Morphological and Phylogenetic Analyses Reveal Two New Species of Sporocadaceae From Hainan, China

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Abstract

Species of Sporocadaceae have often been reported as plant pathogens, endophytes or saprobic, commonly isolated from a wide range of plant hosts. The isolated fungi were studied through a complete examination based on multi-locus phylogeny of a combined dataset of ITS/ *TUB2*/ *TEF1-α*, in conjunction with morphological characteristics. Nine strains isolated from *Schima superba*, *Ficus microcarpa* and *Ilex chinensis* in Hainan Province, China, represented four species, viz, *Monochaetia schimae* sp. nov., *Neopestalotiopsis haikouensis* sp. nov., *Neopestalotiopsis piceana* and *Pestalotiopsis licualacola*.

Introduction

Xylariales, a group of fungi distributed worldwide, whose members show considerable variability in a number of characteristics, including position of ascomata, presence and type of sterile tissues, and habit (Barr 1990). The Sporocadaceae (type genus: *Sporocadus* Corda) is a well-defined family in the Xylariales (Liu et al. 2019). Based on phylogenetic analyses and morphological comparison, Sporocadaceae has delimited thirty genera including *Monochaetia* (Sacc.) Allesch., *Neopestalotiopsis* Maharachch. et al., *Pestalotiopsis* Steyaert, *Pseudopestalotiopsis* Maharachch. et al., etc., which is generally congruent with the classification system proposed by Nag Raj (1993) prior to the DNA phylogeny era. Species of Sporocadaceae are endophytic, plant pathogenic or saprobic, and associated with a wide range of host plants.

Initially, pestalotia-like asexual morphs were classified in Amphisphaeriaceae by Samuels et al. (1987), accommodating 36 genera (Hawksworth et al. 1995). Its ordinal level of classification, the Amphisphaeriales, was introduced by Eriksson & Hawksworth (1986), but treated as a synonym of Xylariales one year later by Eriksson & Hawksworth (1987). Hawksworth et al. (1995) followed and supported this classification by molecular data. The order was recently resurrected to include Amphisphaeriaceae, Clypeosphaeriaceae and another four novel families derived from Amphisphaeriaceae, however, the sequence dataset was largely incomplete and some of the introduced families were not well supported statistically (Senanayake et al. 2015). Subsequently, Bartaliniaceae, Discosiaceae, Pestalotiopsidaceae and Robillardaceae (Crous et al. 2015) were synonymized and revived the older family name Sporocadaceae to accommodate them, together with the Amphisphaeriaceae and Phlogicylindriaceae, Sporocadaceae was accommodated in the Xylariales, however, Amphisphaeriales was not accepted due to a lack of phylogenetic support in their analysis (Jaklitsch et al. 2016).

Presently, agreement on the classification and delimitation of the family itself seems to have been reached after intense debate. Fungi in the Sporocadaceae (e.g. *Bartalinia*, *Pestalotia*, *Pestalotiopsis*, *Robillarda*, *Seimatosporium*, *Seiridium* and *Truncatella*) possess common asexual morphological characters related to their acervular conidiomata, conidiogenesis and conidia (Liu et al. 2019). To date, most phylogenetic studies addressing genera of Sporocadaceae have been based solely on ITS and *LSU* sequences (Barber et al. 2011; Tanaka et al. 2011; Jaklitsch et al. 2016), or on concatenated datasets of more genes but with incomplete datasets (Senanayake et al. 2015; Wijayawardene et al. 2016). Consequently, the taxonomic concept of, and generic delimitation within Sporocadaceae remain unclear.

Most of the genera of Sporocadaceae contain over-lapping morphological characters of conidia such as the number of median cells, colour of median cells, presence of apical and basal appendages (Jeewon et al. 2002). In this study, we made a collection of the established genera *Pestalotiopsis*, *Neopestalotiopsis* and *Monochaetia* species on leaves of *Schima superba*, *Ficus microcarpa* and *Ilex chinensis* in Hainan Province, China and the new species is established with descriptions, illustrations and molecular data based on ITS, *TUB2* and *TEF1-α* loci.

Materials And Methods

Specimen collection and morphological descriptions

The samples were collected from Hainan Province, China. The strains were isolated from diseased leaves of *Schima superba*, *Ficus microcarpa* and *Ilex chinensis* using tissue isolation from surface sterilized leaf tissues were conducted following the protocol of Gao et al. (2014). Tissue fragments (0.5 × 0.5 cm) were taken from the margin of leaf lesions and surface-sterilized by consecutively immersing in 75% ethanol solution for 30 s, 5% sodium hypochlorite solution for 1 min, and then rinsing in sterile

distilled water for 30 s. The pieces were dried with sterilized paper towels and placed on potato dextrose agar (PDA). All the plates were incubated at biochemical incubator at 25°C for 3–4 days, then hyphae were picked out of the periphery of the colonies and inoculated onto new PDA plates. Photographs of the colonies were taken at 7 days and 15 days using a Powershot G7X mark digital camera. Micromorphological characters were observed using Olympus SZX10 stereomicroscope and Olympus BX53 microscope, all fitted with Olympus DP80 high definition colour digital cameras to photo-document fungal structures. All fungal strains were stored in 10% sterilized glycerin at 4 ℃ for further studies. The holotype specimens are deposited in the Herbarium of Plant Pathology, Shandong Agricultural University (HSAUP). Ex-type cultures are deposited in the Shandong Agricultural University Culture Collection (SAUCC). Taxonomic information of the new taxa was submitted to MycoBank (http://www.mycobank.org).

DNA extraction, PCR amplification, and DNA sequencing

Genomic DNA was extracted from fungal mycelium on PDA, using a modified cetyltrimethylammonium bromide (CTAB) protocol as described in Guo et al. (2000). The internal transcribed spacer regions with intervening 5.8S nrRNA gene (ITS), part of the beta-tubulin gene region (*TUB2*), and partial translation elongation factor 1-alpha (*TEF1-α*) genes were amplified and sequenced by using primers pairs ITS4/ITS5 (White et al. 1990), T1/Bt2b (O'Donnell & Cigelnik 1997; Glass & Donaldson 1995), EF1-728F/EF2 (Carbone & Kohn 1999; O'Donnell et al. 1998), respectively.

PCR was performed using an Eppendorf Master Thermocycler (Hamburg, Germany). Amplification reactions were performed in a 50 µL reaction volume, which contained 25 µL Green Taq Mix (Vazyme, Nanjing, China), 2 µL of each forward and reverse primer (10 µM) (Tsingke, Beijing, China), and 2 µL template genomic DNA in amplifier, and were adjusted with distilled deionized water to a total volume of 50 µL. PCR parameters were as follows: 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at a suitable temperature for 30 s, extension at 72°C for 1 min and a final elongation step at 72°C for 7 min. Annealing temperature for each gene were 55°C for ITS, 54°C for *TUB2*, 52°C for *TEF1-α*. The PCR products were visualised on 1% agarose electrophoresis gel. Sequencing was done bi-directionally, conducted by the Tsingke Biotechnology Company Limited (Shanghai, China). Consensus sequences were obtained using MEGA 7.0 or MEGA-X (Kumar et al. 2016). All sequences generated in this study were deposited in GenBank (Table 1).

