Persoonia 39, 2017: 118–142
www.ingentaconnect.com/content/nhn/pimj

RESEARCH ARTICLE

ISSN (Online) 1878-9080
https://doi.org/10.3767/persoonia.2017.39.06

Phylogenetic reassessment of *Nigrospora*: Ubiquitous endophytes, plant and human pathogens

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Key words

Apiosporaceae
Ascomycota
phylogeny
species delimitation
systematics

Abstract Species of *Nigrospora* commonly occur as plant pathogens, endophytes or saprobes, and have been shown to be extremely interesting for the discovery of novel metabolites. The familial placement, as well as phylogenetic relationships among *Nigrospora* species remain ambiguous. In this study, *Nigrospora* (= *Khusia*) is confirmed as a monophyletic genus belonging to *Apiosporaceae* (*Xylariales*), based on a phylogeny inferred from LSU sequence data. A multi-locus phylogeny based on ITS, *TEF1-α* and *TUB2*, in conjunction with morphological characters, host associations, and ecological data was employed for species delimitation in *Nigrospora*, as well as identification of 165 recently collected isolates from China, and three from Europe. In total 13 novelties are proposed including 12 new species and 1 new combination. Five species are re-described based on an examination of type specimens and/or fresh collections. New species described in this paper include: *N. aurantiaca*, *N. bambusae*, *N. camelliae-sinensis*, *N. chinensis*, *N. guilinensis*, *N. hainanensis*, *N. lacticolonia*, *N. osmanthi*, *N. pyriformis*, *N. rubi*, *N. vesicularis* and *N. zimmermanii*. Furthermore, *N. vietnamensis* is transferred to *Arthrinium.* Our results indicate a high level of species diversity within *Nigrospora*, with a general lack in host specificity. Taxa that cluster basal in *Nigrospora* have wide host ranges, whereas those that diverged later tend to have narrow host ranges. The currently available data suggest, therefore, that the general evolutionary direction in the genus *Nigrospora* is from a wide to a narrow host range.

Article info Received: 7 March 2017; Accepted: 6 April 2017; Published: 7 July 2017.

INTRODUCTION

Nigrospora is an important genus of fungal ascomycetes with a cosmopolitan distribution and wide host range. *Nigrospora* species have been isolated as endophytes from leaves and stems of various plants, or as saprobes from detritus, dead larvae or leaf litter (Mason 1927, Wu et al. 2009, Thalavaipandian et al. 2011, Uzor et al. 2015). *Nigrospora* species have also been commonly recorded as plant pathogens on many important economic crops, fruits and ornamentals. Examples include *N. oryzae* causing stem blight on *Brassica juncea* in India (Sharma et al. 2013), *N. sphaerica* causing leaf blight on *Camellia sinensis* in China (Liu et al. 2015) and *N. musae* causing 'squirter' disease on bananas (Jones & Stover 2000). In addition, *N. sphaerica* is an opportunistic pathogen causing onychomycosis in humans (De Hoog et al. 2000, Fan et al. 2009) and corneal ulcer (Kindo et al. 2014).

Nigrospora species are also commonly isolated from the indoor environment. Webster (1952) demonstrated that *N. sphaerica* has a violent spore discharge mechanism, that can forcibly project its spores to a distance of up to 2 cm vertically, and 6.7 cm horizontally. The study by Wu et al. (2004) also showed that *Nigrospora* spores are one of the more dominant groups in the atmosphere, being associated with dust storms. Moreover, some *Nigrospora* spores are responsible for a Type I allergic response, seasonal rhinitis (hay fever), asthma or respiratory allergic diseases (Santo-Pietro 2006, Khan & Karuppayil 2012, Saha & Bhattacharya 2015).

Nigrospora is regarded as extremely interesting as a source of natural products and because of its potential industrial applications (Chen et al. 2016). Metabolites produced by *N. sacchari* showed remarkable herbicidal activity in the treatment of intact greenhouse-grown plants (Fukushima et al. 1998), while Phomalactone produced by *N. spherica* was found to be an active constituent against mosquitoes (Meepagala et al. 2015). Moreover, some extrolites produced by *N. sphaerica* exhibited antibacterial activities against the growth of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Klebsiella pneumonia* cells (Ibrahim et al. 2015).

