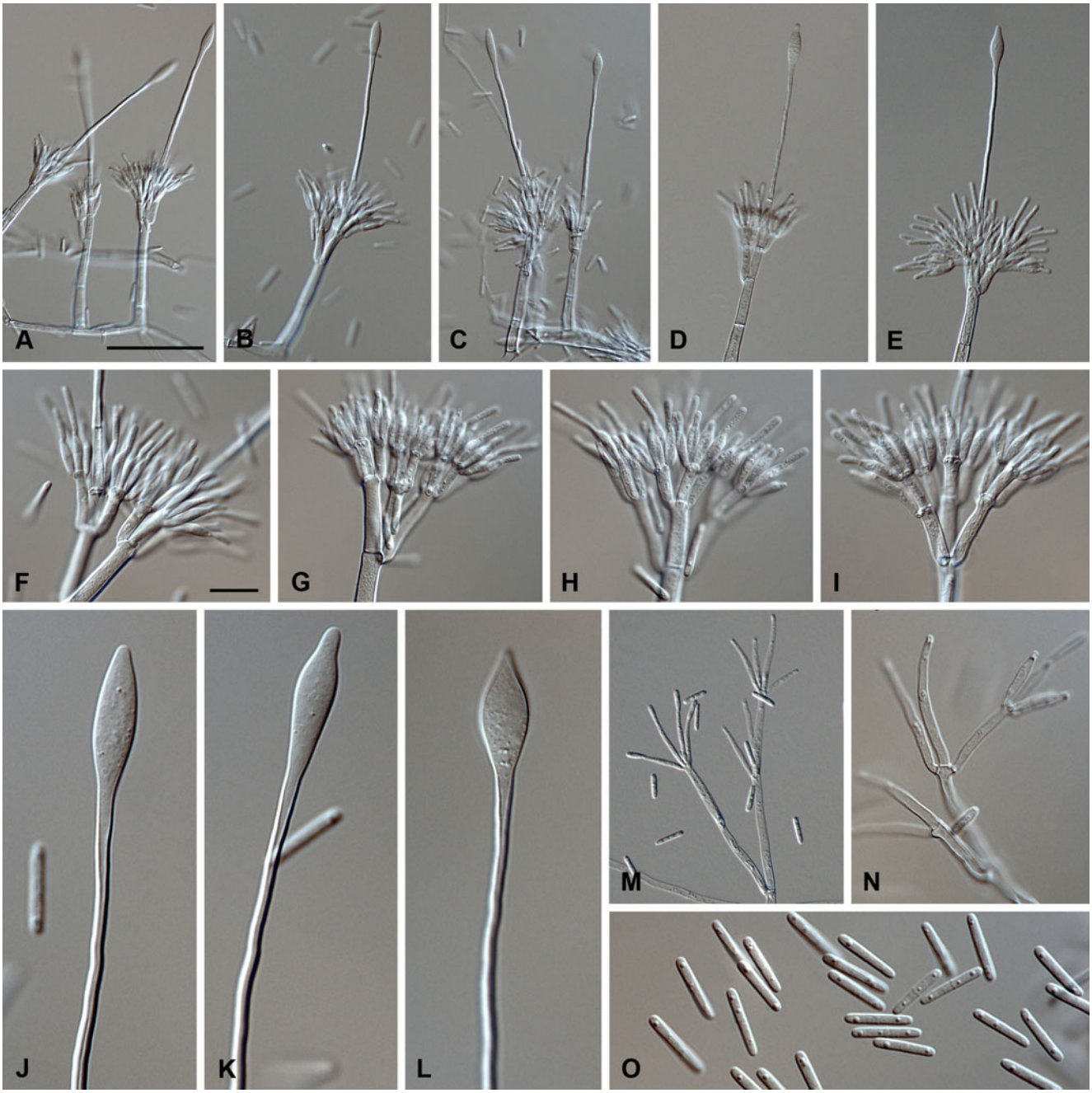

Fig. 2 *Cylindrocladiella australiensis*. **a**–**e**. Penicillate conidiophores. **f**–**i**. Conidiogenous apparatus with conidiophore branches and phialides. **j**–**l**. Terminal vesicles. **m**–**n**. Subverticillate conidiophores. **o**. Conidia. A=50 μm (apply to **b**–**e**, **m**), F=10 μm (apply to **g**–**l**, **n**–**o**)

Culture characteristics – Colonies raised (convex), cottony, with smooth margins, white, buff yellow (19 d) to umber (13i) (reverse); chlamydospores moderate throughout medium, arranged in chains; reaching 90 mm after 7 days on MEA at 24°C in the dark.

Specimens examined – Australia, Queensland, Daydream Island, Whitsundays Island Resort, from soil, 2 Aug. 2009, P.W. Crous, holotype CBS-H20596, culture ex-type CBS 129567=CPC 17507; Australia, Queensland, Lake Barrine, from soil, 18 June 2009, P.W. Crous, culture CBS 129568= CPC 17562.

Notes – *Cylindrocladiella australiensis* can be distinguished from *C. infestans* (av. 15×3 μm) by its smaller conidia and its terminal vesicle shape. The subverticillate conidiophores of *C. australiensis* also form secondary branches not reported for *C. infestans*. Unique fixed nucleotides were also identified for *C. australiensis* for three loci: BTUB positions 186 (T), 296 (C), 350 (T),

381 (C) and 387 (T); HIS3 positions 90 (T) and 387 (T); TEF-1α positions 113 (C), 153 (G), 155 (C), 168 (T), 229 (T), 232 (C), 254 (G), 266 (C), 282 (T), 462 (A) and 468 (C).

Cylindrocladiella clavata L. Lombard & Crous, sp. nov. – MycoBank MB561674, Fig. 3.

Etymology – Named after the clavate shape of its vesicles.

Cylindrocladiellae variabilis morphologice similis, sed vesiculis clavatis distinguitur.

Teleomorph unknown. *Conidiophores* monomorphic, penicillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 3a–e) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 40–86×6–10 μm; stipe extension aseptate, straight, 116–170 μm long, thick-walled with one basal septum, terminating in thin-walled, elongated, clavate vesicles (Fig. 3f–h), 4–7 μm wide. *Penicillate conidiogenous apparatus* (Fig. 3i–k) with primary branches aseptate, 10–23×3–8 μm, secondary branches aseptate, 6–11×2–4 μm, each terminal branch producing 2–4 phialides; phialides doliiform to cymbiform, hyaline, aseptate, 7–12×2–3 μm, apex with minute periclinal thickening and collarette absent. *Subverticillate conidiophores* not observed. *Conidia* (Fig. 3l) cylindrical, rounded at both ends, straight, 1-septate, (10–)13–15(–16)×2–3 μm (av. = 14×2 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with undulate margins, white with buff yellow (19 d) centre, umber (13i) (reverse); chlamydospores extensive throughout medium arranged in chains; reaching 70 mm after 7 days on MEA at 24°C in the dark.

Specimen examined – Australia, Queensland, Byron Bay, from soil, 17 July 2009, P.W. Crous, holotype CBS-H20597, culture ex-type CBS 129564=CPC 17592, Australia, Queensland, Byron Bay, from soil, 17 July 2009, P.W. Crous, culture CBS 129563=CPC 17591.

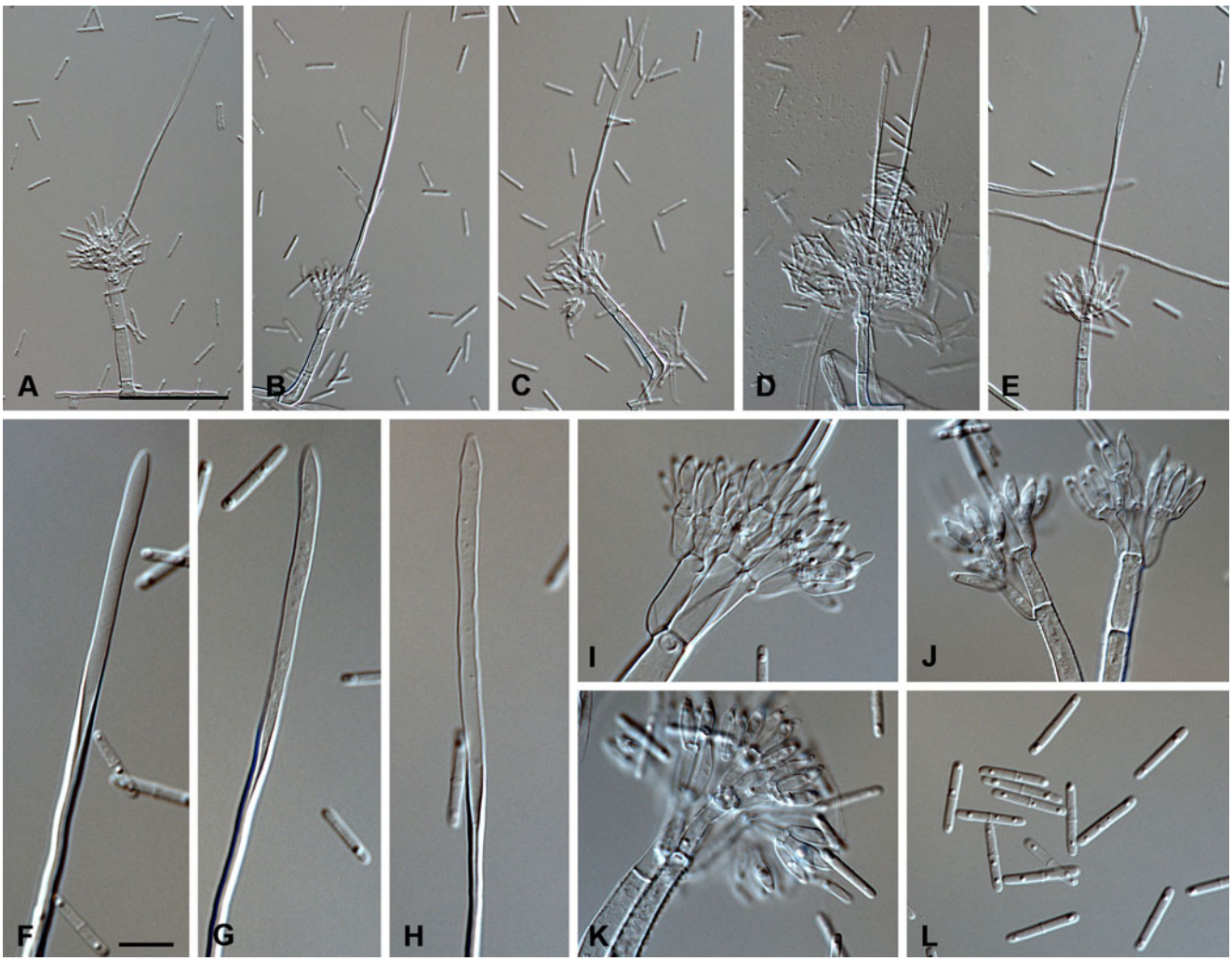

Fig. 3 *Cylindrocladiella clavata*. **a**–**e**. Penicillate conidiophores. **f**–**h**. Terminal vesicles. **i**–**k**. Conidiogenous apparatus with conidiophore branches and phialides. L. Conidia. Scale bars: A=50 μm (apply to **b**–**e**), F=10 μm (apply to **g**–**l**)

Notes – This species can be distinguished from other species in the genus by its elongated clavate terminal vesicles. The conidia are also slightly larger than those of *C. lageniformis* (av. 12×2 μm).

Cylindrocladiella cymbiformis L. Lombard & Crous, sp. nov. – MycoBank MB561666, Fig. 4.

Etymology – Named after its phialides, which are cymbiform in shape.

Cylindrocladiellae elegantis morphologice valde similis, sed conidis majoribus, (15–)16–20(–22)×(2–)3–5(–6) μm, distinguitur.

Teleomorph unknown. *Conidiophores* monomorphic, penicillate, mononematous, hyaline; comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle (Fig. 4a–e); stipe septate, hyaline, smooth, 44–84×2–4 μm; stipe extension aseptate, straight, 107–175 μm long, thick-walled with one basal septum, terminating in thin-walled, lageniform to broadly clavate vesicles (Fig. 4j–l), 6–8 μm wide. *Penicillate conidiogenous apparatus* (Fig. 4f–i) with primary branches aseptate, 12–28×4–6 μm, secondary branches aseptate, 11–19×2–5 μm, each terminal branch producing 2–4 phialides; phialides cymbiform, hyaline, aseptate, 11–19×2–5 μm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* not observed. *Conidia* (Fig. 4m) cylindrical, rounded at both ends, straight, 1-septate, (15–)16–20(–22)×(2–)3–5(–6) μm (av. = 18×3 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with undulate margins, white, buff yellow (19 d) (reverse); chlamydospores sparse throughout medium, arranged in chains; reaching 45 mm after 7 days on MEA at 24°C in the dark.