Table 1
Species and GenBank accession numbers of DNA sequences used in this study. New sequences in bold.

Species	Strain	Host/substrate	Country	GenBank accession number		
				ITS	*TUB2*	*TEF1*
Bartalinia robillardoides	CBS 122705 T	*Leptoglossus occidentalis*	Italy	LT853104	LT853252	LT853202
Ciliochorella phanericola	MFLUCC 14-0984 T	*Phanera purpurea*	Thailand	KX789680	KX789682	–
	MFLUCC 12-0310	*Phanera purpurea*	Thailand	KF827444	KF827478	KF827477
Monochaetia castaneae	CFCC 54354 = SM9-1 T	*Castanea* sp.	China	MW166222	MW218515	MW199741
	SM9-2	*Castanea* sp.	China	MW166223	MW218516	MW199742
M. dimorphospora	NBRC 9980	*Castanea pubinervis*	Japan	LC146750	–	–
M. ilexae	KUMCC 15-0520 T	*Ilex* sp.	China	KX984153	–	–
	CBS 101009	Air	Japan	MH553953	MH554612	MH554371
M. junipericola	CBS 143391 T	*Juniperus communis*, twig	Germany	MH107900	MH108045	MH108021
M. kansensis	PSHI2004Endo1030	*Cyclobalaopsis myrsinaefolia*	China	DQ534044	DQ534047	–
	PSHI2004Endo1031	*Cyclobalaopsis myrsinaefolia*	China	DQ534045	DQ534048	–
M. monochaeta	CBS 546.80	Culture contaminant	Netherlands	MH554056	MH554732	MH554491
	CBS 199.82 T	*Quercus pubescens*	Italy	MH554018	MH554694	–
	CBS 115004	*Quercus robur*	Netherlands	AY853243	MH554639	MH554398
M. quercus	CBS 144034 T	*Quercus eduardi*	Mexico	MH554171	MH554844	MH554606
M. schimae	**SAUCC212201 T**	Schima superba	**China**	**MZ577565**	**OK104867**	**OK104874**
	SAUCC212202	Schima superba	**China**	**MZ577566**	**OK104868**	**OK104875**
	SAUCC212203	Schima superba	**China**	**MZ577567**	**OK104869**	**OK104876**
M. sinensis	HKAS 10065 T	*Quercus* sp.	China	MH115995	MH115999	–
Neopestalotiopsis acrostichi	MFLUCC 17-1754 T	*Acrostichum aureum*	Thailand	MK764272	MK764338	MK764316
N. aotearoa	CBS 367.54 T	*Canvas*	New Zealand	KM199369	KM199454	KM199526
N. asiatica	CFCC 54339 = SM32	*Castanea mollissima*	China	MW166224	MW218517	MW199743

Isolates marked with “T” are ex-type or ex-epitype strains.

Species	Strain	Host/substrate	Country	GenBank accession number		
				ITS	*TUB2*	*TEF1*
	SM7	*Castanea mollissima*	China	MW166225	MW218518	MW199744
N. brachiata	MFLUCC 17-1555 T	*Rhizophora apiculata*	Thailand	MK764274	MK764340	MK764318
N. brasiliensis	COAD 2166 T	*Psidium guajava*	Brazil	MG686469	MG692400	MG692402
	CFCC 54341 = ZY4	*Castanea mollissima*	China	MW166229	MW218522	MW199748
	ZY4-2D	*Castanea mollissima*	China	MW166230	MW218523	MW199749
N. cubana	CBS 600.96 T	leaf litter	Cuba	KM199347	KM199438	KM199521
N. egyptiaca	CBS 140162 T	*Mangifera indica*	Egypt	KP943747	KP943746	KP943748
N. eucalypticola	CBS 264.37 T	*Eucalyptus globulus*	–	KM199376	KM199431	KM199551
N. formicarum	CBS 362.72 T	dead ant	Ghana	KM199358	KM199455	KM199517
	CBS 115.83	Plant debris	Cuba	KM199344	KM199444	KM199519
N. haikouensis	**SAUCC212271 T**	Ilex chinensis	**China**	**OK087294**	**OK104870**	**OK104877**
	SAUCC212272	Ilex chinensis	**China**	**OK087295**	**OK104871**	**OK104878**
N. honoluluana	CBS 114495 T	*Telopea* sp.	USA	KM199364	KM199457	KM199548
N. iraniensis	CBS 137768 T	*Fragaria ananassa*	Iran	KM074048	KM074057	KM074051
N. javaensis	CBS 257.31 T	*Cocos nucifera*	Indonesia	KM199357	KM199437	KM199543
N. mesopotamica	CBS 336.86 T	*Pinus brutia*	Iraq	KM199362	KM199441	KM199555
N. musae	MFLUCC 15-0776 T	*Musa* sp.	Thailand	KX789683	KX789686	KX789685
N. natalensis	CBS 138.41 T	*Acacia mollissima*	South Africa	KM199377	KM199466	KM199552
N. pandanicola	KUMCC 17-0175 T	Pandanaceae	China	–	MH412720	MH388389
N. petila	MFLUCC 17-1738 T	*Rhizophora mucronata*	Thailand	MK764276	MK764342	MK764320
N. phangngaensis	MFLUCC 18-0119 T	Pandanaceae	Thailand	MH388354	MH412721	MH388390
N. piceana	CBS 394.48 T	*Picea* sp.	UK	KM199368	KM199453	KM199527
	CBS 254.32	*Cocos nucifera*	Indonesia	KM199372	KM199452	KM199529
	SAUCC210112	Ficus microcarpa	**China**	**OK149224**	**OK206434**	**OK206436**
	SAUCC210113	Ficus microcarpa	**China**	**OK149225**	**OK206435**	**OK206437**
N. protearum	CBS 114178 T	*Leucospermum cuneiforme* cv. “Sunbird”	Zimbabwe	MK764278	MK764344	MK764322

Isolates marked with “T” are ex-type or ex-epitype strains.