The generic name *Nigrospora* was first introduced by Zimmerman (1902) for *N. panici*, which was isolated as an endophyte from leaves of *Panicum amphibium* in Java, Indonesia. Later, Mason (1927) transferred several black-spored hyphomycetes occurring on monocotyledonous hosts to *Nigrospora*, including *N. oryzae* (= *Monotospora oryzae*), *N. sphaerica* (= *Trichosporum sphaericum*), *N. arundinacea* (= *Hadrotrichum arundinaceum*) and *N. sacchari* (= *Glenospora sacchari*). Mason (1927) further pointed out that the Indonesian fungus, *N. javanica* (Palm 1918), occurs on maize, rice and wheat, and is a synonym of *N. panici*. However, type specimens from both taxa have been lost, and thus a direct morphological comparison and molecular analysis is not possible. *Nigrospora gallarum* (= *Basisporium gallarum*) and *N. gorlenkoana* were previously regarded as synonyms of *N. oryzae* in MycoBank due to their similar conidial morphology. Presently, there are 15 recognised species listed in MycoBank, but the familial placement of the genus remains unresolved. Barnett & Hunter (1998) placed *Nigrospora* in *Dematiaceae* (*Moniliales*) based

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Table 1 Strains of the *Nigrospora* species used in this study with details about host and location, and GenBank accessions of the sequences generated.