Specimen examined – Australia, Queensland, Brisbane, from soil, 11 July 2009, P.W. Crous, holotype CBS-H20598,

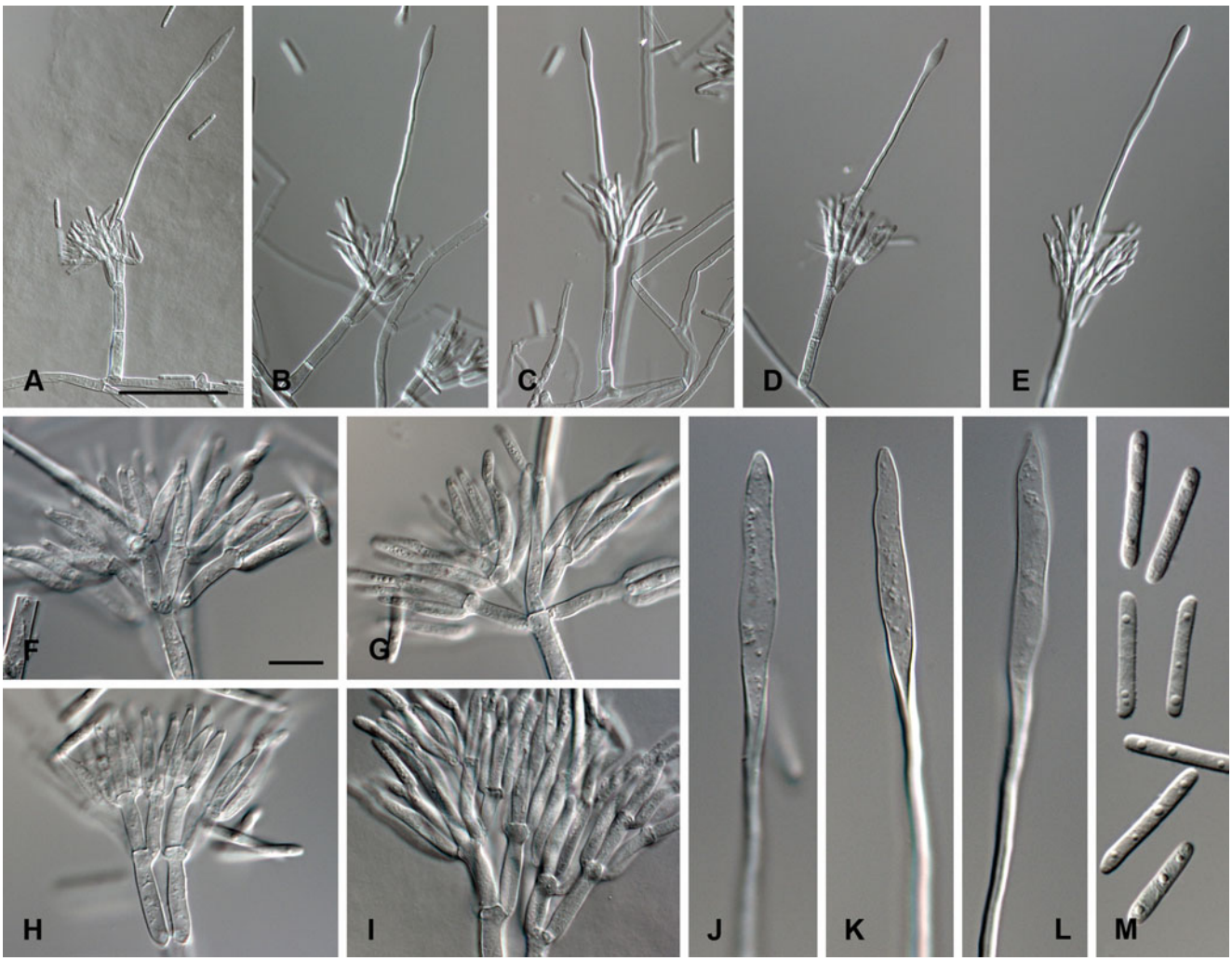

Fig. 4 *Cylindrocladiella cymbiformis*. **a**–**e**. Penicillate conidiophores. **f**–**i**. Conidiogenous apparatus with conidiophore branches and phialides. **j**–**l**. Terminal vesicles. **m**. Conidia. Scale bars: A=50 μm (apply to **b**–**e**), F=10 μm (apply to **g**–**m**)

culture ex-type CBS 129553=CPC 17393; Australia, Queensland, Brisbane, from soil, 11 July 2009, P.W. Crous, culture CBS 129554=CPC 17392.

Notes – Based on phylogenetic inference, *C. cymbiformis* is placed in the *C. elegans* species complex, and closely related to *C. novaezelandiae*. Morphologically, this species has larger conidia (av. 18×3 μm) than *C. elegans* (av. 14.5×2 μm) and *C. novaezelandiae* (av. 14.5×2 μm) (Crous 2002), and stipe extensions are also much longer. Only cymbiform phialides were observed for *C. cymbiformis,* whereas both *C. elegans* and *C. novaezelandiae* also produce doliiform to reniform phialides. Furthermore, no subverticillate conidiophores were observed for *C. cymbiformis*, but have been reported for *C. elegans*.

Cylindrocladiella ellipsoidea L. Lombard & Crous, sp. nov. – MycoBank MB561681, Fig. 5.

Etymology – Named after the characteristic ellipsoid shape of its vesicles.

Cylindrocladiellae infestantis morphologice similis, sed vesiculis clavatis vel ellipsoideis distiunguitur.

Teleomorph unknown. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 5a–e) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 41–83×6–8 μm; stipe extension aseptate, straight, 77–155 μm long, thick-walled with one basal septum, terminating in thin-walled, clavate to ellipsoidal vesicles (Fig. 5j–n), 5–8 μm wide. *Penicillate conidiogenous apparatus* (Fig. 5f–i) with primary branches aseptate, 11–20×3–6 μm, secondary branches aseptate, 9–12×3–5 μm, each terminal branch producing 2–4 phialides; phialides doliiform to cymbiform, hyaline, aseptate, 9–12×2–4 μm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* (Fig. 5o–p) in moderate numbers, comprising of a septate stipe, primary and secondary branches terminating in 2–3 phialides; primary branches straight, hyaline, 0–1-septate, 23–40×3–6 μm, secondary branches rare, straight, hyaline, aseptate, 16–31×4 μm; phialides cymbiform to cylindrical, hyaline, aseptate, 27–52×2–4 μm, apex with minute periclinal thickening and collarette. *Conidia* (Fig. 5q) cylindrical, rounded at both ends, straight, 1-septate, (14–)16–18(–19)×3–4 μm (av. = 17×4 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with smooth to undulate margins, white, buff yellow (19 d) to umber (13i) (reverse); chlamydospores moderate throughout media arranged in chains; reaching 60 mm after 7 days on MEA at 24°C in the dark.

Specimen examined – Australia, Queensland, Lake Barrine, from soil, 18 June 2009, P.W. Crous, holotype CBS-H20599, culture ex-type CBS 129573=CPC 17560; Australia, Queensland, Lake Barrine, from soil, 18 June 2009, P.W. Crous, culture CBS 129572=CPC 17558; Australia, Queensland, Lake Barrine, from soil, 18 June 2009, P.W. Crous, culture CPC 17559.

Notes – *Cylindrocladiella ellipsoidea* produces subverticillate conidiophores with secondary branches, which has not reported been observed for other species in the *C. infestans* complex. Furthermore, *C. ellipsoidea* can also be distinguished for others in the complex based on their terminal vesicle shape. Unique fixed nucleotides were also identified for *C. ellipsoidea* for two loci: HIS3 positions 124 (C), 130 (A), 134 (C), 314 (A) and 349 (A); TEF-1α position 210 (indel).

Cylindrocladiella hawaiiensis L. Lombard & Crous, sp. nov. – MycoBank MB561677, Fig. 6.

Etymology – Named after Hawaii, where this fungus was collected.

Cylindrocladiellae infestantis morphologice similis, sed vesiculis clavatis distinguitur.

Teleomorph unknown. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 6a–c) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 47–80×5–6 μm; stipe extension aseptate, straight, 80–116 μm long, thick-walled with one basal septum, terminating in thin-walled, clavate vesicles (Fig. 6d–e), 5–7 μm wide. *Penicillate conidiogenous apparatus* (Fig. 6f–j) with primary branches aseptate, 11–19×4–5 μm, secondary branches aseptate, 8–19×3–4 μm, each terminal branch producing 2–4 phialides; phialides cymbiform to cylindrical, hyaline, aseptate, 8–18×2–3 μm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* (Fig. 6k–l) abundant, comprising of a septate stipe, and primary branches terminating in 2–3 phialides; primary branches straight, hyaline, 0–1-septate, 23–38×3–5 μm; phialides cymbiform to cylindrical, hyaline, aseptate, 19–41×2–4 μm, apex with minute periclinal thickening and collarette. *Conidia* (Fig. 6m) cylindrical, rounded at both ends, straight, 1-septate, (10–)12–14×2–4 μm (av. = 13×3 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with smooth margins, white, buff yellow (19 d) (reverse); chlamydospores sparse throughout medium, arranged in chains; reaching 65 mm after 7 days on MEA at 24°C in the dark.

Specimens examined – Hawaii, from soil, 1 Aug. 2005, Y. Degawa, holotype CBS-H20600, culture ex-type CBS 129569=CPC 12272; Hawaii, Kaua'i Island, Secret waterfall, from soil, 8 Aug. 2005, Y. Degawa, culture CBS 118704.

Notes – *Cylindrocladiella hawaiiensis* produces clavate terminal vesicles, distinguishing it from *C. infestans*, which

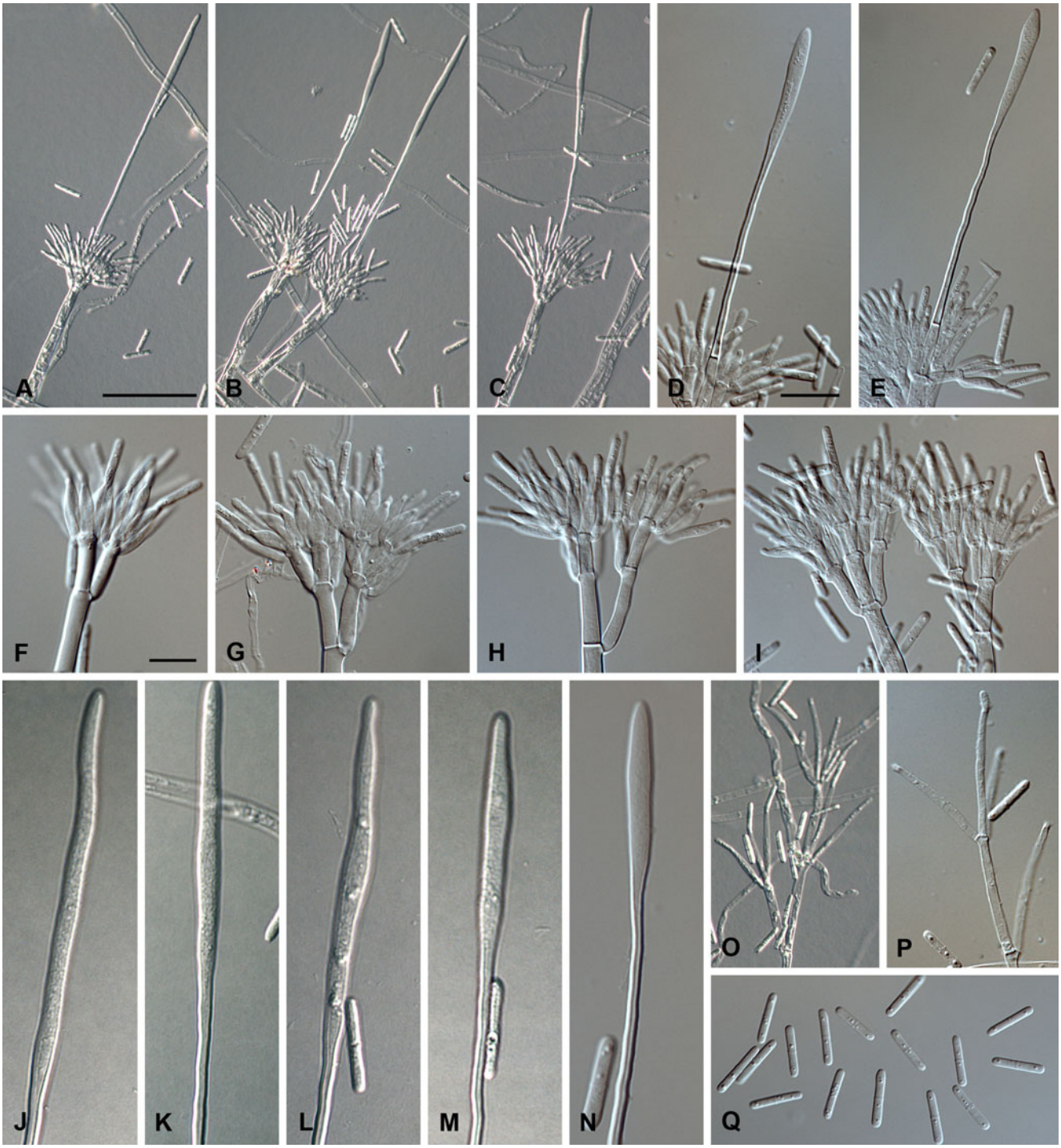

Fig. 5 *Cylindrocladiella ellipsoidea*. **a**–**e**. Penicillate conidiophores. **f**–**i**. Conidiogenous apparatus with conidiophore branches and phialides. **j**–**n**. Terminal vesicles. **o**–**p**. Subverticillate conidiophores. **q**. Conidia. Scale bars: A=50 μm (apply to **b**–**c**), D=20 μm (apply to **e**, **o**), F=10 μm (apply to **g**–**n**, **p**–**q**)

has cylindrical terminal vesicles. Conidia of *C. hawaiiensis* (av. = 13×3 μm) are also smaller than those of *C. infestans* (av. 15×3 μm). Unique fixed nucleotides were also identified for *C. hawaiiensis* for two loci: BTUB positions 91 (A), 113 (C), 121 (T), 138 (T), 367 (T), 368 (T), 369 (C) and 375 (G); HIS3 positions 43 (T), 50 (T), 88 (A), 91 (indel), 116 (A), 229 (A), 235 (G), 248 (A), 278 (A), 456 (indel), 462 (T) and 469 (A).