Species	Strain	Host/substrate	Country	GenBank accession number		
				ITS	*TUB2*	*TEF1*
N. rhizophorae	MFLUCC 17-1550 T	*Rhizophora mucronata*	Thailand	KM199360	KM199430	KM199524
N. rosae	CBS 124745	*Paeonia suffruticosa*	USA	KM199359	KM199429	KM199523
	CBS 101057 T	*Rosa* sp.	New Zealand	KY885239	KY885245	KY885243
N. rosicola	CFCC 51992 T	*Rosa chinensis*	China	KY885239	KY885245	KY885243
	CFCC 51993	*Rosa chinensis*	China	KY885240	KY885246	KY885244
N. saprophytica	CBS 115452 T	*Magnolia* sp.	China	KM199345	KM199433	KM199538
N. sichuanensis	CFCC 54338 = SM15-1 T	*Castanea mollissima*	China	MW166231	MW218524	MW199750
	SM15-1C	*Castanea mollissima*	China	MW166232	MW218525	MW199751
N. sonneratae	MFLUCC 17-1745 T	Sonneronata alba	Thailand	MK764280	MK764346	MK764324
N. surinamensis	CBS 450.74 T	soil under *Elaeis guineensis*	Suriname	KM199351	KM199465	KM199518
N. thailandica	MFLUCC 17-1730 T	*Rhizophora mucronata*	Thailand	MK764281	MK764347	MK764325
N. vitis	MFLUCC 15-1265 T	*Vitis vinifera* cv. “Summer black"	China	KU140694	KU140685	KU140676
N. zimbabwana	CBS 111495 T	*Leucospermum cunciforme* cv. “Sunbird”	Zimbabwe	JX556231	KM199456	KM199545
Nonappendiculata quercina	CBS 116061 T	*Quercus suber*	Italy	MH553982	MH554641	MH554400
	CBS 270.82	*Quercus pubescens*	Italy	MH554025	MH554701	MH554459
Pestalotiopsis australasiae	CBS 114126 T	*Knightia* sp.	New Zealand	KM199297	KM199409	KM199499
P. australis	CBS 114193 T	*Grevillea* sp.	Australia	KM199332	KM199383	KM199475
P. grevilleae	CBS 114127 T	*Grevillea* sp.	Australia	KM199300	KM199407	KM199504
P. hollandica	CBS 265.33 T	*Sciadopitys verticillata*	The Netherlands	KM199328	KM199388	KM199481
P. kenyana	CBS 442.67 T	*Coffea* sp.	Kenya	KM199302	KM199395	KM199502
P. knightiae	CBS 114138 T	*Knightia* sp.	New Zealand	KM199310	KM199408	KM199497
P. licualacola	HGUP4057 T	*Licuala grandis*	China	KC492509	KC481683	KC481684
	SAUCC210087	*Ilex chinensis*	**China**	**OK087323**	**OK104872**	**OK104879**
	SAUCC210088	*Ilex chinensis*	**China**	**OK087324**	**OK104873**	**OK104880**

Isolates marked with “T” are ex-type or ex-epitype strains.

Species	Strain	Host/substrate	Country	GenBank accession number		
				ITS	*TUB2*	*TEF1*
P. oryzae	CBS 353.69 T	*Oryza sativa*	Denmark	KM199299	KM199398	KM199496
P. parva	CBS 278.35	*Leucothoe fontanesiana*	–	KM199313	KM199405	KM199509
P. portugalica	CBS 393.48 T	–	Portugal	KM199335	KM199422	KM199510
P. spathuliappendiculata	CBS 144035 T	*Phoenix canariensis*	Australia	MH554172	MH554845	MH554607
Pseudopestalotiopsis cocos	CBS 272.29 T	*Cocos nucifera*	Indonesia	KM199378	KM199467	KM199553
Pse. elaeidis	CBS 413.62 T	*Elaeis guineensis*	Nigeria	MH554044	MH554720	MH554479
Pse. indica	CBS 459.78 T	*Rosa sinensis*	India	KM199381	KM199470	KM199560
Seiridium papillatum	CBS 340.97 T	*Eucalyptus delegatensis*	Australia	LT853102	LT853250	MH554468
Seir. phylicae	CBS 133587 T	*Phylica arborea*	Tristan da Cunha	LT853091	LT853238	LT853188
Isolates marked with “T” are ex-type or ex-epitype strains.						

Phylogenetic analyses

Newly generated sequences in this study were aligned with additional related sequences downloaded from GenBank (Table 1) using MAFFT 7 online service with the Auto strategy (Katoh et al. 2019, http://mafft.cbrc.jp/alignment/server/). To establish the identity of the isolates at species level, phylogenetic analyses were conducted first individually for each locus and then as combined analyses of three loci (ITS, *TUB2* and *TEF1-α*). Phylogenetic analyses were based on maximum likelihood (ML) and Bayesian inference (BI) for the multi-locus analyses. For BI, the best evolutionary model for each partition was determined using MrModeltest v. 2.3 (Nylander 2004) and incorporated into the analyses. ML and BI were run on the CIPRES Science Gateway portal (https://www.phylo.org/) (Miller et al. 2012) using RaxML-HPC2 on XSEDE (8.2.12) (Stamatakis 2014) and MrBayes on XSEDE (3.2.7a) (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003; Ronquist et al. 2012), respectively. Four Markov chains were run for two runs from random starting trees for 5 million generations (ITS + *TUB2* + *TEF1-α*) until the split deviation frequency value < 0.01, and trees were sampled every 1000 generation. The first quarter generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated.

The resulting trees were plotted using FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree) and edited with Adobe Illustrator CC 2019. New sequences generated in this study were deposited at GenBank (https://www.ncbi.nlm.nih.gov; Table 1).

Results

Phylogeny

Nine strains of Sporocadaceae isolated from plant hosts from Hainan, China, were grown in culture and used for analyses of molecular sequence data. The combined dataset of ITS + *TUB2* + *TEF1-α* has an aligned length of 2285 total characters (ITS: 1–616, *TUB2*: 617–1570, *TEF1-α*: 1571–2285) including gaps, of which 1119 characters are constant, 285 variable characters are parsimony-uninformative, and 881 are parsimony-informative. For the BI and ML analyses, the substitution model SYM+I+G for ITS, HKY+G for *TUB2* and GTR+I+G for *TEF1-α* were selected and incorporated into the analyses. The MCMC analysis of the three concatenated genes run for 2,650,000 generations, resulting in 2651 trees. The initial 662 trees representative of the analysis burn-in phase was discarded, while the remaining trees were used to calculate posterior probabilities in the majority rule consensus trees (Fig. 1; first value: PP ≥ 0.50 shown). The alignment contained a total of 1453 unique site patterns (ITS: 269,

TUB2: 670, *TEF1-α*: 514). The ML tree topology confirmed the tree topologies obtained from the BI analyses, and therefore, only the ML tree is presented (Fig. 1).

ML bootstrap support values (≥ 50%) and Bayesian posterior probability (≥ 0.50) are shown as first and second position above nodes, respectively. The 81 strains were assigned to 59 species clades based on the three gene loci phylogeny (Fig. 1). Based on the multi-locus phylogeny and morphology, nine isolates were assigned to four species, including *Monochaetia schimae* sp. nov., *Neopestalotiopsis haikouensis* sp. nov., *Neopestalotiopsis piceana*, *Pestalotiopsis licualacola*, respectively.

Taxonomy

Monochaetia schimae Z.X. Zhang, X.G. Zhang & J.W. Xia, **sp. nov.** Fig. 2.

MycoBank 841381

Etymology: Name refers to the genus of the host plant *Schima superba*.