Species	Accession numbers[1,2]	Host	Locality	GenBank accession numbers[3]			
				ITS	LSU	*TUB2*	*TEF1-α*
N. aurantiaca	CGMCC 3.18130* = LC 7302	*Nelumbo* sp. (leaf)	China	KX986064	KX986098	KY019465	KY019295
	LC 7034	*Musa paradisiaca*	China	KX986093	–	KY019598	KY019394
*N. bambusa*e	CGMCC 3.18327* = LC 7114	Bamboo (leaf)	China	KY385307	–	KY385319	KY385313
	LC 7244	Bamboo (leaf)	China	KY385306	–	KY385320	KY385314
	LC 7245	Bamboo (leaf)	China	KY385305	–	KY385321	KY385315
N. camelliae-sinensis	LC 2710	*Castanopsis* sp.	China	KX985957	–	KY019484	KY019310
	LC 3287	*Camellia sinensis*	China	KX985975	–	KY019502	KY019323
	LC 3496	*Camellia sinensis*	China	KX985985	–	KY019510	KY019327
	CGMCC 3.18125* = LC 3500	*Camellia sinensis*	China	KX985986	KX986103	KY019460	KY019293
	LC 4460	*Castanopsis* sp.	China	KX986015	–	KY019538	KY019353
	LC 6304	*Camellia sinensis*	China	KX986045	–	KY019566	KY019370
	LC 6984	*Musa paradisiaca* (leaf)	China	KX986080	–	KY019587	KY019387
	LC 6684	*Camellia sinensis*	China	KX986046	–	KY019570	KY019449
	LC 6989	*Musa paradisiaca* (leaf)	China	KX986083	–	KY019590	KY019453
	LC 6992	*Musa paradisiaca* (leaf)	China	KX986084	–	KY019591	KY019389
	LC 7018	*Musa paradisiaca* (leaf)	China	KX986089	–	KY019595	KY019392
	LC 7044	*Musa paradisiaca* (leaf)	China	KX986095	–	KY019600	KY019395
N. chinensis	LC 2696	*Lindera aggregata*	China	KX985947	–	KY019474	KY019424
	LC 3085	*Camellia sinensis*	China	KX985970	–	KY019497	KY019427
	LC 3175	*Camellia sinensis*	China	KX985972	–	KY019499	KY019428
	LC 3275	*Camellia sinensis*	China	KX985973	–	KY019500	KY019429
	LC 3286	*Camellia sinensis*	China	KX985974	–	KY019501	KY019430
	LC 3293	*Camellia sinensis*	China	KX985977	–	KY019504	KY019431
	LC 3400	*Camellia sinensis*	China	KX985979	–	KY019505	KY019432
	LC 3441	*Camellia sinensis*	China	KX985981	–	KY019507	KY019433
	LC 3493	*Camellia sinensis*	China	KX985984	–	KY019509	KY019434
	LC 4364	*Aucuba japonica*	China	KX986011	–	KY019534	KY019435
	LC 4433	*Castanopsis* sp.	China	KX986013	–	KY019536	KY019436
	LC 4463	Unknown host plant	China	KX986016	–	KY019539	KY019437
	LC 4554	Unknown host plant	China	KX986018	–	KY019541	KY019439
	LC 4555	Unknown host plant	China	KX986019	–	KY019542	KY019440
	LC 4558	Unknown host plant	China	KX986020	–	KY019543	KY019441
	LC 4565	*Itea* sp.	China	KX986021	–	KY019544	KY019442
	CGMCC 3.18127* = LC 4575	*Machilus brevifl*ora	China	KX986023	KX986107	KY019462	KY019422
	LC 4593	*Machilus duthiei*	China	KX986024	–	KY019546	KY019443
	LC 4619	*Osmanthus* sp.	China	KX986025	–	KY019547	KY019444
	LC 4660	*Quercus* sp.	China	KX986026	–	KY019548	KY019445
	LC 4673	*Smilax ocreata*	China	KX986028	–	KY019550	KY019446