Cylindrocladiella kurandica L. Lombard & Crous, sp. nov. – MycoBank MB561683, Fig. 7.

Etymology – Named after the Kuranda, the town where this fungus was collected.

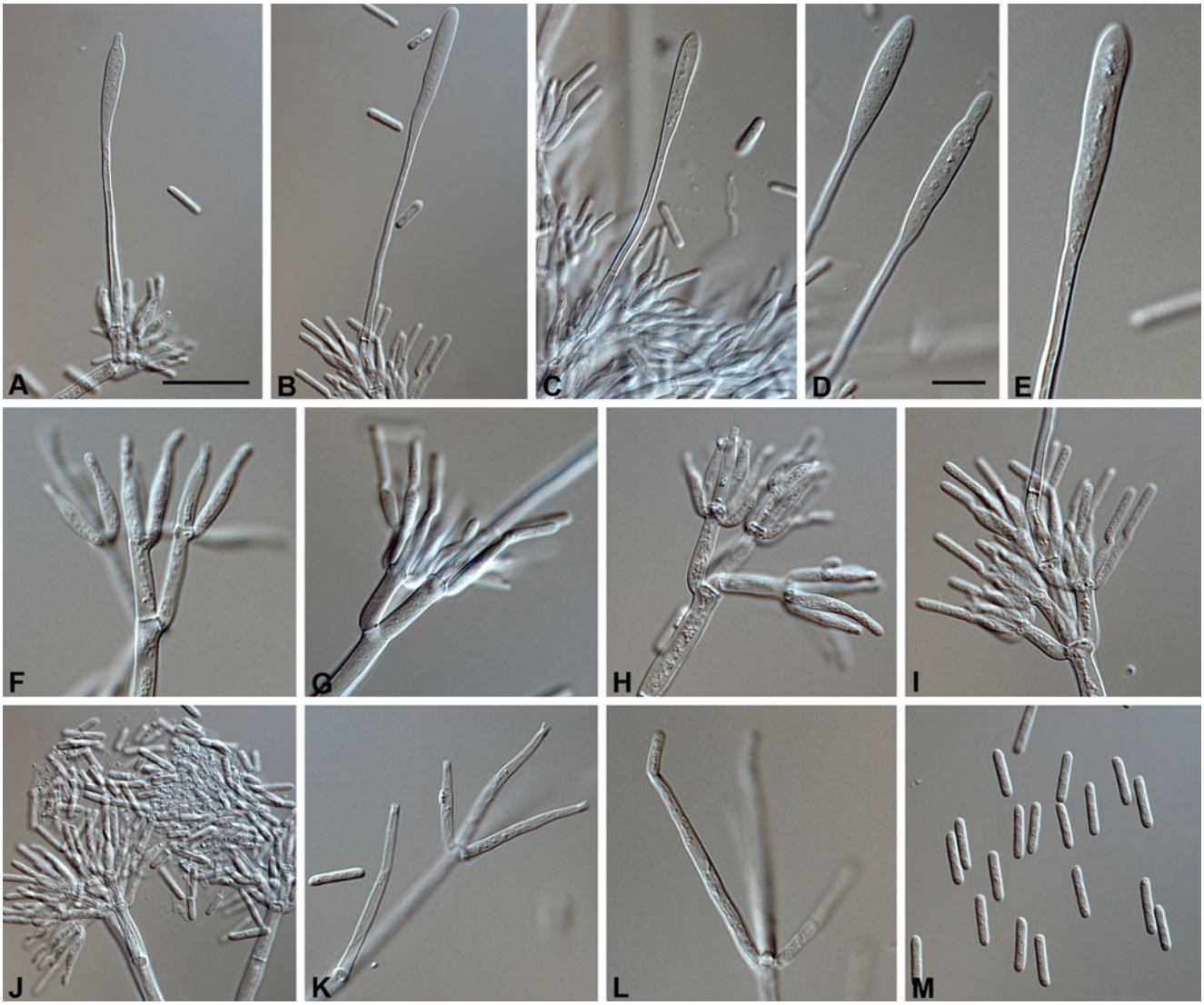

Fig. 6 *Cylindrocladiella hawaiiensis*. **a**–**c**. Penicillate conidiophores. **d**–**e**. Terminal vesicles. **f**–**j**. Conidiogenous apparatus with conidiophore branches and phialides. **k**–**l**. Subverticillate conidiophores. **m**. Conidia. A=20 μm (apply to **b**, **j**), D=10 μm (apply to **e**–**i**, **k**–**m**)

Cylindrocladiellae infestantis morphologice valde similis et vix distinguibilis, sed characteribus sequentibus nucleotiditis fixationibus in positionibus diversis [BTUB 97 (T), 395 (A) et 482 (T); HIS3 22 (T), 50 (A) et 315 (T); TEF-1α 107 (C)] genetice distinguitur.

Teleomorph unknown. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 7a–e) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 54–87×5–9 μm; stipe extension aseptate, straight, 153–219 μm long, thick-walled with one basal septum, terminating in thin-walled, ellipsoidal to lanceolate vesicles (Fig. 7j–l), 6–9 μm wide. *Penicillate conidiogenous apparatus* (Fig. 7f–i) with primary branches aseptate, 12–24×3–7 μm, secondary branches aseptate, 8–15×2–4 μm, each terminal branch producing 2–4 phialides; phialides doliiform to cymbiform, hyaline, aseptate, 8–14×2–4 μm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* (Fig. 7m–o) in moderate numbers, comprising of a septate stipe, and primary branches terminating in 2–3 phialides; primary branches straight, hyaline, 0–1-septate, 20–48×2–4 μm; phialides cymbiform to cylindrical, hyaline, aseptate, 18–35×2–5 μm, apex with minute periclinal thickening and collarette. *Conidia* (Fig. 7p) cylindrical, rounded at both ends, straight, 1-septate, (10–)12–14(–16)×2–4 μm (av. = 13×3 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised, cottony, with undulate margins, white with straw (21 d) tint in patches, umber (13i) (reverse); chlamydospores extensive

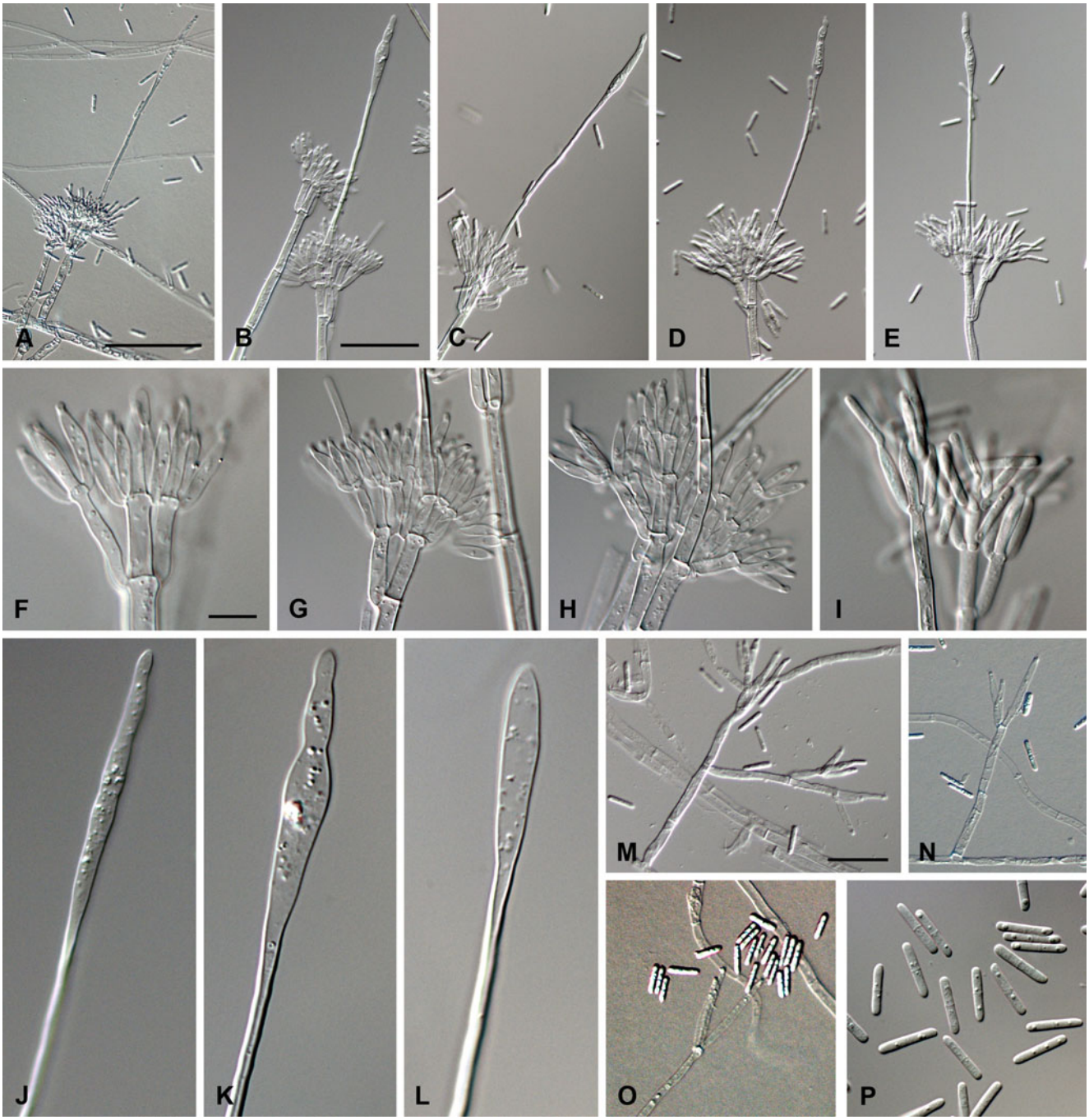

Fig. 7 *Cylindrocladiella kurandica*. **a**–**e**. Penicillate conidiophores. **f**–**i**. Conidiogenous apparatus with conidiophore branches and phialides. **j**–**l**. Terminal vesicles. **m**–**o**. Subverticillate conidiophores. **p**. Conidia. Scale bars: A=50 μm, B=20 μm (apply to **c**–**e**, **m**–**o**), F=10 μm (apply to **g**–**l**, **p**)

throughout medium, arranged in chains; reaching 65 mm after 7 days on MEA at 24°C in the dark.

Specimen examined – Australia, Queensland, Kuranda, from soil, 13 Aug 2009, P.W. Crous, holotype CBS-H20601, culture ex-type CBS 129577=CPC 17551; Australia, Queensland, Kuranda, from soil, 13 Aug 2009, P.W. Crous, culture CBS 129576=CPC 17547; Australia, Queensland, Kuranda, from soil, 13 Aug 2009, P.W. Crous, culture CPC 17549.

Notes – *Cylindrocladiella kurandica* is difficult to distinguish from *C. longistipitata* and other species in the *C. infestans* complex, and therefore phylogenetic inference is required for an accurate identification. *Cylindrocladiella kurandica* can be distinguished from other species in the *C. infestans*

complex by different unique fixed nucleotides for three loci: BTUB positions 97 (T), 395 (A) and 482 (T); HIS3 positions 22 (T), 50 (A) and 315 (T); TEF-1α position 107 (C).

Cylindrocladiella lanceolata L. Lombard & Crous, sp. nov. – MycoBank MB561675, Fig. 8.

Etymology – Named after the lanceolate shape of its vesicles.

Cylindrocladiellae lageniformis morphologice similis, sed vesiculis lanceolatis distinguitur.

Teleomorph unknown. *Conidiophores* monomorphic, penicillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 8a–e) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 31–77× 5–10 μm; stipe extension aseptate, straight, 76–173 μm long, thick-walled with one basal septum, terminating in thin-walled, lanceolate vesicles (Fig. 8f–h), 5–7 μm wide. *Penicillate conidiogenous apparatus* (Fig. 8i–k) with primary branches aseptate, 12–30×3–8 μm, secondary branches aseptate, 7–17×3–6 μm, each terminal branch producing 2–4 phialides; phialides reniform to doliiform to cymbiform, hyaline, aseptate, 7–13×2–3 μm, apex with minute periclinal thickening and collarette absent. *Subverticillate conidiophores* not observed. *Conidia* (Fig. 8l) cylindrical, rounded at both ends, straight, 1-septate, (13–)15–17(–20)×2–3 μm (av. = 16×3 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with smooth margins, white, umber (13i) (reverse); chlamydospores extensive throughout medium, arranged in chains; reaching 55 mm after 7 days on MEA at 24°C in the dark.

Specimens examined – Australia, Queensland, Brisbane, from soil, 18 July 2009, P.W. Crous, holotype CBS-H20602, culture ex-type CBS 129566=CPC 17567, CBS 129565= CPC 17566; South Africa, KwaZulu-Natal, Kwambonambi, Mondi Sawmill, from *Eucalyptus* sp., 1 May 1990, P.W. Crous, culture CBS 114950=CPC 396.