Type: China, Hainan Province: East Harbour National Nature Reserve, on diseased leaves of *Schima superba*, 23 May 2021, Z.X. Zhang (holotype HSAUP212201; ex-type living culture SAUCC212201). GenBank deposition numbers of sequences derived from type: ITS, MZ577565; *TUB2*, OK104867; *TEF1-α*, OK104874.

Description: *Colonies* on PDA 39.0–45.0 mm in diameter after 15 days at 25°C in darkness, growth rate 2.5–3.0 mm diam/day, irregularly circular, raised, dense surface with lobate edge, zonate with different sector light brown at the margin, brown at the center; reverse brown at the margin, dark brown at the center. Sexual morph: Undetermined. Asexual morph: Leaf spots irregular, pale brown in center, brown to tan at margin. *Conidiomata* solitary, scattered, black, raising above surface of culture medium, subglobose, exuding black conidial droplets from central ostioles after 10 days in light at 25°C. *Conidiophores* cylindrical, hyaline, smooth-walled. *Conidiogenous cells* 9.0–16.5 × 1.2–2.2 µm, phialidic, ampulliform, discrete, hyaline, smooth, thin-walled. *Conidia* 18.0–24.0 × 4.5–6.0 µm, mean ± SD = 20.5 ± 1.1 × 5.5 ± 0.4 µm, fusiform, tapering at both ends, 4-septate; apical cell 2.0–4.0 µm long, conic, hyaline and smooth-walled; three median cells together 12.5–15.5 µm long, mean ± SD = 14.2 ± 0.7 µm, doliiform, brown, rough-walled, upper second cell 3.8–5.3 µm long, upper third cell 3.4–5.0 µm long, upper fourth cell 4.4–5.4 µm long; basal cell 2.2–4.5 µm long, conical, hyaline and smooth-walled; apical appendage 7.0–12.5 µm long (mean = 9.2 µm), single, central, tubular, filiform; basal appendage 2.5–5.0 µm long, single, tubular, filiform.

Additional specimen examined: China, Hainan Province: East Harbour National Nature Reserve, 23 May 2021, Z.X. Zhang. On diseased leaves of *Schima superba* (Theaceae), paratype HSAUP212202, ex-paratype culture SAUCC212202; on diseased leaves of *Schima superba* (Theaceae), paratype HSAUP212203, ex-paratype culture SAUCC212203.

Notes: *Monochaetia schimae* is introduced based on the multi-locus phylogenetic analysis, with three isolates clustering separately in a well-supported clade (ML/BI = 100/1). *M. schimae* is most closely related to *M. ilexae* (CBS 101009), but distinguished based on ITS, *TUB2* and *TEF1-α* loci by 94 nucleotide differences in the concatenated alignment, in which 18/526 are distinct in the ITS region, 32/698 in the *TUB2* region and 44/462 in the *TEF1-α* region. Morphologically, *M. schimae* differ from *M. ilexae* in its smaller conidia (18.0–24.0 × 4.5–6.0 vs. 20.0–27.0 × 3.0–5.0 µm). Therefore, we establish this fungus as a novel species.

Neopestalotiopsis haikouensis Z.X. Zhang, X.G. Zhang & J.W. Xia, **sp. nov.** Fig. 3.

MycoBank 841382

Etymology: Named after the host collection, Haikou City.

Type: China, Hainan Province, Haikou City: East Harbour National Nature Reserve, on diseased leaves of *Ilex chinensis*. 23 May 2021, Z.X. Zhang (holotype HSAUP212271; ex-type living culture SAUCC212271). GenBank deposition numbers of sequences derived from type: ITS, OK087294; *TUB2*, OK104870; *TEF1-α*, OK104877.

Description: *Colonies* on PDA incubated at 25°C in the dark with an average radial growth rate of 7.0–9.0 mm/day and occupying an entire 90 mm Petri dish in 7 d; edge undulate, white to grey white, with moderate aerial mycelium on the surface, with black, gregarious conidiomata; reverse similar in colour. Sexual morph: Undetermined. Asexual morph: Leaf spots irregular, grey white in center, brown to tan at margin. *Conidiomata* globose to clavate, solitary or confluent, embedded or semi-immersed to erumpent, dark brown, exuding globose, dark brown to black conidial masses. *Conidiophores* indistinct, often reduced to *conidiogenous cells*. *Conidiogenous cells* discrete, subcylindrical to ampulliform, hyaline, 5.0–10.0 × 2.0–6.0 µm, apex 1.0–2.0 µm diam. *Conidia* fusoid, ellipsoid, straight to slightly curved, 4-septate, 16.0–22.0 × 4.5–7.0 µm, mean ± SD = 20.0 ± 1.8 × 5.5 ± 0.4 µm; basal cell conic with a truncate base, hyaline, rugose and thin-walled, 3.0–4.5µm long; three median cells doliiform, 11.5–15.0 µm long, mean ± SD = 13.2 ± 1.0 µm, wall rugose, versicoloured, septa darker than the rest of the cell, second cell from the base pale brown, 3.5–5.5 µm long; third cell honey-brown, 4.0–6.0 µm long; fourth cell brown, 3.8–5.7 µm long; apical cell 2.5–5.5 µm long, hyaline, cylindrical to subcylindrical, thin- and smooth-walled; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, 13.5–24.0 µm long, mean ± SD = 19.1 ± 3.5 µm; basal appendage 2.0–7.0 µm long, single, tubular, unbranched, centric.

Additional specimen examined: China, Hainan Province: East Harbour National Nature Reserve, 23 May 2021, Z.X. Zhang. On diseased leaves of *Ilex chinensis*, paratype HSAUP212272, ex-paratype culture SAUCC212272.

Notes: Phylogenetic analysis of a combined three gene showed that *Neopestalotiopsis haikouensis* formed an independent clade (Fig. 1) and is phylogenetically distinct from *N. aotearoa* (CBS 367.54), *N. piceana* (CBS 254.32), *N. brachiate* (MFLUCC 17-1555), *N. phangngaensis* (MFLUCC 18-0119), *N. rhizophorae* (MFLUCC 17-1550), *N. petila* (MFLUCC 17-1738) and *N. rosicola* (CFCC 51993). *N. haikouensis* can be distinguished from the phylogenetically most closely related species *N. aotearoa* by shorter and wider conidia (16.0–22.0 × 4.5–7.0 vs. 20.0–27.0 × 3.0–5.0 µm), and several loci (7/532 in the ITS region, 4/771 *TUB2* and 6/487 *TEF1-α*). Therefore, we establish this fungus as a novel species.

Neopestalotiopsis piceana Maharachch., K.D. Hyde & Crous, Studies in Mycology 79:146. (2014) Fig. 4.