	LC 6631	*Camellia sinensis*	China	KX986043	–	KY019569	KY019448
	LC 6851	Unknown host plant	China	KX986049	–	KY019579	KY019450
	LC 6972	*Musa paradisiaca*	China	KX986078	–	KY019585	KY019451
	LC 6998	*Musa paradisiaca* (leaf)	China	KX986086	–	KY019593	KY019391
	LC 7026	*Musa paradisiaca* (leaf)	China	KX986090	–	KY019596	KY019393
N. gorlenkoana	CBS 480.73*	*Vitis vinifera*	Kazakhstan	KX986048	KX986109	KY019456	KY019420
*N. guilinensi*s	LC 7301	*Nelumbo* sp. (stem)	China	KX986063	–	KY019608	KY019404
	CGMCC 3.18124* = LC 3481	*Camellia sinensis*	China	KX985983	KX986113	KY019459	KY019292
N. hainanensis	CGMCC 3.18129* = LC 7030	*Musa paradisiaca* (leaf)	China	KX986091	KX986112	KY019464	KY019415
	LC 6979	*Musa paradisiaca* (leaf)	China	KX986079	–	KY019586	KY019416
	LC 7031	*Musa paradisiaca* (leaf)	China	KX986092	–	KY019597	KY019417
	LC 7042	*Musa paradisiaca* (leaf)	China	KX986094	–	KY019599	KY019418
N . lacticolonia	CGMCC 3.18123* = LC 3324	*Camellia sinensis*	China	KX985978	KX986105	KY019458	KY019291
	LC 7009	*Musa paradisiaca* (leaf)	China	KX986087	–	KY019594	KY019454
N. musae	CBS 319.34*	*Musa paradisiaca* (fruit)	Australia	KX986076	KX986110	KY019455	KY019419
	LC 6385	*Camellia sinensis*	China	KX986042	–	KY019567	KY019371
N. oryzae	LC 6759	*Oryza sativa*	China	KX986054	–	KY019572	KY019374
	LC 6760	*Oryza sativa*	China	KX986055	–	KY019573	KY019375
	LC 6761	*Oryza sativa*	China	KX986056	–	KY019574	KY019376
	LC 6762	*Oryza sativa*	China	KX986057	–	KY019575	KY019377
	LC 6763	*Oryza sativa*	China	KX986058	–	KY019576	KY019378
	LC 6764	*Oryza sativa*	China	KX986059	–	KY019577	KY019379
	LC 6765	*Oryza sativa*	China	KX986060	–	–	KY019380
	LC 6893	*Oryza sativa*	China	KX986050	–	KY019580	KY019382
	LC 7293	*Nelumbo* sp. (leaf)	China	KX985931	–	KY019601	KY019396
	LC 7297	*Nelumbo* sp. (leaf)	China	KX985936	–	KY019605	KY019400
	LC 6029	*Nelumbo* sp. (leaf)	China	KX985938	–	KY019564	KY019368
	LC 7299	*Nelu*mbo sp. (leaf)	China	KX98606	–	KY019607	KY019402
	LC 7300	*Nelumbo* sp. (leaf)	China	KX986062	–	–	KY019403
	LC 7305	*Nelumbo* sp. (leaf)	China	KX986067	–	KY019611	KY019407
	LC 7306	*Nelumbo* sp. (leaf)	China	KX986068	–	KY019612	KY019408
	LC 7307	*Nelumbo* sp. (leaf)	China	KX986069	–	KY019613	KY019409
	LC 7308	*Nelumbo* sp. (leaf)	China	KX986070	–	KY019614	KY019410
	LC 7309	*Nelumbo* sp. (leaf)	China	KX986071	–	KY019615	KY019411
	LC 7310	*Nelumbo* sp. (leaf)	China	KX986072	–	KY019616	KY019412
	LC 7311	*Nelumbo* sp. (leaf)	China	KX986073	–	KY019617	KY019413
	LC 6766	*Oryza sativa* L.	China	KX986074	–	KY019578	KY019381
	LC 6566	*Oryza sativa* L.	China	KX986075	–	KY019568	KY019372
	LC 2689	*Rhododendron* sp.	China	KX985942	–	KY019469	KY019423