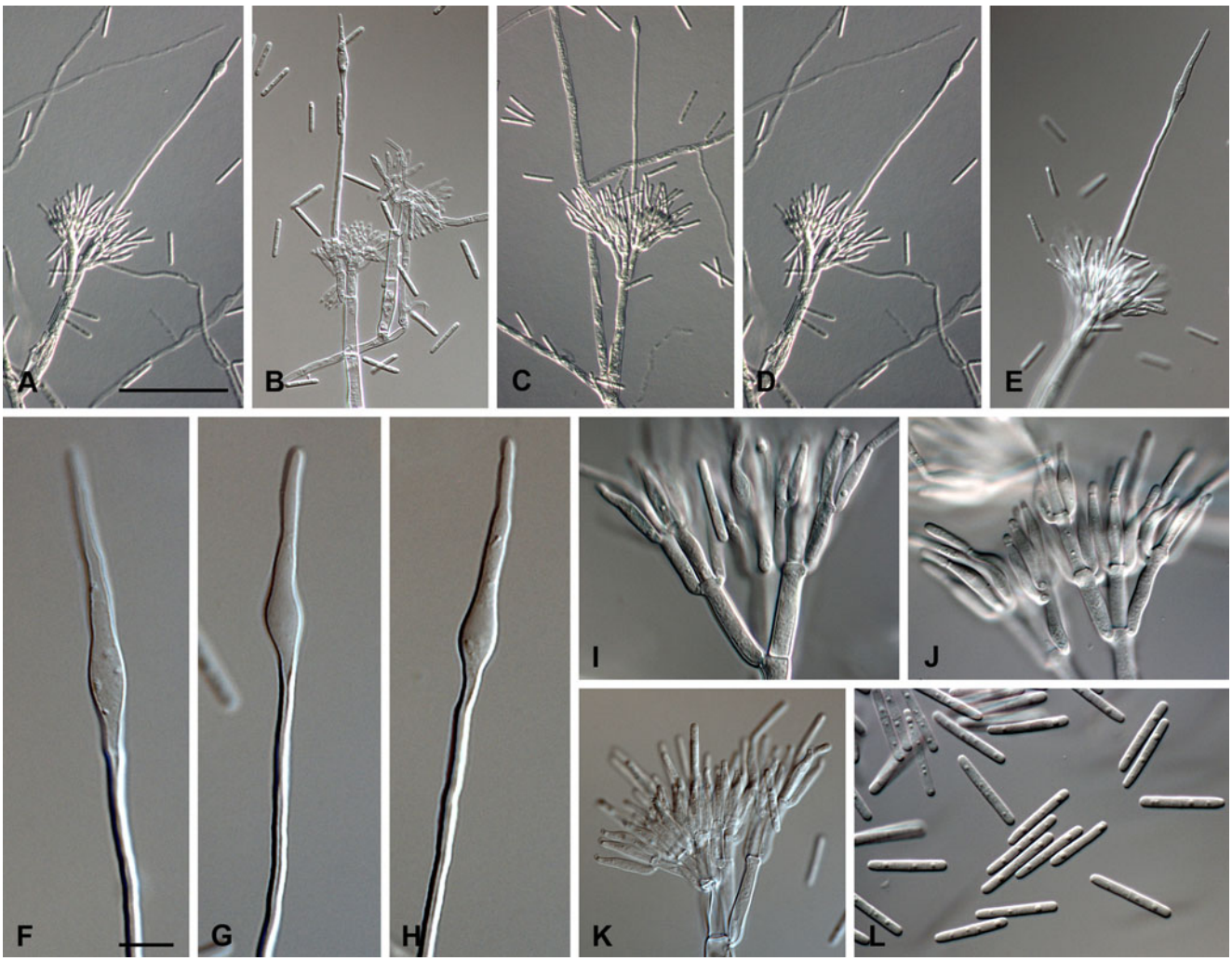

Fig. 8 *Cylindrocladiella lanceolata*. **a**–**e**. Penicillate conidiophores. **f**–**h**. Terminal vesicles. **i**–**k**. Conidiogenous apparatus with conidiophore branches and phialides. L. Conidia. Scale bars: A=50 μm (apply to **b**–**e**), F=10 μm (apply to **g**–**l**)

Note – *Cylindrocladiella lanceolata* can be distinguished from *C. lageniformis* by its lanceolate terminal vesicles and conidium dimensions.

Cylindrocladiella longiphialidica L. Lombard & Crous, sp. nov. – MycoBank MB561669, Fig. 9.

Etymology – Named after its characteristically long phialides.

Cylindrocladiellae camelliae morphologice valde similis, sed phialidibus conidiophorum subverticillatorum longioribus distinguitur.

Fig. 9 *Cylindrocladiella longiphialidica*. **a**–**e**. Penicillate conidiophores. **f**–**i**. Conidiogenous apparatus with conidiophore branches and phialides. **j**–**n**. Terminal vesicles. **o**–**q**. Subverticillate conidiophores. R. Conidia. Scale bars: A=50 μm (apply to **b**–**e**), F=10 μm (apply to **g**–**r**)

Teleomorph unknown. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 9a–e) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 43–107×6–9 μm; stipe extension aseptate, straight, 114–189 μm long, thick-walled with one basal septum, terminating in thin-walled, ellipsoidal to lanceolate vesicles (Fig. 9j–n), 5–8 μm wide. *Penicillate conidiogenous apparatus* (Fig. 9f–i) with primary branches aseptate, 11–33×3–7 μm, secondary branches aseptate, 9–26×3–5 μm, with each terminal branch producing 2–4 phialides; phialides doliiform to reniform to cymbiform, hyaline, aseptate, 8–13×2–4 μm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* (Fig. 9o–q) abundant, comprising of a septate stipe, and primary branches terminating in 1–3 phialides; primary branches straight, hyaline, 0–1-septate, 28–68×4–6 μm; phialides cymbiform to cylindrical, hyaline, aseptate, 20–79×2–5 μm, apex with minute periclinal thickening and collarette. *Conidia* (Fig. 9r) cylindrical, rounded at both ends, straight, 1-septate, 12–14×2–3 μm (av. = 13×3 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with undulate margins, white centre becoming buff yellow (19 d) towards the margins, buff yellow (19 d) (reverse); chlamydospores extensive throughout medium, arranged in chains; reaching 55 mm after 7 days on MEA at 24°C in the dark.

Specimens examined – Thailand, Chiang Mai, from soil, Oct. 2010, P.W. Crous, holotype CBS-H20603, culture ex-type CBS 129557=CPC 18839; Thailand, Chiang Mai, from soil, Oct. 2010, P.W. Crous culture, CBS 129558=CPC 18841.

Notes – *Cylindrocladiella longiphialidica* is morphologically similar to *C. nederlandica*, *C. pseudocamelliae* and *C. camelliae*, but can be distinguished from these species by its longer phialides on the subverticillate conidiophores.

Cylindrocladiella longistipitata L. Lombard & Crous, sp. nov. – MycoBank MB561679, Fig. 10.

Etymology – Named after its characteristically long stipe extensions on its conidiophores.

Cylindrocladiellae infestantis morphologice similis, sed extensionibus stipitis longioribus.

Teleomorph unknown. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 10a–e) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 54–80×5–9 μm; stipe extension aseptate, straight, 130–216 μm long, thick-walled with one basal septum, terminating in thin-walled, cylindrical to lanceolate vesicles (Fig. 10j–n), 5–7 μm wide. *Penicillate conidiogenous apparatus* (Fig. 10f–i) with primary branches aseptate, 13–20×3–5 μm, secondary branches aseptate, 9–13×3–5 μm, each terminal branch producing 2–4 phialides; phialides cymbiform to cylindrical, hyaline, aseptate, 10–16×2–4 μm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* (Fig. 10o–p) in moderate numbers, comprising of a septate stipe, and primary branches terminating in 2–3 phialides; primary branches straight, hyaline, 0–1-septate, 21–40×4 μm; phialides cymbiform to cylindrical, hyaline, aseptate, 18–31×2–4 μm, apex with minute periclinal thickening and collarette. *Conidia* (Fig. 10q) cylindrical, rounded at both ends, straight, 1-septate, (12–)14–16(–17)×2–4 μm (av. = 15×3 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with smooth to undulate margins, white, umber (13i) (reverse); chlamydospores extensive throughout medium, arranged in chains; reaching 45 mm after 7 days on MEA at 24°C in the dark.

Specimens examined – China, Hong Kong, from soil, Nov. 1993, M.J. Wingfield, holotype CBS-H20604, culture ex-type CBS 116075=CPC 708; Australia, Queensland, Topaz, Atherton Tablelands, from *Opisthiolepsis heterophylla*, 2 Apr. 2001, C. Pearce & B. Paulus, culture CBS 112953=CPC 4720.

Notes – *Cylindrocladiella longistipitata* can be distinguished from other species in the *C. infestans* complex by its longer stipe extension and terminal vesicle morphology. Furthermore, it has unique fixed nucleotides for three loci: BTUB position 363 (A); HIS3 positions 37 (C) and 400 (T); TEF-1α positions 44 (A) and 45 (T).

Cylindrocladiella natalensis L. Lombard & Crous, sp. nov. – MycoBank MB561670, Fig. 11.

Etymology – Named after the Province in South Africa where this fungus was first collected, KwaZulu-Natal.

Cylindrocladiellae elegantis morphologice valde similis, sed conidiis majoribus, (12–)14–16(–17)×2–3 μm, distinguitur.

Teleomorph unknown. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 11a–e) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 88–135×5–8 μm; stipe extension aseptate, straight, 82–127 μm long, thick-walled with one basal septum, terminating in thin-walled, ellipsoidal to fusoid vesicles (Fig. 11j–m), 6–8 μm wide. *Penicillate conidiogenous apparatus* (Fig. 11f–i) with primary branches aseptate, 13–29×2–5 μm, secondary branches aseptate, 8–17×3–4 μm, each terminal branch producing 2–4 phialides; phialides cymbiform to cylindrical, hyaline, aseptate, 9–14×2–3 μm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores*

Fig. 10 *Cylindrocladiella longistipitata*. **a**–**e**. Penicillate conidiophores. **f**–**i**. Conidiogenous apparatus with conidiophore branches and phialides. **j**–**n**. Terminal vesicles. **o**–**p**. Subverticillate conidiophores. Q. Conidia. A=50 μm (apply to **b**–**e**), F=10 μm (apply to **g**–**q**)

(Fig. 11n–o) in moderate numbers, comprising of a septate stipe, and primary branches terminating in 2–3 phialides; primary branches straight, hyaline, 0–1-septate, 23–39×2–4 μm; phialides cymbiform to cylindrical, hyaline, aseptate, 19–34×2–4 μm, apex with minute periclinal thickening and collarette. *Conidia* (Fig. 11p) cylindrical, rounded at both ends, straight, 1-septate, (12–)14–16(–17)×2–3 μm (av. = 15×3 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with smooth to undulate margins, white, buff yellow (19 d) (reverse); chlamydospores sparse throughout medium, arranged in chains; reaching 70 mm after 7 days on MEA at 24°C in the dark.

Specimens examined – South Africa, KwaZulu-Natal, from *Arachis hypogaea*, 1 Feb. 1991, M.J. Wingfield, holotype CBS-H20605, culture ex-type CBS 114943= CPC 456, CBS 114945=CPC 459; Australia, Queensland,

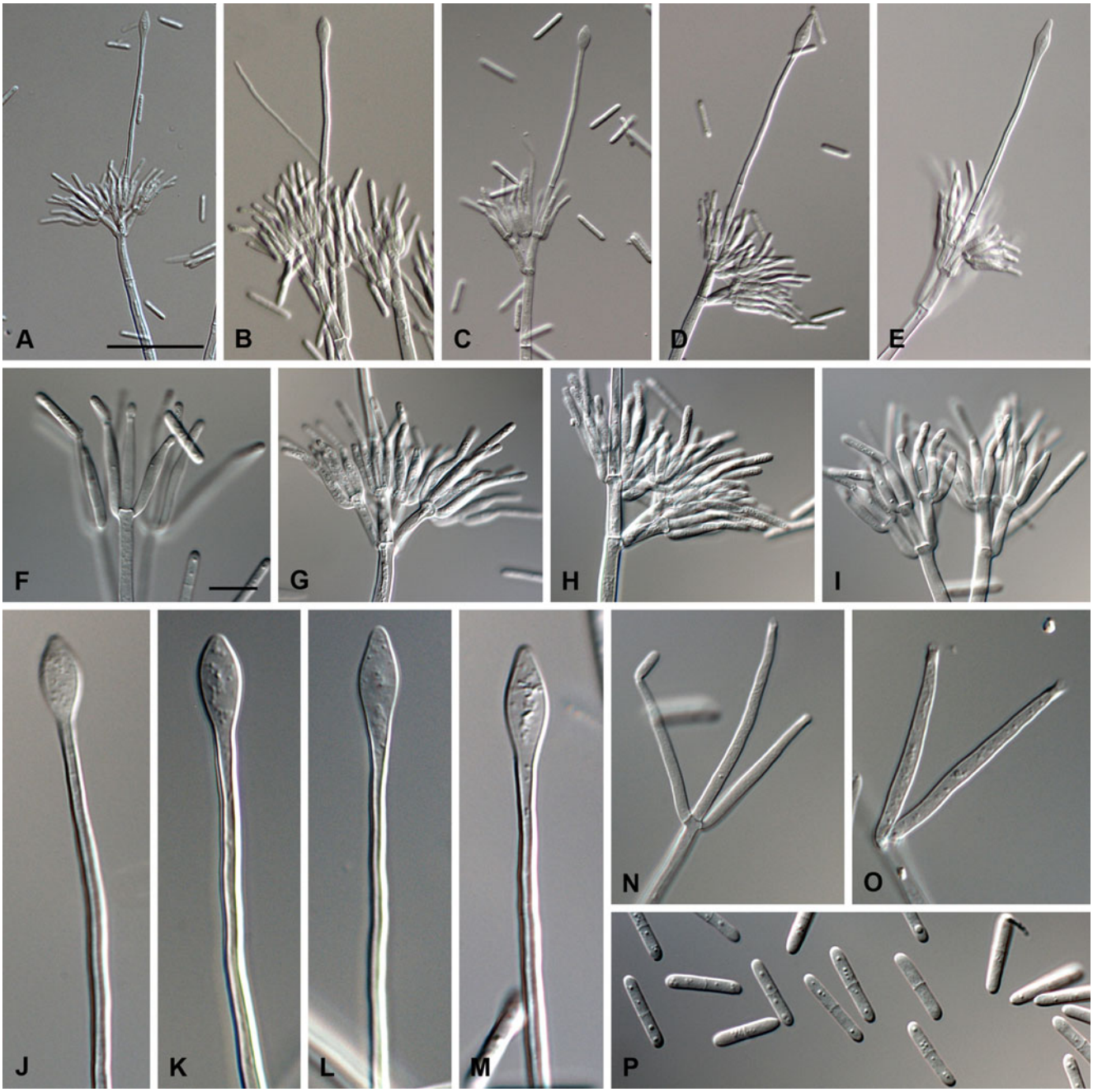

Fig. 11 *Cylindrocladiella natalensis*. **a**–**e**. Penicillate conidiophores. **f**–**i**. Conidiogenous apparatus with conidiophore branches and phialides. **j**–**m**. Terminal vesicles. **n**–**o**. Subverticillate conidiophores. P. Conidia. Scale bars: A = 50 μm (apply to **b**–**e**), F = 10 μm (apply to **g**–**p**)

Byron Bay, from soil, 17 July 2009, P.W. Crous, culture CPC 17395.