Description: *Colonies* on PDA reaching 70.0–80.0 mm diam after 7 d at 25°C, edge entire, whitish to pale honeycoloured, with sparse aerial mycelium on the surface, with black, gregarious conidiomata; reverse similar in colour. Sexual morph: Undetermined. Asexual morph: Leaf spots irregular, pale brown in center, brown to tan at margin. *Conidiomata* (on PDA) pycnidial, globose to clavate, solitary, semi-immersed, brown to black; exuding globose, dark brown to black conidial masses. Conidiophores reduced to conidiogenous cells. *Conidiogenous cells* discrete, ampulliform to lageniform, hyaline, smooth and thin walled, simple, 4.0–12.0 × 2.0–10.0 µm, apex 2.0–5.0 µm diam. *Conidia* ellipsoid to clavate, straight to slightly curved, 4-septate, 19.5–26.5 ×5.5–7.0 µm, mean ± SD = 22.7 ± 0.8 × 6.1 ± 0.4 µm; somewhat constricted at septa; basal cell obconic with truncate base, rugose and thin-walled, 2.7–5.0 µm long; three median cells 12.0–16.0 µm long, mean ± SD = 14.7 ± 0.9 µm, doliiform, verruculose, versicoloured, septa darker than the rest of the cell second cell from base pale brown, 4.0–5.7 µm long; third cell dark brown, 3.5–5.2 µm long; fourth cell brown, 3.8–5.8 µm long; apical cell obconic, hyaline, thin and smooth-walled, 2.5–5.2 µm long; with 3 tubular apical appendages, arising from the apical crest, flexuous, unbranched, 21.0–32.0 µm long, mean ± SD = 24.8 ± 3.5 µm; basal appendage single, tubular, unbranched, centric, 2.7–6.5 µm long.

Specimens examined: China, Hainan Province: Five Fingers Group Scenic Area, 20 May 2021, Z.X. Zhang. On diseased leaves of *Ficus microcarpa*, paratype HSAUP210112, ex-paratype culture SAUCC210112; on diseased leaves of *Ficus microcarpa*, paratype HSAUP210113, ex-paratype culture SAUCC210113.

Notes: In the present study, two strains (SAUCC210112 and SAUCC210113) from symptomatic leaves of *Ficus microcarpa* were similar to *Neopestalotiopsis piceana* (CBS 394.48) (Maharachch. et al. 2014) based on phylogeny (Fig. 1). Morphologically, our strains were similar to *N. piceana*, which was originally described with an asexual morph on wood of *Picea* sp., *Cocos nucifera* and fruit of *Mangifera indica* in China, but the sexual morph of *N. piceana* was undetermined. We therefore identify our strains as *N. piceana*.

Pestalotiopsis licualacola K. Geng, Y. Song, K.D. Hyde & Yong Wang bis, Phytotaxa 88 (3):51. (2013) Fig. 5.

Description: *Colonies* on PDA incubated at 25°C in the dark with an average radial growth rate of 7.0–9.0 mm/day and occupying an entire 90 mm Petri dish in 7 d, with edge undulate, whitish, aerial mycelium on surface, fruiting bodies black, concentric; reverse of culture yellow to pale brown. Sexual morph: Undetermined. Asexual morph: Leaf spots irregular, pale brown in center, brown to tan at margin. *Conidiomata* (pycnothyria) solitary, scattered, black, raising above surface of culture medium, subglobose. *Conidiophores* cylindrical, hyaline, smooth-walled. *Conidiophores* most often indistinct. *Conidiogenous cells* discrete, hyaline, simple, filiform, 5.5–10.0 µm long. *Conidia* 18.0–24.5 × 4.0–5.5 µm, mean ± SD = 20.5 ± 1.9 × 5.3 ± 0.3 µm, fusiform, straight to slightly curved, 4-septate, smooth, greyish brown; basal cell conical, hyaline, thin-walled, 2.8–6 µm long; with three median cells, dark brown, concolorous, septa and periclinal walls darker than the rest of the cell, together 11.5–16.0 µm long, mean ± SD = 13.2 ± 1.2 µm; second cell from base 3.4–5.5 µm; third cell 3.3–4.7 µm; fourth cell 3.5–5.1 µm; apical cell hyaline, conic to subcylindrical, 3.1–5.3 µm; with 1–3 tubular apical appendages (mostly 1) without knobs, arising from the apex of the apical cell, 10.0–20.5 µm long, mean ± SD = 16.0 ± 4.0 µm; basal appendage filiform, short.

Specimens examined: China, Hainan Province: East Harbour National Nature Reserve, 23 May 2021, Z.X. Zhang. On diseased leaves of *Ilex chinensis*, paratype HSAUP210087, ex-paratype culture SAUCC210087; on diseased leaves of *Ilex chinensis*, paratype HSAUP210088, ex-paratype culture SAUCC210088.

Notes: In the present study, two strains (SAUCC210087 and SAUCC210088) from symptomatic leaves of *Ilex chinensis* were similar to *Pestalotiopsis licualacola* (HGUP4057) (Geng et al. 2013) based on phylogeny (Fig. 1). Morphologically, our strains were similar to *P. licualacola*, which was originally described with an asexual morph on leaves of *Licuala grandis* in China, but the sexual morph of *P. licualacola* was undetermined. We therefore identify our strains as *P. licualacola*.

Discussion

In the study of its phylogenetic affinity and position in the Ascomycota hierarchical system, Liu et al. (2019) placed a series of coelomycetous fungi with appendage-bearing conidia in the newly introduced family Sporocadaceae (type genus: *Sporocadus* Corda). In most genera, conidiomata pycnidial, acervular or stromatic, semi-immersed or immersed, scattered, gregarious or confluent, glabrous; conidiophores branched or reduced to conidiogenous cells, mostly hyaline, smooth; conidiogenous cells ampulliform, lageniform, cylindrical or subcylindrical, hyaline, sometimes pale brown; conidia septate, smooth, undulate or verruculose, fusoid, subcylindrical or cylindrical, straight or curved; end cells mostly hyaline, or sometimes pale brown; median cells pale brown to dark brown, or sometime almost colourless; appendages on the end cells present, or absent in some genera, if present, tubular, filiform, straight or flexuous, attenuated or not, branched or unbranched.

In the previous study, Liu et al. (2019) revealed three major clades based on the ITS/*LSU*/*RPB2* phylogeny, corresponding to the three previously proposed families “Bartaliniaceae, Discosiaceae and Pestalotiopsidaceae”. Because these family names were considered synonyms of Sporocadaceae by Jaklitsch et al. (2016), these groups are referred to as Clade 1 (Discosiaceae), Clade 2 (Pestalotiopsidaceae) and Clade 3 (Bartaliniaceae) for convenience. We picked Clade 2 of the family Sporocadaceae in this study, including *Pestalotiopsis* Steyaert, *Neopestalotiopsis* Maharachch. et al., *Pseudopestalotiopsis* Maharachch. et al., *Monochaetia* (Sacc.) Allesch., *Ciliochorella* Syd., *Seiridium* Nees and *Nonappendiculata* Liu et al. (Liu et al. 2019). Based on ITS/*TUB2*/*TEF1-α* molecular data, phylogenetic analyses revealed that the retrieved three isolates (SAUCC212201, SAUCC212202 and SAUCC212203) are include in *Monochaetia*, four isolates (SAUCC212271, SAUCC212272, SAUCC210112 and SAUCC210113) are include in *Neopestalotiopsis* and two isolates (SAUCC210087 and SAUCC210088) are include in *Pestalotiopsis*. Owing to different nucleotides in the concatenated alignment and morphology, three isolates (SAUCC212201, SAUCC212202 and SAUCC212203) of *Monochaetia* were delimited as a new species, namely *M. schimae* and two isolates (SAUCC212271 and SAUCC212272) of *Neopestalotiopsis* were delimited as a new species, namely *N. haikouensis*. Besides, two isolates (SAUCC210112 and SAUCC210113) of *Neopestalotiopsis* clustered in *N. piceana* and two isolates (SAUCC210087 and SAUCC210088) of *Pestalotiopsis* clustered in *P. licualacola*.