Table 1 (cont.)

Species	Accession numbers[1,2]	Host	Locality	GenBank accession numbers[3]			
				ITS	LSU	*TUB2*	*TEF1-α*
N. oryzae (cont.)	LC 2693	*Neolitsea* sp.	China	KX985944	KX986101	KY019471	KY019299
	LC 2695	*Rubus reflexus*	China	KX985946	–	KY019473	KY019301
	LC 2699	*Hamamelis mollis*	China	KX985949	–	KY019476	KY019303
	LC 2702	*Rubus* sp.	China	KX985950	–	KY019477	KY019304
	LC 2704	*Rhododendron* sp.	China	KX985951	–	KY019478	KY019425
	LC 2706	*Rhododendron* sp.	China	KX985953	–	KY019480	KY019306
	LC 2707	*Rhododendron simiarum*	China	KX985954	–	KY019481	KY019307
	LC 2708	*Rhododendron* sp.	China	KX985955	–	KY019482	KY019308
	LC 2709	*Rhododendron simiarum*	China	KX985956	–	KY019483	KY019309
	LC 2712	*Castanopsis* sp.	China	KX985958	–	KY019485	KY019311
	LC 2724	*Symplocos zizyphoid*es	China	KX985959	–	KY019486	KY019312
	LC 2744	*Symplocos zizyphoides*	China	KX985961	–	KY019488	KY019314
	LC 2749	*Ternstroemi*a sp.	China	KX985962	–	KY019489	KY019315
	LC 2752	*Osmanthus* sp.	China	KX985963	–	KY019490	KY019316
	LC 2972	*Tutcheria microcarpa*	China	KX985967	–	KY019494	KY019320
	LC 2991	*Cleyera japonica*	China	KX985969	–	KY019496	KY019321
	LC 3690	*Symplocos zizyphoides*	China	KX985987	–	KY019511	KY019328
	LC 3695	*Osmanthus fragrans*	China	KX985988	–	KY019512	KY019329
	LC 4260	*Rhododendron* sp.	China	KX985991	–	KY019515	KY019332
	LC 4265	*Rhododendron* sp.	China	KX985994	–	KY019518	KY019335
	LC 4273	*Cephalotaxus sinensis*	China	KX985995	–	KY019519	KY019336
	LC 4275	*Rhododendron* sp.	China	KX985997	–	KY019521	KY019338
	LC 4281	*Rhododendron* sp.	China	KX985999	–	KY019523	KY019340
	LC 4294	*Daphniphyllum macropodum*	China	KX986002	–	KY019526	KY019343
	LC 4295	*Daphniphyllum macropodum*	China	KX986003	–	KY019527	KY019344
	LC 4320	*Daphniphyllum oldhamii*	China	KX986006	–	KY019530	KY019347
	LC 4327	*Camellia* sp.	China	KX986007	–	KY019531	KY019348
	LC 4338	*Camellia* sp.	China	KX986008	–	KY019532	KY019349
	LC 4345	*Camellia* sp.	China	KX986009	–	KY019533	KY019350
	LC 4679	*Osmanthus* sp.	China	KX986029	–	KY019551	KY019356
	LC 4680	*Camellia sinensis*	China	KX986030	–	KY019552	KY019357
	LC 4961	*Pittosporum illicioides*	China	KX986031	–	KY019553	KY019358
	LC 5181	*Pentactina rupicola*	China	KX986032	–	KY019554	KY019359
	LC 5243	Submerged wood	China	KX986033	–	KY019555	KY019360
	LC 5964	Submerged wood	China	KX986037	–	KY019559	KY019447
	LC 5965	Submerged wood	China	KX986038	–	KY019560	KY019364
	LC 5982	Submerged wood	China	KX986040	–	KY019562	KY019366
	LC 5999	Submerged wood	China	KX986041	–	KY019563	KY019367
	LC 6923	*Oryza sativa* L.	China	KX986051	–	KY019581	KY019383
	LC 6955	*Oryza sativa* L.	China	KX986052	–	KY019582	KY019384
	LC 6957	*Oryza sativa* L.	China	KX986053	–	KY019583	KY019385
N. osmanthi	CGMCC 3.18126* = LC 4350	*Osmanthus* sp.	China	KX986010	KX986106	KY019461	KY019421
	LC 4487	*Hedera nepalensis*	China	KX986017	–	KY019540	KY019438
N. pyriformis	CGMCC 3.18122* = LC 2045	*Citrus sinensis*	China	KX985940	KX986100	KY019457	KY019290
	LC 2688	*Lindera aggregata*	China	KX985941	–	KY019468	KY019297
	LC 2690	*Rosa* sp.	China	KX985943	–	KY019470	KY019298
	LC 2694	*Rubus reflexus*	China	KX985945	–	KY019472	KY019300
	LC 3099	*Camellia sinensis*	China	KX985971	–	KY019498	KY019322
	LC 3292	*Camellia sinensis*	China	KX985976	–	KY019503	KY019324
	LC 4669	*Castanopsis* sp.	China	KX986027	–	KY019549	KY019355
	LC 6985	*Musa paradisiaca* (leaf)	China	KX986081	–	KY019588	KY019388
	LC 6988	*Musa paradisiaca* (leaf)	China	KX986082	–	KY019589	KY019452
N. rubi	CGMCC 3.18326* = LC 2698	*Rub*us sp.	China	KX985948	KX986102	KY019475	KY019302
N. sphaerica	LC 7294	*Nelumbo* sp. (leaf)	China	KX985932	–	KY019602	KY019397
	LC 7295	*Nelumbo* sp. (leaf)	China	KX985933	–	KY019603	KY019398
	LC 7296	*Nelumbo* sp. (leaf)	China	KX985934	–	KY019604	KY019399
	LC 7312	*Nelumbo* sp. (leaf)	China	KX985935	–	KY019618	KY019414
	LC 7298	*Nelumbo* sp. (leaf)	China	KX985937	KX986097	KY019606	KY019401
	LC 7303	*Nelumbo* sp. (leaf)	China	KX986065	–	KY019609	KY019405
	LC 7304	*Nelumbo* sp. (leaf)	China	KX986066	–	KY019610	KY019406
	LC 2705	*Rosa* sp.	China	KX985952	–	KY019479	KY019305
	LC 2839	*Harpullia longipetala*	China	KX985964	–	KY019491	KY019317
	LC 2840	*Harpullia longipetala*	China	KX985965	–	KY019492	KY019318
	LC 2958	*Cleyera japonica*	China	KX985966	–	KY019493	KY019319
	LC 2983	*Camellia* sp.	China	KX985968	–	KY019495	KY019426
	LC 3420	*Camellia sinensis*	China	KX985980	–	KY019506	KY019325
	LC 3477	*Camellia sinensis*	China	KX985982	–	KY019508	KY019326
	LC 4174	*Rhododendron arboreum*	China	KX985989	–	KY019513	KY019330
	LC 4241	*Deutzia* sp.	China	KX985990	–	KY019514	KY019331
	LC 4263	*Rhododendron arboreum*	China	KX985992	–	KY019516	KY019333
	LC 4264	*Rhododendron arboreum*	China	KX985993	–	KY019517	KY019334
	LC 4274	*Rhododendron arboreum*	China	KX985996	–	KY019520	KY019337
	LC 4278	*Rhododendron arboreum*	China	KX985998	–	KY019522	KY019339
	LC 4291	*Rhododendron arboreum*	China	KX986000	–	KY019524	KY019341
	LC 4293	*Rhododendron arboreum*	China	KX986001	–	KY019525	KY019342
	LC 4303	*Rhododendron arboreum*	China	KX986004	–	KY019528	KY019345
	LC 4307	*Rhododendron arboreum*	China	KX986005	–	KY019529	KY019346