Note – *Cylindrocladiella natalensis* can be distinguished from other species in this genus by its conidium dimensions and shape of the terminal vesicle.

Cylindrocladiella nederlandica L. Lombard & Crous, sp. nov. – MycoBank MB561667, Fig. 12.

Etymology – Named after the Netherlands, the country where this fungus was collected.

Cylindrocladiellae camelliae morphologice valde similis, sed phialidibus majoribus, 14–30 × 3–5 μm, distinguitur.

Teleomorph unknown. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 12a–e) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 41–124 × 4–10 μm; stipe extension aseptate, straight, 102–158 μm long, thick-walled with one basal septum,

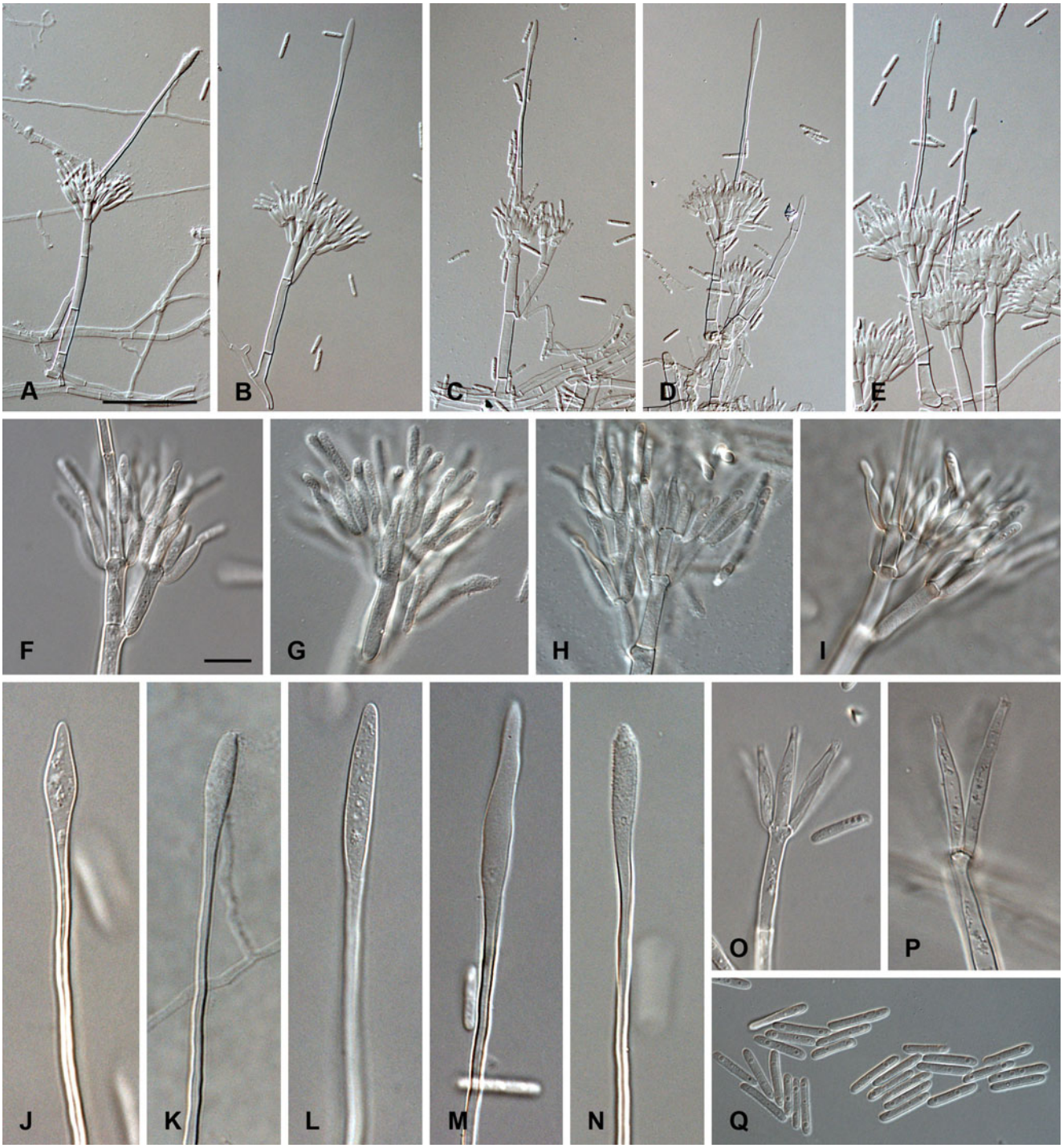

Fig. 12 *Cylindrocladiella nederlandica*. **a**–**e**. Penicillate conidiophores. **f**–**i**. Conidiogenous apparatus with conidiophore branches and phialides. **j**–**n**. Terminal vesicles. **o**–**p**. Subverticillate conidiophores. Q. Conidia. Scale bars: A=50 μm (apply to **b**–**e**), F=10 μm (apply to **g**–**q**)

terminating in thin-walled, lageniform to ellipsoidal vesicles (Fig. 12j–n), 4–9 μm wide. *Penicillate conidiogenous apparatus* (Fig. 12f–i) with primary branches aseptate, 12–31×3–7 μm, secondary branches aseptate, 8–18×2–5 μm, each terminal branch producing 2–4 phialides; phialides doliiform to reniform to cymbiform, hyaline, aseptate, 8–14×2–4 μm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* (Fig. 12o–p) abundant, comprising of a septate stipe, and primary branches terminating in 1–3 phialides; primary branches straight, hyaline, 0–1-septate, 18–32×3–5 μm; phialides cymbiform to cylindrical, hyaline, aseptate, 14–30×3–5 μm, apex with minute periclinal thickening and collarette. *Conidia* (Fig. 12q) cylindrical, rounded at

both ends, straight, 1-septate, (10–)12–14(–15)×2–4 μm (av. = 13×2 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with smooth to undulate margins, white, buff yellow (19 d) to umber (13i) (reverse); chlamydospores moderate throughout medium, arranged in chains; reaching 55 mm after 7 days on MEA at 24°C in the dark.

Specimens examined – The Netherlands, from *Pelargonium* sp., Mar. 1991, J.W. Veenbaas-Rijks, holotype CBS-H5129, culture ex-type CBS 152.91=PD 90/2015; The Netherlands, Aalsmeer, from *Kalanchoë* sp., Feb. 1995, J.W. Veenbaas-Rijks, culture CBS 143.95=PD 94/1353; The Netherlands, stem of *Rhododendron*, Mar. 1994, culture CBS 146.94=PD 39/1776.

Notes – Morphologically, isolates of *C. nederlandica* are very similar to *C. camelliae*, with a slight difference in terminal vesicle shape. The phialides on the subverticillate conidiophores of *C. nederlandica* (14–30×3–5 μm) are larger than those of *C. camelliae* (15–26×2–3.5 μm; Crous 2002).

Cylindrocladiella pseudocamelliae L. Lombard & Crous, sp. nov. – MycoBank MB561668, Fig. 13.

Etymology – Named after its morphological similarity to *Cylindrocladiella camelliae*.

Cylindrocladiellae camelliae morphologice similis, sed vesiculis divergentibus.

Teleomorph unknown. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 13a–e) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 65–137×6–10 μm; stipe extension aseptate, straight, 106–188 μm long, thick-walled with one basal septum, terminating in thin-walled, ellipsoidal to lageniform to lanceolate vesicles (Fig. 13j–n), 6–10 μm wide. *Penicillate conidiogenous apparatus* (Fig. 13f–i) with primary branches aseptate, 12–27×3–6 μm, secondary branches aseptate, 8–18×2–5 μm, each terminal branch producing 2–4 phialides; phialides doliiform to reniform to cymbiform, hyaline, aseptate, 10–17×2–3 μm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* (Fig. 13o–p) abundant, comprising of a septate stipe, and primary branches terminating in 1–3 phialides; primary branches straight, hyaline, 0–1-septate, 15–32×3–6 μm; phialides cymbiform to cylindrical, hyaline, aseptate, 19–31×3–5 μm, apex with minute periclinal thickening and collarette. *Conidia* (Fig. 13q) cylindrical, rounded at both ends, straight, 1-septate, (9–)11–15(–16)×2–4 μm (av. = 13×3 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with smooth margins, white, buff yellow (19 d) to umber (13i) (reverse); chlamydospores moderate throughout medium, arranged in chains; reaching 90 mm after 7 days on MEA at 24°C in the dark.

Specimens examined – Thailand, Chiang Mai, from soil, Oct. 2010, P.W. Crous, holotype CBS-H20606, culture ex-type CBS 129555=CPC 18825; Thailand, Chiang Mai, from soil, Oct. 2010, P.W. Crous, culture CBS 129556=CPC 18832; Thailand, Chiang Mai, from soil, Oct. 2010, P.W. Crous, culture CPC 18838.

Notes – As with *C. nederlandica*, *C. pseudocamelliae* is morphologically similar to *C. camelliae*. However, *C. pseudocamelliae* can be distinguished from both the other species by its longer stipe extension and the shape of its terminal vesicle.

Cylindrocladiella pseudohawaiiensis L. Lombard & Crous, sp. nov. – MycoBank MB561678, Fig. 14.

Etymology – Named after its morphological similarity to *Cylindrocladiella hawaiiensis*.

Cylindrocladiellae infestantis morphologice valde similis, sed conidiis minoribus, (11–)12–14(–15)×2–4 μm, distinguitur.

Teleomorph unknown. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 14a–c) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 31–62×5–8 μm; stipe extension aseptate, straight, 70–97 μm long, thick-walled with one basal septum, terminating in thin-walled, clavate to ellipsoidal vesicles (Fig. 14d–f), 6–8 μm wide. *Penicillate conidiogenous apparatus* (Fig. 14g–i) with primary branches aseptate, 9–19×3–5 μm, secondary branches aseptate, 9–11×4 μm, each terminal branch producing 2–4 phialides; phialides cymbiform to cylindrical, hyaline, aseptate, 10–15×2–4 μm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* (Fig. 14j–k) in moderate numbers, comprising of a septate stipe, and primary branches terminating in 2–3 phialides; primary branches straight, hyaline, 0–1-septate, 16–40×4 μm; phialides cymbiform to cylindrical, hyaline, aseptate, 17–28×3–4 μm, apex with minute periclinal thickening and collarette. *Conidia* (Fig. 14l) cylindrical, rounded at both ends, straight, 1-septate, (11–)12–14(–15)×2–4 μm (av. = 13×3 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with smooth margins, white, buff yellow (19 d) to umber (13i) (reverse); chlamydospores extensive throughout medium, arranged in chains; reaching 75 mm after 7 days on MEA at 24°C in the dark.