In addition, members of Sporocadaceae are of particular interest with regard to the production of secondary metabolites, e.g. *Pestalotiopsis*, *Bartalinia* and *Morinia* (Collado et al. 2006; Gangadevi & Muthumary 2008; Liu et al. 2009). *Pestalotiopsis fici* was shown to possess a very high number of gene clusters involved in bioactive compound synthesis (Wang et al. 2016). Because

genera in this family of fungi share the same evolutionary history, it is important to found novel species and screen for novel metabolites in future studies.

Declarations

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Author contribution Conceived and designed the experiments: JW Xia. Performed the experiments: ZX Zhang. Analyzed the data: TC Mu, SB Liu and RY Liu. Wrote the paper: ZX Zhang, XG Zhang and JW Xia. All authors read and approved the final manuscript.

Data availability The data, including the sequences on GenBank and specimen data on MycoBank will be available to any researcher wishing to use them for non-commercial purposes, without breaching participant confidentiality.

Declarations Conflict of interest The authors declare no competing interests.

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Figures

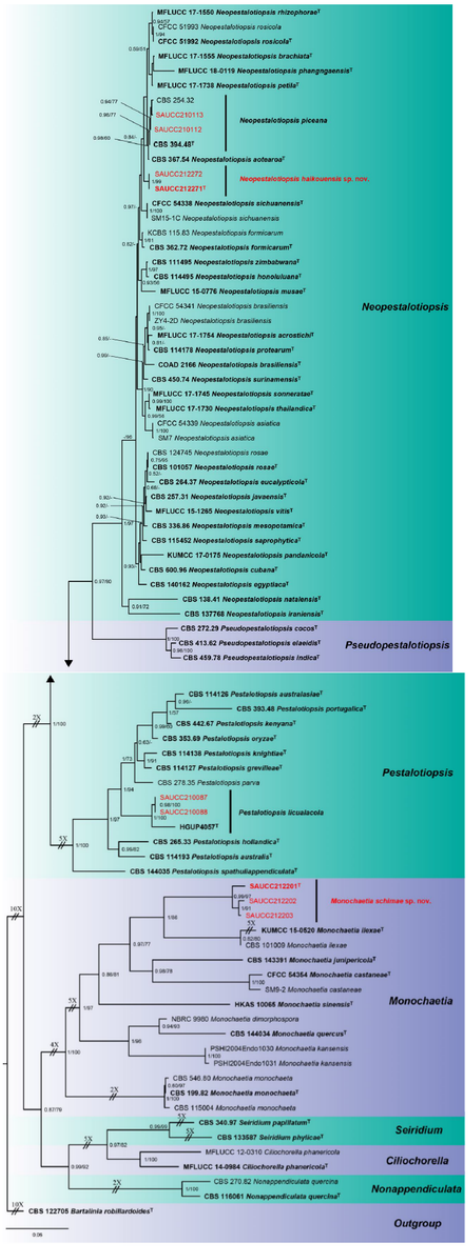

Figure 1

Phylogram of Sporocadaceae based on combined ITS, TUB2 and TEF1-α genes. The BI and ML bootstrap support values above 0.50 BYPP and 50% are shown at the first and second position, respectively. The tree is rooted to Bartalinia robillardoides (CBS 122705), ex-type or ex-epitype cultures are indicated in bold face. Strains from the current study are in red. Some branches were shortened to fit them to the page – these are indicated by two diagonal lines with the number of times a branch was shortened indicated next to the lines.