Table 1 (cont.)

Species	Accession numbers[1,2]	Host	Locality	GenBank accession numbers[3]			
				ITS	LSU	*TUB2*	*TEF1-α*
N. sphaerica (cont.)	LC 4372	*Rhododendron arboreum*	China	KX986012	–	KY019535	KY019351
	LC 4447	Unknown host plant	China	KX986014	–	KY019537	KY019352
	LC 5901	Submerged wood	China	KX986034	–	KY019556	KY019361
	LC 5932	Submerged wood	China	KX986035	–	KY019557	KY019362
	LC 5944	Submerged wood	China	KX986036	–	KY019558	KY019363
	LC 5966	Submerged wood	China	KX986039	–	KY019561	KY019365
	LC 6294	*Camellia sinensis*	China	KX986044	–	KY019565	KY019369
	LC 6969	*Musa paradisiaca* (leaf)	China	KX986077	–	KY019584	KY019386
	LC 6996	*Musa paradisiaca* (leaf)	China	KX986085	–	KY019592	KY019390
Nigrospora sp. 1	LC 2725	*Symplocos zizyphoides*	China	KX985960	KX986104	KY019487	KY019313
	LC 4566	*Lithocarpus* sp.	China	KX986022	–	KY019545	KY019354
Nigrospora sp. 2	LC 6704	*Camellia sinensis*	China	KX986047	KX986108	KY019571	KY019373
N. vesicularis	LC 0322	Unknown host plant	Thailand	KX985939	–	KY019467	KY019296
	CGMCC 3.18128* = LC 7010	*Musa paradisiaca* (leaf)	China	KX986088	KX986099	KY019463	KY019294
N. zimmermanii	CBS 167.26	Unknown	Unknown	KY385308	–	KY385318	KY385312
	CBS 290.62*	*Saccharum officinarum* (leaf)	Ecuador	KY385309	–	KY385317	KY385311
	CBS 984.69	*Saccharum officinarum* (leaf)	Brazil	KY385310	–	KY385322	KY385316
A. vietnamensis	IMI 99670*	*Citrus sinensis*	Vietnam	KX986096	KX986111	KY019466	–

[1] CGMCC = China General Microbiological Culture Collection, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; CBS = Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; IMI = Culture Collection of CABI Europe UK Centre, Egham, UK; LC = working collection of Lei Cai, housed at the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.

[2] * = ex-type culture.

[3] ITS = internal transcribed spacers and intervening 5.8S nrDNA; LSU = 28S nrRNA gene; *TUB2* = Beta-tubulin; *TEF1-α*: translation elongation factor 1-alpha.

on its conidial characters, while Kirk et al. (2008) assigned *Nigrospora* and its *Khuskia* sexual morph to the *Trichosphaeriaceae* (*Trichosphaeriales*).

The objectives of the present study were therefore to:

1. resolve the higher order phylogenetic placement of *Nigrospora*;
2. infer the phylogenetic and evolutionary relationships of *Nigrospora* species based on multi-locus DNA sequence data (ITS, *TEF1-α*, *TUB2*) analyses; and
3. identify 165 *Nigrospora* strains collected in China and three strains from Europe to species level.