Specimens examined – Brazil, Sao Paulo, Aracruz nursery, from *Eucalyptus* cutting, 1992, A.C. Alfenas, holotype CBS-H20607, culture ex-type CBS 210.94=PPRI 4450=UFV 125=IMI 361579; Madagascar, Isoamala-Beraketa, Mount

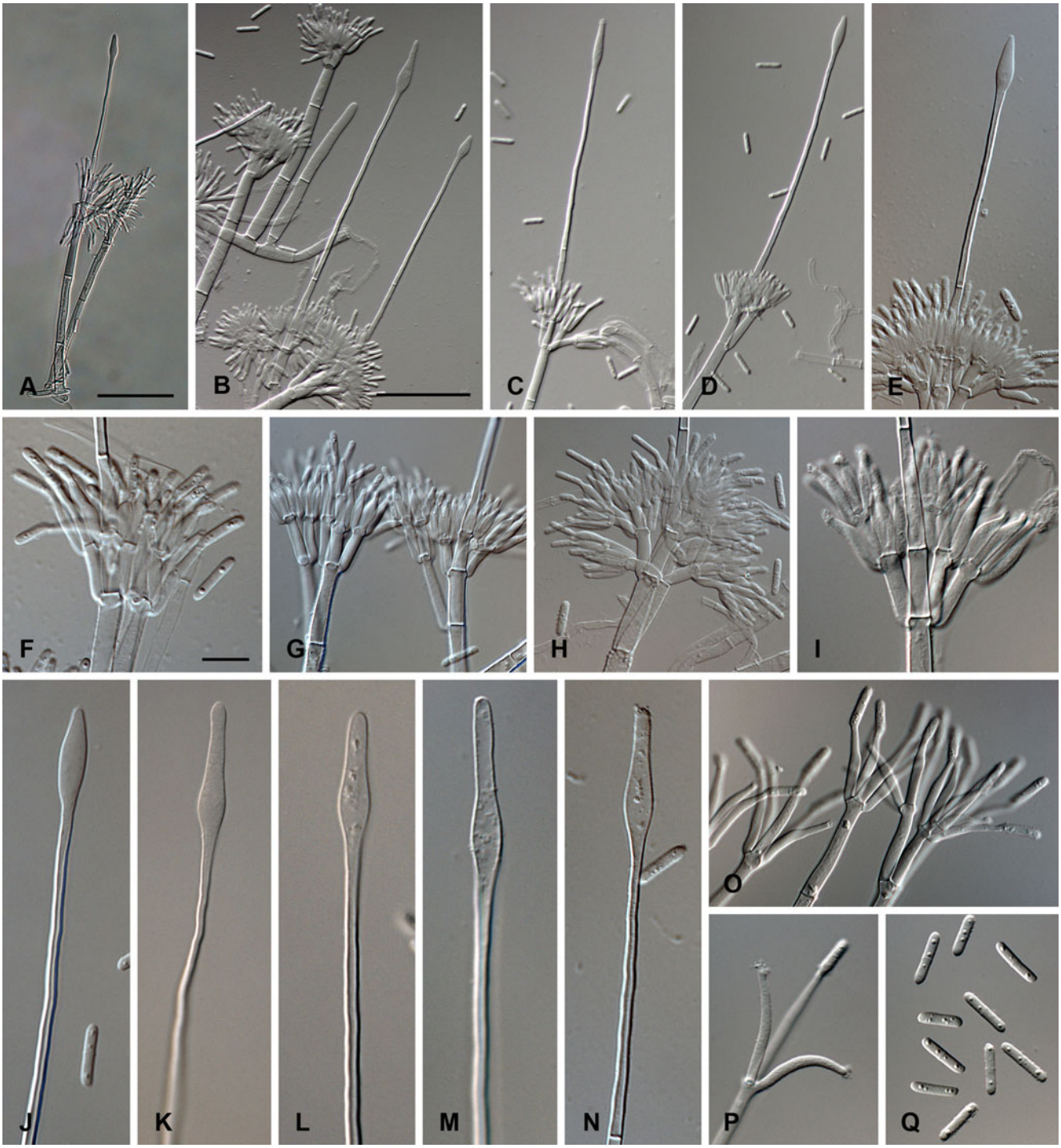

Fig. 13 *Cylindrocladiella pseudocamelliae*. **a**–**e**. Penicillate conidiophores. **f**–**i**. Conidiogenous apparatus with conidiophore branches and phialides. **j**–**n**. Terminal vesicles. **o**–**p**. Subverticillate conidiophores. Q. Conidia. Scale bars: A=50 μm, B=50 μm (apply to **c**–**e**), F=10 μm (apply to **g**–**q**)

Tolongo, substrate unknown, 7 Mar. 1994, collector unknown, culture CBS 115610=CPC 909=Fox 409.

Notes – Morphologically *C. pseudohawaiiensis* is difficult to distinguish from *C. hawaiiensis*, and therefore phylogenetic inference is required. It can be distinguished from *C. infestans* by its smaller conidium dimensions and terminal vesicle shape. *Cylindrocladiella pseudohawaiiensis* can also be distinguished from other species in the *C. infestans* complex by different unique fixed nucleotides for three loci: BTUB positions 127 (A) and 384 (G); HIS3 positions 23 (C), 29 (C), 33 (A), 77 (G), 283 (indel), 285 (C), 288 (A), 314 (T), 349 (T) and 463 (T); TEF-1α

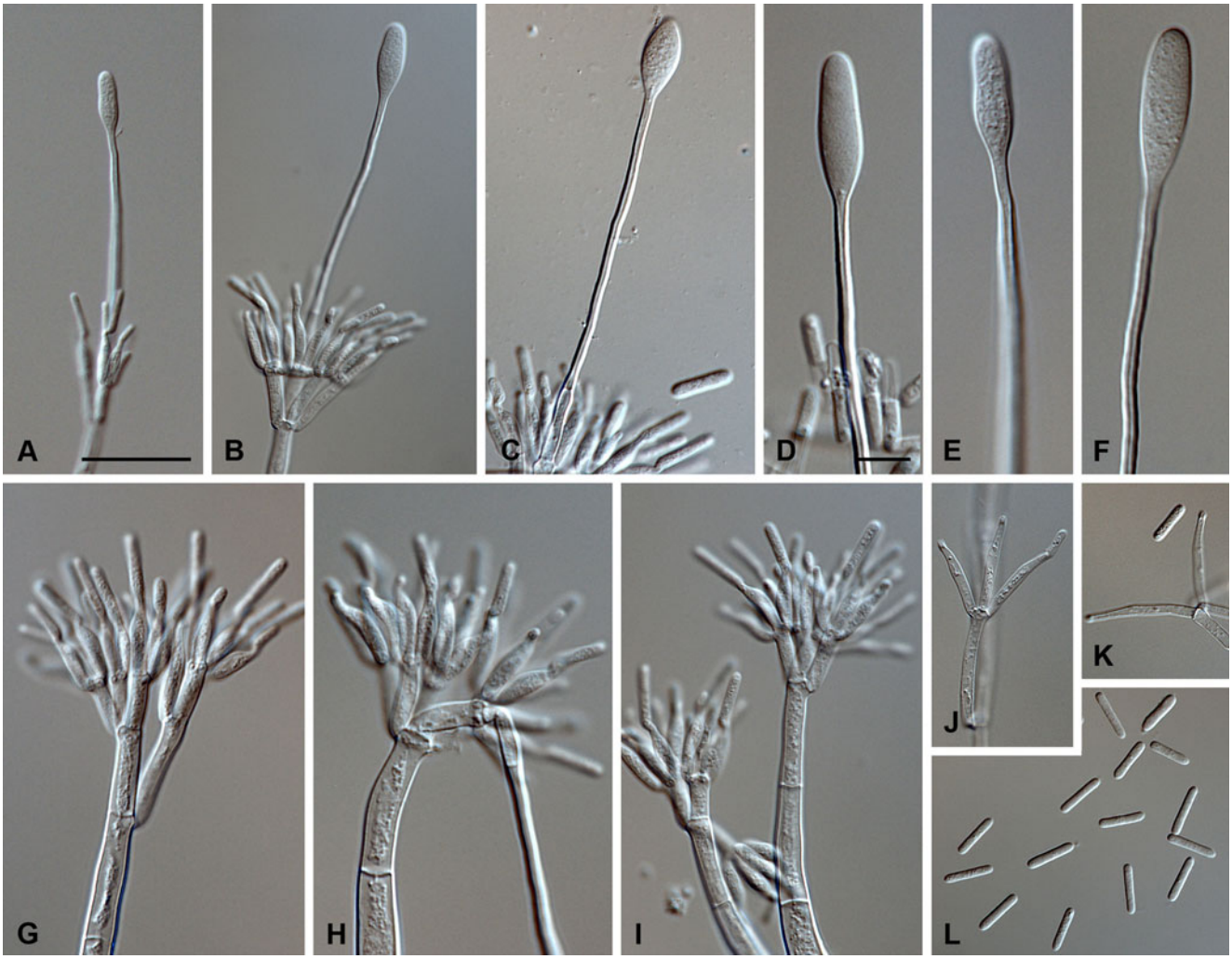

Fig. 14 *Cylindrocladiella pseudohawaiiensis*. **a**–**c**. Penicillate conidiophores. **d**–**f**. Terminal vesicles. **g**–**i**. Conidiogenous apparatus with conidiophore branches and phialides. **j**–**k**. Subverticillate conidiophores. L. Conidia. A=20 μm (apply to **b**–**c**), D=10 μm (apply to **e**–**l**)

positions 153 (T), 244 (T), 288 (T), 289 (A), 290 (T), 337 (C), 465 (A), 471 (G), 478 (T) and 482 (G).

Cylindrocladiella pseudoparva L. Lombard & Crous, sp. nov. – MycoBank MB561672, Fig. 15.

Etymology – Named after its morphological similarity to *Cylindrocladiella parva*.

Cylindrocladiellae parvae morphologice valde similis, sed ramis primariis conidiophorum majoribus distinguitur.

Teleomorph unknown. *Conidiophores* monomorphic, penicillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 15a–e) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 31–86×5–9 μm; stipe extension aseptate, straight, 111–164 μm long, thick-walled with one basal septum, terminating in thin-walled, clavate to ellipsoidal to pyriform vesicles (Fig. 15f–h), 5–7 μm wide. *Penicillate conidiogenous apparatus* (Fig.15i–k) with primary branches aseptate, 16–32×3–6 μm, secondary branches aseptate, 8–18×3–5 μm, each terminal branch producing 2–4 phialides; phialides doliiform to cymbiform, hyaline, aseptate, 10–17×2–4 μm, apex with minute periclinal thickening and collarette absent. *Subverticillate conidiophores* not observed. *Conidia* (Fig. 15l) cylindrical, rounded at both ends, straight, 1-septate, 16–18(–20)×2–4 μm (av. = 17×3 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with smooth margins, white with buff yellow (19 d) centre, umber (13i) (reverse); chlamydospores extensive throughout medium, arranged in chains; reaching 50 mm after 7 days on MEA at 24°C in the dark.

Specimens examined – The Netherlands, Apeldoorn, Paleis Het Loo, from soil, Apr. 2010, P.W. Crous, holotype CBS-H20608, culture ex-type CBS 129560=CPC 18149; New Zealand, South Auckland, Karaka, Karaka road, from *Vitis riparia*, 16 Apr. 2007, K. Paice, culture CBS 122594; Switzerland, Mohlin Canton, Basel, from root of *Quercus*

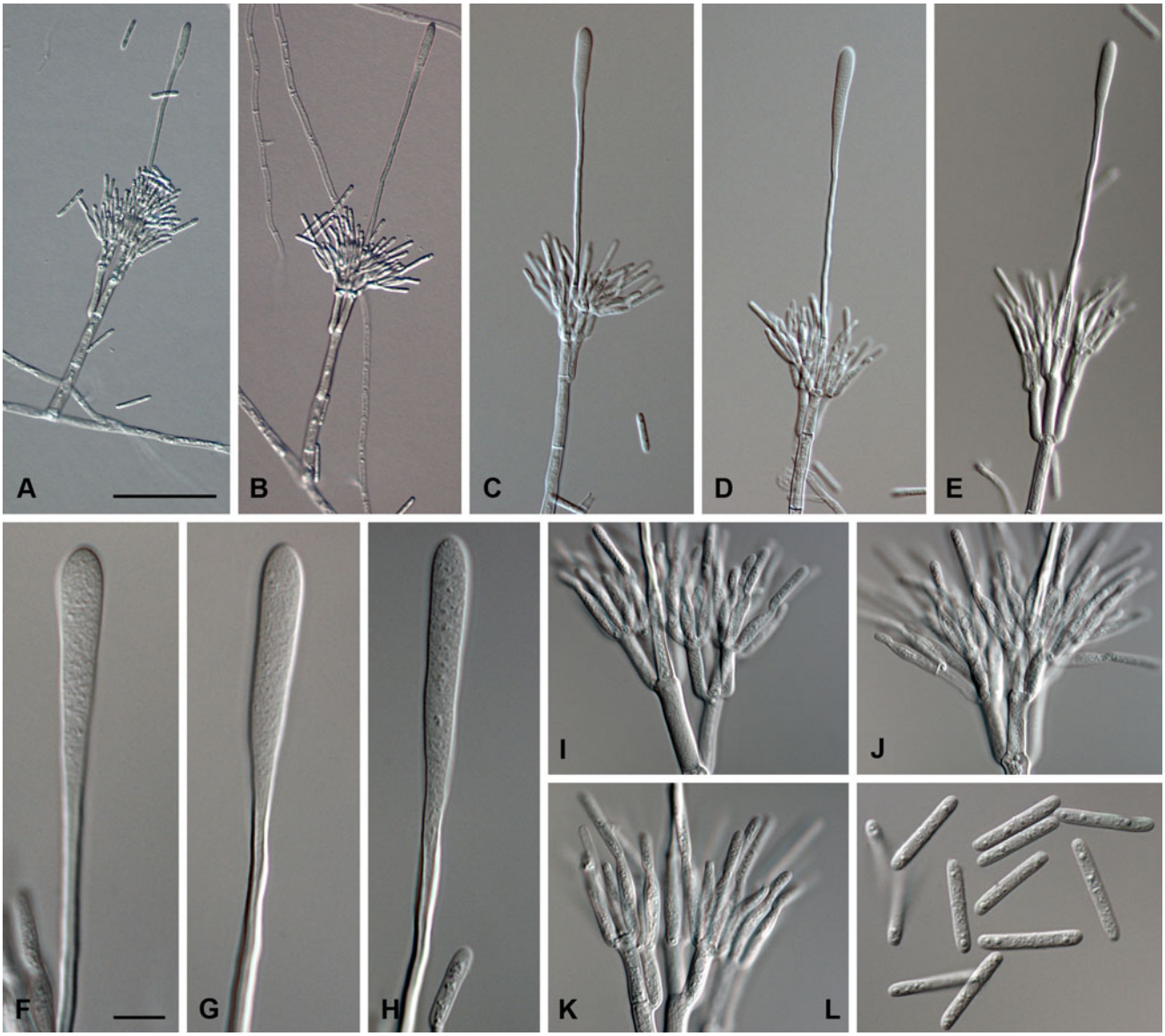

Fig. 15 *Cylindrocladiella pseudoparva*. **a**–**e**. Penicillate conidiophores. **f**–**h**. Terminal vesicles. **i**–**k**. Conidiogenous apparatus with conidiophore branches and phialides. L. Conidia. Scale bars: A=50 μm (apply to **b**–**e**), F=10 μm (apply to **g**–**l**)

sp., 16 Mar. 1994, L. Petrini, culture CBS 113624=CPC 752.