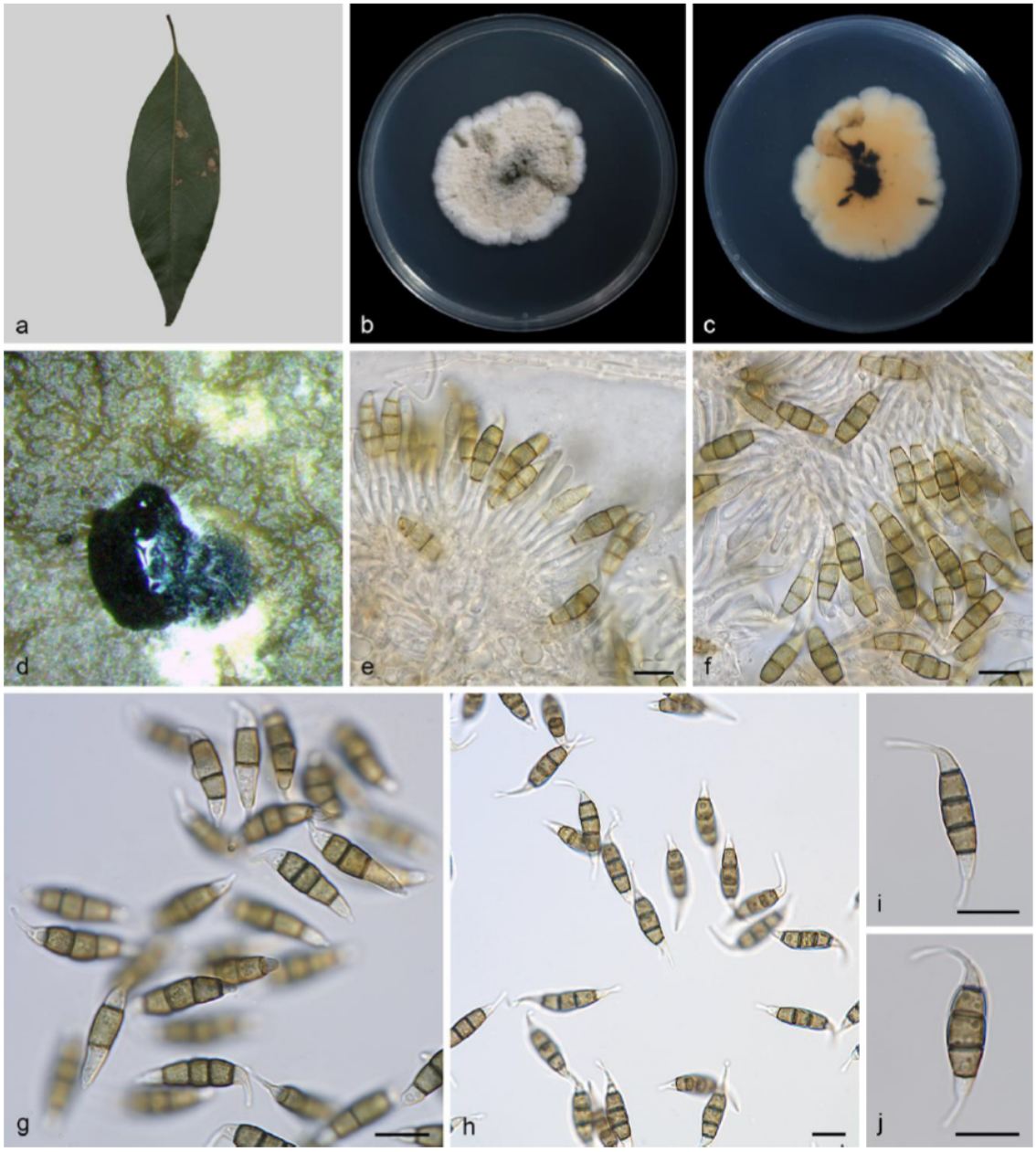

Figure 2

Monochaetia schimae (SAUCC212201). a diseased leaf of Schima superba b surface of colony after 15 days on PDA c reverse of colony after 15 days on PDA d conidiomata e, f conidiogenous cells with conidia g–j conidia. Scale bars: 10 μm (e–j).

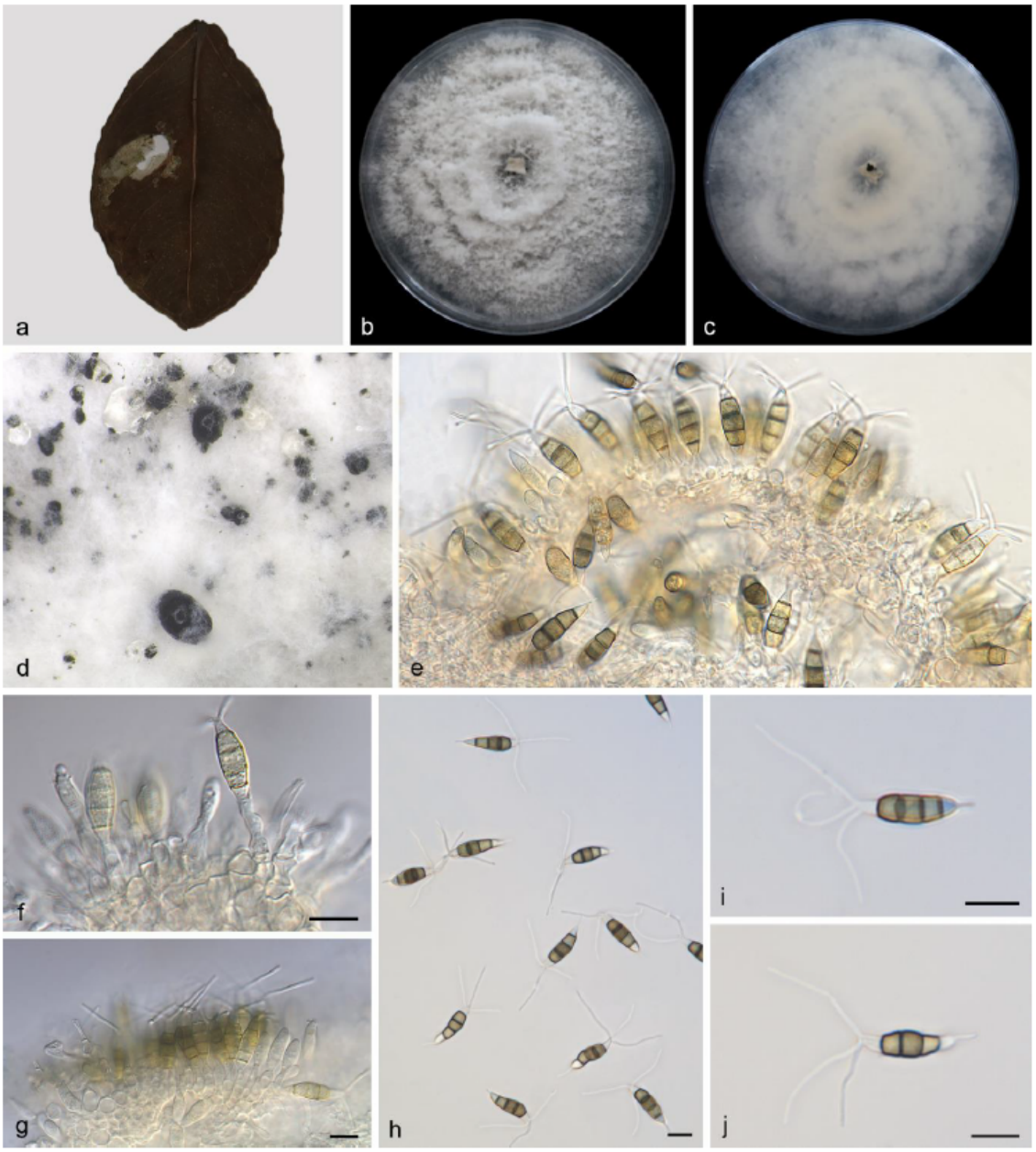

Figure 3

Neopestalotiopsis haikouensis (SAUCC212271). a diseased leaf of Ilex chinensis b surface of colony after 7 days on PDA c reverse of colony after 7 days on PDA d conidiomata e–g conidiogenous cells with conidia h–j conidia. Scale bars: 10 μm (e–j).