Notes – *Cylindrocladiella pseudoparva* can be distinguished from *C. parva* and *C. stellenboschensis* by having larger primary, and smaller secondary branches. However, phylogenetic inference will be required to accurately identify it. *Cylindrocladiella pseudoparva* differs from other species in the *C. parva* complex by unique fixed nucleotides in two loci: BTUB position 199 (G) and 358 (A); HIS3 position 226 (T), 302 (A), 372 (T) and 436 (C).

Cylindrocladiella queenslandica L. Lombard & Crous, sp. nov. – MycoBank MB561682, Fig. 16.

Etymology – Named after Queensland, the state in Australia from where it was collected.

Cylindrocladiellae infestantis morphologice valde similis, sed conidiis minoribus, (9–)10.5–13.5(–15)×2–4 μm, distinguitur.

Teleomorph unknown. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 16a–e) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 41–82×6–9 μm; stipe extension aseptate, straight, 117–180 μm long, thick-walled with one basal septum, terminating in thin-walled, cylindrical to lanceolate vesicles (Fig. 16j–m), 5–8 μm wide. *Penicillate conidiogenous apparatus* (Fig. 16f–i) with primary branches aseptate, 13–23×3–7 μm, secondary branches aseptate, 9–12×2–4 μm, each terminal branch

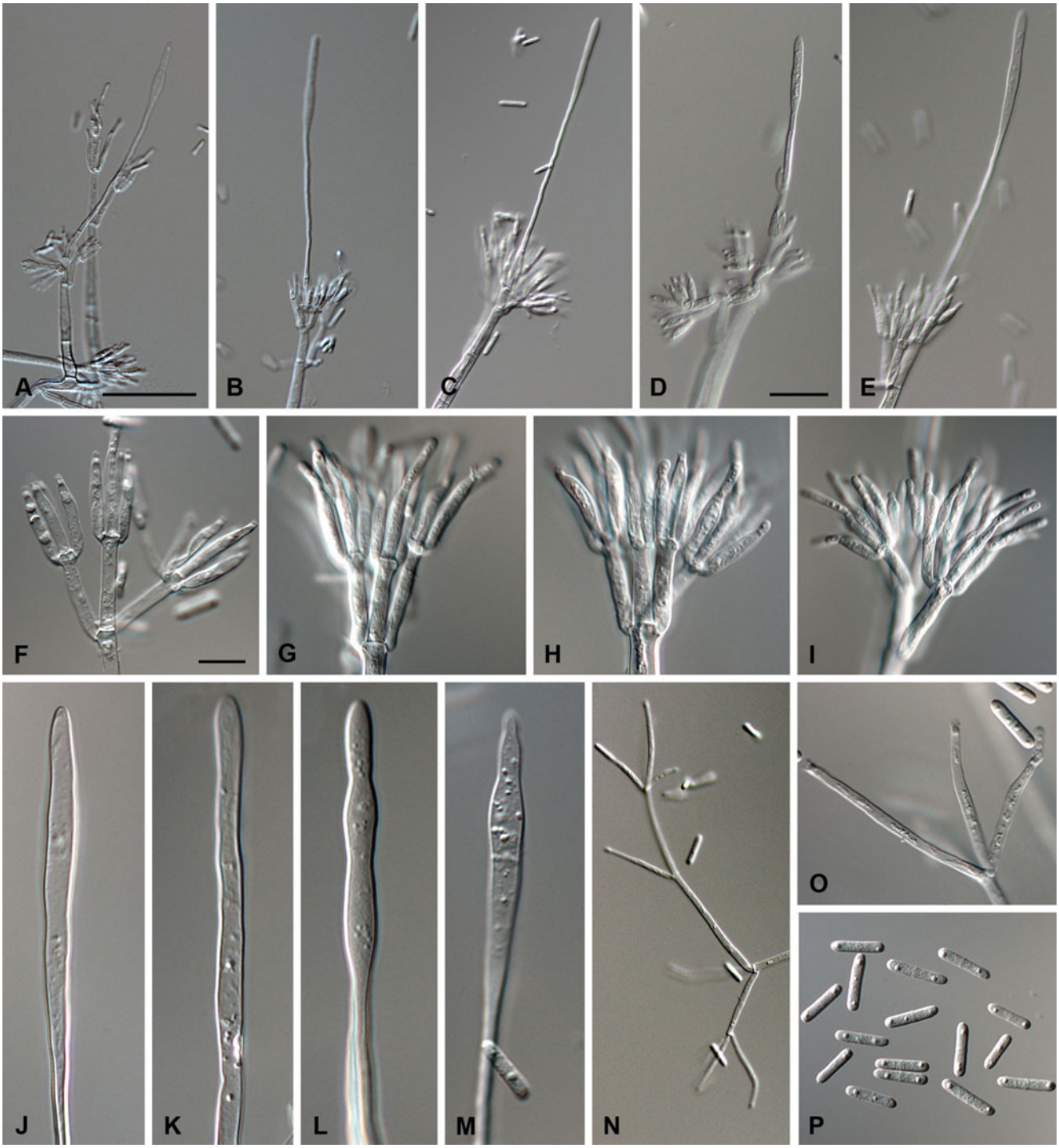

Fig. 16 *Cylindrocladiella queenslandica*. **a**–**e**. Penicillate conidiophores. **f**–**i**. Conidiogenous apparatus with conidiophore branches and phialides. **j**–**m**. Terminal vesicles. **n**–**o**. Subverticillate conidiophores. P. Conidia. Scale bars: A=50 μm (apply to **b**–**c**), D=20 μm (apply to **e**, **n**), F=10 μm (apply to **g**–**m**, **o**–**p**)

producing 2–4 phialides; phialides reniform to doliiform to cymbiform, hyaline, aseptate, 7–15×2–4 μm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* (Fig. 16n–o) in moderate numbers, comprising of a septate stipe, and primary branches terminating in 2–3 phialides; primary branches straight, hyaline, 0–1-septate, 22–50×3–4 μm; phialides cymbiform to cylindrical, hyaline, aseptate, 17–41×2–6 μm, apex with minute periclinal thickening and collarette. *Conidia* (Fig. 16p) cylindrical, rounded at both ends, straight, 1-septate, (9–)10.5–13.5

(–15)×2–4 μm (av. = 12×3 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with smooth to undulate margins, white with straw (21 d) tint in patches, buff yellow (19 d) to umber (13i) (reverse); chlamydospores moderate throughout medium, arranged in chains; reaching 90 mm after 7 days on MEA at 24°C in the dark.

Specimen examined – Australia, Queensland, from soil, 18 June 2009, P.W. Crous, holotype CBS-H20609, culture ex-type CBS 129574=CPC 17568; Australia, Queensland, from soil, 18 June 2009, P.W. Crous, culture CBS 129575=CPC 17569.

Notes – *Cylindrocladiella queenslandica* can be distinguished from other species in the *C. infestans* complex based on its smaller conidia, and unique fixed nucleotides for three loci: BTUB position 201 (T); HIS3 positions 110 (G) and 310 (G); TEF-1α positions 35 (A) and 455 (T).

Cylindrocladiella stellenboschensis L. Lombard & Crous, sp. nov. – MycoBank MB561671, Fig. 17.

Etymology – Named after the town from which this species was first collected, Stellenbosch, South Africa.

Cylindrocladiellae parvae morphologice valde similis, sed conidiis majoribus, (14–)17–19(–21)×2–4 μm, distinguitur.

Teleomorph unknown. *Conidiophores* monomorphic, penicillate, mononematous and hyaline. *Penicillate*

Fig. 17 *Cylindrocladiella stellenboschensis* **a**–**d**. Penicillate conidiophores. **e**–**i**. Conidiogenous apparatus with conidiophore branches and phialides. **j**–**m**. Terminal vesicles. N. Conidia. Scale bars: A=50 μm (apply to **b**–**d**), E=10 μm (apply to **f**–**n**)

conidiophores (Fig. 17a–c) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 37–65×6–9 μm; stipe extension aseptate, straight, 109–169 μm long, thick-walled with one basal septum, terminating in thin-walled, clavate to naviculate vesicles (Fig. 17h–j), 5–7 μm wide. *Penicillate conidiogenous apparatus* (Fig. 17d–g) with primary branches aseptate, 13–28×3–5 μm, secondary branches aseptate, 10–16×3–6 μm, each terminal branch producing 2–4 phialides; phialides doliiform to cymbiform, hyaline, aseptate, 12–21×2–4 μm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* not observed. *Conidia* (Fig. 17k–n) cylindrical, rounded at both ends, straight, 1-septate, (14–)17–19(–21)×2–4 μm (av. = 18×3 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with smooth margins, white with straw (21 d) tint in patches, umber (13i) (reverse); chlamydospores extensive throughout medium, arranged in chains; reaching 60 mm after 7 days on MEA at 24°C in the dark.

Specimens examined – South Africa, Western Cape Province, Stellenbosch, Stellenbosch Botanical Gardens, from leaf litter, 31 Aug. 1992, P.W. Crous, holotype CBS-H20610, culture ex-type CBS 110668=CPC 517; Canada, Toronto, Queens Park North, from leaf litter, 24 Apr. 2008, P.W. Crous, culture CPC 15200; Switzerland, Therwil Canton, Basel, from root of *Quercus* sp., 16 Mar. 1994, L. Petrini, culture CBS 116170=CPC 753.

Notes – This species can be distinguished from *C. parva* by its larger conidia and shape of the terminal vesicle. Furthermore, collarettes are also present on its phialides, whereas these are rare or absent for *C. parva*. *Cylindrocladiella stellenboschensis* differs from other lineages in the *C. parva* complex by unique fixed nucleotides in one locus: BTUB position 112 (A), 162 (G), 172 (A), 268 (C), 352 (T), 361 (C), 366 (G), 370 (T), 371 (G), 378 (A), 382 (A), 396 (A) and 495 (C).

Cylindrocladiella thailandica L. Lombard & Crous, sp. nov. – MycoBank MB561680, Fig. 18.

Etymology – Named after the country where it was collected, Thailand.

Cylindrocladiellae infestantis morphologice similis, sed extensionibus stipitis longioribus, 123–183 μm.

Teleomorph unknown. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 18a–d) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 49–80×5–9 μm; stipe extension aseptate, straight, 123–183 μm long, thick-walled with one basal septum, terminating in thin-walled, cylindrical to lanceolate vesicles (Fig. 18i–m), 5–7 μm wide. *Penicillate conidiogenous apparatus* (Fig. 18e–h) with primary branches aseptate, 11–24×4–8 μm, secondary branches aseptate, 7–14×2–5 μm, each terminal branch producing 2–4 phialides; phialides reniform to doliiform to cymbiform, hyaline, aseptate, 8–13×2–4 μm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* (Fig. 18n–p) in moderate numbers, comprising of a septate stipe, and primary branches terminating in 2–3 phialides; primary branches straight, hyaline, 0–1-septate, 40×3 μm; phialides cymbiform to cylindrical, hyaline, aseptate, 19–38×2 μm, apex with minute periclinal thickening and collarette. *Conidia* (Fig. 18q) cylindrical, rounded at both ends, straight, 1-septate, (13–)14–16(–18)×2–4 μm (av. = 15×3 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with smooth margins, white with buff yellow (19 d) centre, umber (13i) (reverse); chlamydospores extensive throughout medium, arranged in chains; reaching 70 mm after 7 days on MEA at 24°C in the dark.

Specimens examined – Thailand, Chiang Mai, from soil, Oct. 2010, P.W. Crous, holotype CBS-H20611, culture ex-type CBS 129571=CPC 18835; Chiang Mai, from soil, Oct. 2010, P.W. Crous, CBS 129570=CPC 18834; Chiang Mai, from soil, Oct. 2010, P.W. Crous, CPC 18831.

Notes – Morphologically, *C. thailandica* is similar to *C. infestans* and *C. longistipitata*, with the exception that the stipe extensions are longer than those of *C. infestans* but shorter than those of *C. longistipitata*. *Cylindrocladiella thailandica* can also be distinguished from other species in the *C. infestans* complex by different unique fixed nucleotides for two loci: BTUB position 160 (G); HIS3 positions 27 (C), 30 (A), 60-63 (indel), 70 (A) and 117 (A).

Cylindrocladiella variabilis L. Lombard & Crous, sp. nov. – MycoBank MB561673, Fig. 19.

Etymology – Named after its highly variable vesicle morphology.

Cylindrocladiellae lageniformis morphologice similis, sed vesiculis divergentibus.