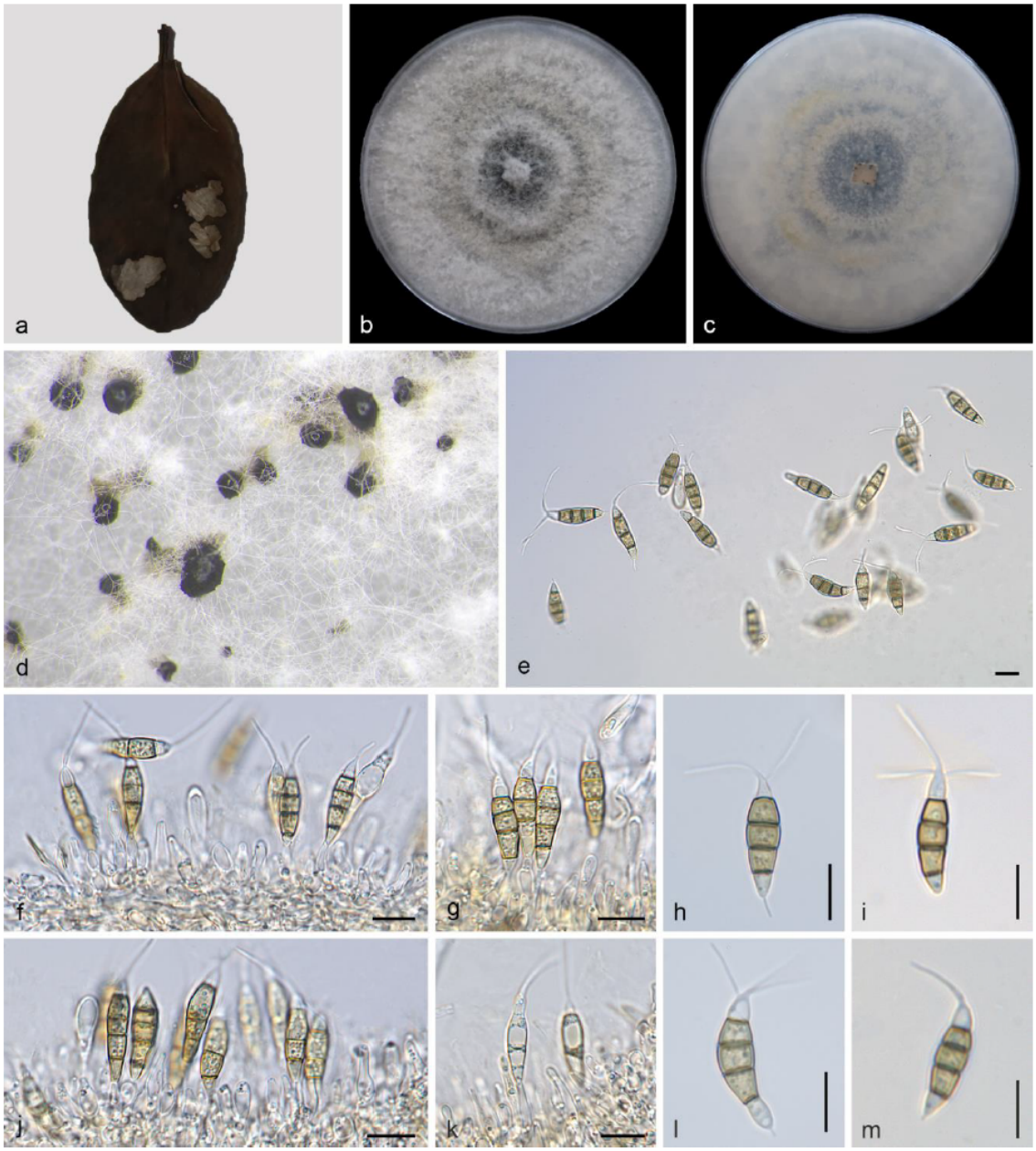

Figure 4

Neopestalotiopsis piceana (SAUCC210112). a diseased leaf of Ficus microcarpa b surface of colony after 7 days on PDA c reverse of colony after 7 days on PDA d conidiomata f, g, j, k conidiogenous cells with conidia e, h, i, l, m conidia. Scale bars: 10 μm (e–m).

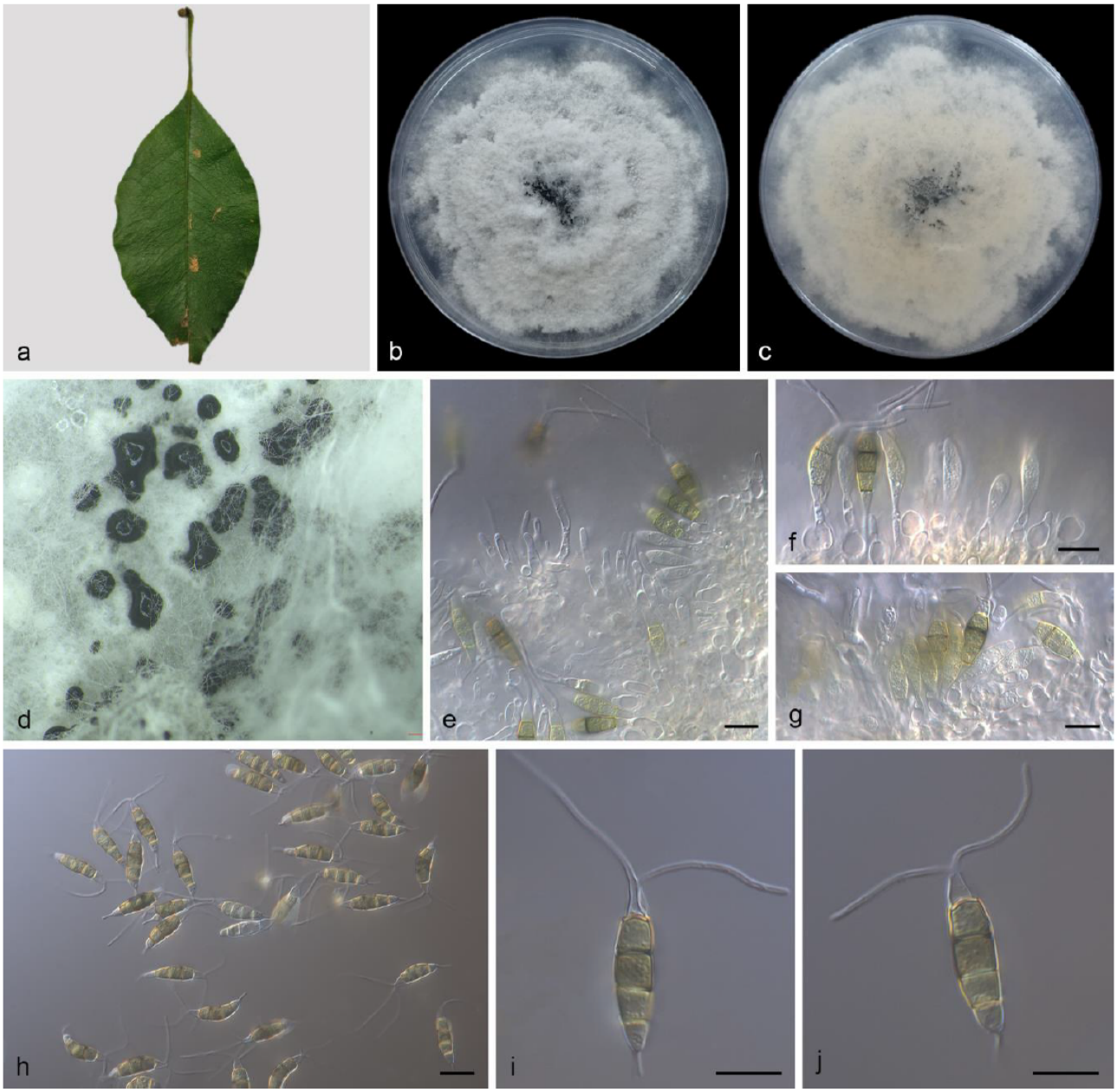

Figure 5

Pestalotiopsis licualacola (SAUCC210087). a diseased leaf of Ilex chinensis b surface of colony after 7 days on PDA c reverse of colony after 7 days on PDA d conidiomata e–g conidiogenous cells with conidia h–j conidia. Scale bars: 10 μm (e–j).