Teleomorph unknown. *Conidiophores* monomorphic, penicillate, mononematous and hyaline. *Penicillate conidiophores* (Fig. 19a–e) comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 41–91×5–9 μm; stipe extension aseptate, straight, 67–106 μm long, thick-walled with one basal septum, terminating in thin-walled, clavate to fusoid to ovoid vesicles (Fig. 19j–m), 5–10 μm wide. *Penicillate conidiogenous apparatus* (Fig. 19f–i) with primary branches aseptate, 12–23×3–7 μm, secondary branches aseptate, 9–14×3–6 μm, each terminal branch producing 2–4 phialides; phialides doliiform to cymbiform, hyaline, aseptate, 7–17×2–6 μm, apex with minute periclinal thickening and collarette absent. *Subverticillate conidiophores* not observed. *Conidia*

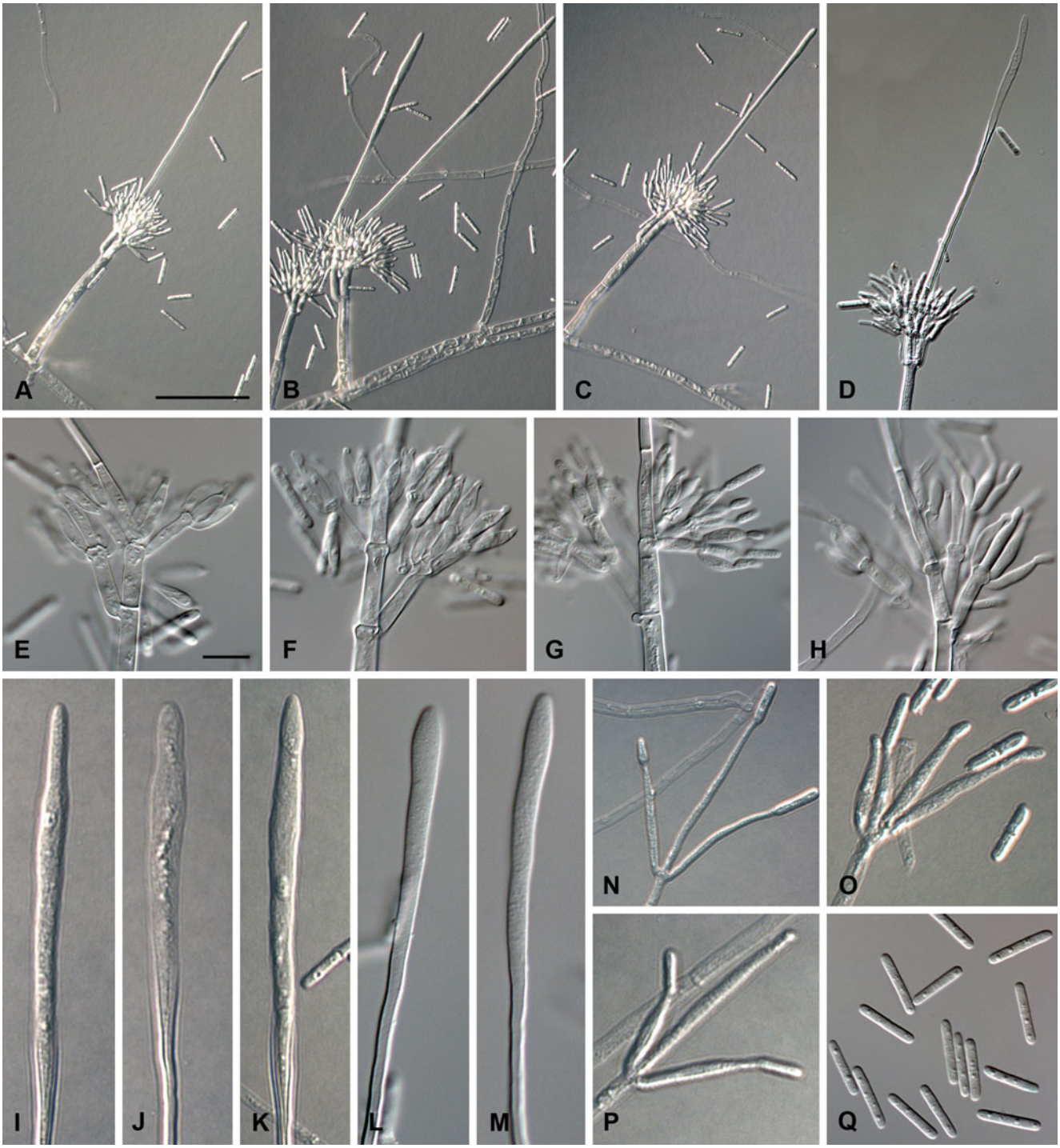

Fig. 18 *Cylindrocladiella thailandica*. **a**–**d**. Penicillate conidiophores. **e**–**h**. Conidiogenous apparatus with conidiophore branches and phialides. **i**–**m**. Terminal vesicles. **n**–**p**. Subverticillate conidiophores. Q. Conidia. A = 50 μm (apply to **b**–**d**), F = 10 μm (apply to **e**–**q**)

(Fig. 19n) cylindrical, rounded at both ends, straight, 1-septate, (9–)11–13(–14)×2–3 μm (av. = 12×3 μm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies raised (convex), cottony, with smooth margins, white, umber (13i) (reverse); chlamydospores extensive throughout medium, arranged in chains; reaching 60 mm after 7 days on MEA at 24°C in the dark.

Specimens examined – Australia, Queensland, Daydream island, Whitsundays Island Resort, from soil, 2 Aug. 2009, P.W. Crous, holotype CBS-H20612, culture ex-type CBS

Fig. 19 *Cylindrocladiella variabilis*. **a**–**e**. Penicillate conidiophores. **f**–**i**. Conidiogenous apparatus with conidiophore branches and phialides. **j**–**m**. Terminal vesicles. N. Conidia. Scale bars: A=20 μm (apply to **b**), C=50 μm (apply to **d**), E=10 μm (apply to **f**–**n**)

129561=CPC 17505, CPC 17504; Australia, Queensland, Lake Barrine, from soil, 18 June 2009, P.W. Crous, culture CBS 129562=CPC 17563.

Notes – *Cylindrocladiella variabile* can be distinguished from *C. lageniformis* by the high variability of its terminal vesicle shape. This species does not produce subverticillate conidiophores, whereas *C. lageniformis* produces them in moderate numbers (Crous 2002).

Cylindrocladiella pseudoinfestans L. Lombard & Crous, nom. nov. – MycoBank MB561684

Basionym: *Nectricladiella infestans* Crous & C.L. Schoch, Studies in Mycology 45: 55. 2000.

Etymology – Named after its morphological similarity to *C. infestans*.

Notes – *Cylindrocladiella pseudoinfestans* is introduced as a new name for *N. infestans* in the genus *Cylindrocladiella*. *Nectricladiella infestans* was incorrectly linked to its purported anamorph, *C. infestans* (Schoch et al. 2000), to which it is morphologically similar. *Cylindrocladiella pseudoinfestans* can be distinguished from other species in the *C. infestans* complex by different unique fixed nucleotides for three loci: BTUB position 395 (A); HIS3 positions 22 (T), 41 (G), 47 (A), 50 (A), 72 (T) and 272 (C); TEF-1α positions 268 (A), 272 (G), 478 (A) and 480 (C).

Discussion

In this study, several *Cylindrocladiella* isolates from numerous hosts and countries collected over the past two decades were shown to include a number of novel species. These species were recognised using phylogenetic inference and, where possible, supported by morphological features. The taxonomic status of several phylogenetic species identified in this study remains unresolved due to either representation by only a single isolate (e.g. CBS 116095) or culture sterility (e.g. clade containing CBS 115673). Naming these novel species in the anamorph genus *Cylindrocladiella* and not the teleomorph genus *Nectricladiella* follows the "strict priority" option as applied by Gräfenhan et al. (2011), which continued the approach of Lombard et al. (2009, 2010a–c), and Schroers et al. (2011) of naming fungi in the *Hyprocreales* with the oldest generic name, irrespective of its morph typification. Consequently, the novel species found in this study were named in the genus *Cylindrocladiella* (Boesewinkel 1982) rather than in the teleomorph genus *Nectricladiella* (Schoch et al. 2000).

Five species complexes could be identified in this study based on phylogenetic inference supported by morphological characterisation. Although previous authors (Victor et al. 1998, Schoch et al. 2000, Crous 2002, van Coller et al. 2005) acknowledged the presence of species complexes in the genus *Cylindrocladiella*, their sample sizes were small. In our study, a larger sample size, obtained from various culture collections, allowed a multi-gene analysis to more clearly identify species complexes in *Cylindrocladiella*.

The *Cylindrocladiella camelliae* species complex was shown to consist of several phylogenetic species, four of which were described as *C. longiphialidica*, *C. natalensis*, *C. nederlandica* and *C. pseudocamelliae*. Each of these four new species was distinguished from *C. camelliae* and each other by the morphology and dimensions of conidia, subverticillate conidiophores and stipe extensions. Geographical distribution of the various species in the *C. camelliae* complex reflected the cosmopolitan nature of this group of fungi. *Cylindrocladiella nederlandica* and *C. natalensis* were isolated from diseased plant material, and *C. pseudocamelliae* and *C. longiphialidica* were only isolated from soil, and their significance as plant pathogens still needs to be determined.

Cylindrocladiella cymbiformis is a newly described species closely related to both *C. novaezelandiae*, as well as novel lineages in the newly identified *C. elegans* species complex. *Cylindrocladiella cymbiformis* is not a cryptic species in the *C. elegans* complex as it can be distinguished from both *C. novaezelandiae* and *C. elegans* by its larger conidium dimensions and shorter stipe extensions. All isolates in this study representing the *C. elegans* complex originated from South Africa, whereas *C. cymbiformis* is described here from soil samples collected in Australia. Cryptic species were not resolved in the *C. elegans* complex as the cultures were sterile.

Past studies have presented evidence of cryptic speciation within *C. infestans* (Victor et al. 1998, Schoch et al. 2000, Crous 2002, van Coller et al. 2005). In an attempt to resolve taxa in this complex, a large sample of *C. infestans senso lato* isolates was included in this study. Based on phylogenetic inference and morphological characterisation, a total of 12 cryptic species were identified. Of these, eight were described as novel taxa. All eight of these newly named species may be regarded as phylogenetic species, as morphological characters are limited to distinguish them from each other. These species are recognised using the genealogical concordance phylogenetic species recognition (GCPSR) criteria (Taylor et al. 2000) based on DNA sequence data for the five loci used in this study. As has been done for other fungal groups (O'Donnell et al. 2004, Grünig et al. 2008, Pavlic et al. 2009, Lombard et al. 2010b), these species are chiefly characterised by fixed single nucleotide polymorphisms (SNPs).

Schoch et al. (2000) described *Nectricladiella infestans* as the teleomorph state of *C. infestans sensu lato* from an isolate collected in Madagascar that produced perithecia in culture. With additional sequence data and isolates, van Coller et al. (2005) showed this isolate represented a cryptic species distinct from *C. infestans senso stricto*. This was further supported by the phylogenetic inference in this study, and based on GCPSR, *Nectricladiella infestans* has been provided with a new name, *C. pseudoinfestans*.

Cylindrocladiella clavata, *C. lanceolata* and *C. variabilis* are newly described here, closely related to *C. lageniformis*. They can be distinguished from each other and *C. lageniformis* based on the absence of subverticillate conidiophores, terminal vesicle morphology and conidium dimensions. All three of these species, with the exception of *C. lanceolata*, are presently only known from soil samples collected in Australia. *Cylindrocladiella lanceolata* was also isolated from a diseased *Eucalyptus* cutting in South Africa, adding another *Cylindrocladiella* species recorded from that country (Crous et al. 1993, Crous et al. 1994, Crous 2002, van Coller et al. 2005).

Phylogenetic inference applied in this study also identified a number of cryptic species within a large sample of *C. parva sensu lato* isolates. Only two of these cryptic species could be named here, as most isolates were sterile. *Cylindrocladiella stellenboschensis* and *C. pseudoparva* are difficult to distinguish from each other or from *C. parva* by morphology alone. These two species are recognised as phylogenetic species described according to the GCPSR criteria using fixed SNPs.

Isolates of *C. peruviana* used in this study also included cryptic species that could not be named. As with the *C.*

elegans complex, isolates representing these cryptic species were sterile and their taxonomy remains unresolved.

Traditionally, DNA sequence data for the ITS and BTUB gene regions were used to explore the phylogenetic relationship between *Cylindrocladiella* spp. (Victor et al. 1998, Schoch et al. 2000). Van Coller et al. (2005) introduced HIS3 sequence data for this group of fungi, increasing the gene regions that provide the most valuable information on the relationships among *Cylindrocladiella* spp. Data for these three gene regions have been available only for a small sample of *Cylindrocladiella* isolates. This present study has attempted to address this problem and also introduced partial TEF-1α gene region sequences for all known *Cylindrocladiella* spp. Phylogenetic analysis of the individual gene regions showed that the TEF-1α gene region provided the best resolution to distinguish between *Cylindrocladiella* spp., followed by BTUB and HIS3. As was found with *Calonectria* spp. (Lombard et al. 2010b), the ITS and LSU gene regions provided limited information to distinguish between *Cylindrocladiella* spp.

Identification of a large number of cryptic species within the genus *Cylindrocladiella* based on phylogenetic inference and morphological comparisons, highlights how little attention this group has received in the past. Although *Cylindrocladiella* spp. are generally not regarded as important plant pathogens, correct identification is essential for disease control and biosecurity implications. This study has revealed the importance of combining morphological and phylogenetic data to understand the taxonomic issues surrounding this group of fungi.

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