MATERIALS AND METHODS

Collection, isolation and herbarium specimens

Diseased and healthy plant tissues were collected from *Camellia sinensis*, *Musa paradisiaca* and several other unidentified plant hosts in eight Chinese provinces (Fujian, Guangxi, Guizhou, Hainan, Hubei, Jiangxi, Tibet and Yunnan). Isolates associated with leaf spots were cultured using both single spore and tissue isolation methods. The single spore isolation protocol of Zhang et al. (2013) was adopted by using quarter strength potato dextrose agar (1/4 PDA; 9.75g Difco PDA, 15g Difco agar and 1L distilled water) with antibiotics (Sodium ampicillin and Streptomycin sulfate). Fungal endophytes were isolated by cutting four fragments (2 × 2 mm) per leaf from the apex, base and lateral sides; samples were surface sterilised with 75 % ethanol for 1 min, 5 % NaClO for 30 s; and then rinsed in sterile distilled water for 1 min. Leaf pieces were dried between sterilised paper towels and then plated onto 1/4 PDA.

All cultures are preserved in the LC culture collection (personal culture collection of Lei Cai housed in the Institute of Microbiology, Chinese Academy of Sciences). Type specimens were deposited in the Mycological Herbarium of Microbiology Institute, Chinese Academy of Sciences, Beijing, China (HMAS), with ex-type living cultures deposited in the China General Microbiological Culture Collection Center (CGMCC) and the Agricultural Culture Collection of China (ACCC). New descriptions and nomenclature were deposited in MycoBank (www.MycoBank.org; Crous et al. 2004).

Loan requests of type specimens were sent to 22 herbaria, viz. B, BIOT, BO, BZ, FIPIA, IARI, IPA, K, KRB, LE, LIL, LP, MEL, MELU, PAD, PAS, PDA, PEUFR, RO, UFP, URM, VLA. Four types of *Nigrospora* species were received from K, i.e. *N. oryzae* (= *Monotospora oryzae*, IMI 99832), *N. sphaerica* (= *Trichosporum sphaericum*, IMI 103253), *N. arundinacea* (= *Hadrotrichum arundinaceum*, K(M) 203264) and *Khuskia oryzae* (sexual morph of *N. oryzae*, IMI 79239).

Morphology

Cultures were incubated on PDA for 7 d at 25 °C to measure diagonal growth. To enhance sporulation, 5 mm diam plugs from the margin of actively growing cultures were transferred to the centre of 9 cm diam Petri dishes containing synthetic nutrient-poor agar medium (SNA; Nirenberg 1976) at 28 °C. Morphological descriptions were based on cultures sporulating on SNA. The shape and size of microscopic structures were observed using a light microscope and colonies were assessed according to the colour charts of Rayner (1970). At least 50 conidiogenous cells and conidia were measured to calculate the mean size.

DNA extraction, PCR amplification and sequencing

Fresh fungal mycelia grown on PDA for 7 d at 25 °C were scraped from the colony margin and used for genomic DNA extraction using a modified CTAB protocol as described in Guo et al. (2000). PCR amplification and sequencing of the large subunit (LSU) rDNA using the primer pair LR0R/LR5 and the 5.8S nuclear ribosomal gene with the two flanking transcribed spacers (ITS) using primer pair ITS1/ITS4 was performed (Vilgalys & Hester 1990, White et al. 1990). Part of the translation elongation factor 1-alpha (*TEF1-α*) was amplified and sequenced using primer pair EF1-728F (Carbone & Kohn 1999) and EF-2 (O'Donnell et al. 1998). Bt-2a and Bt-2b (Glass & Donaldson 1995) were used for the Beta-tubulin fragment (*TUB2*). PCR was performed in a 25 µL reaction containing 18.95 µL double distilled water, 2.5 µL 10 × PCR buffer, 0.3 µL dNTP mix (2.5 mM), 1 µL per primer (10 mM), 1 µL DNA template, 0.25 µL Taq DNA polymerase (Genstar). Amplification conditions for ITS, LSU and *TEF1-α* followed Crous et al. (2013) and for *TUB2*, Lee et al. (2004). Purification and sequencing of PCR amplicons were carried out at the SinoGenoMax Company,

Beijing. DNA sequences were generated with upper surface and reverse primers to obtain consensus sequences analysed with MEGA v. 6.0 (Tamura et al. 2013).

Phylogenetic analysis

LSU sequences of *Nigrospora* and similar sequences from related genera obtained from GenBank (Table 1, 2) were analysed to resolve the higher order phylogenetic placement of *Nigrospora*. Single locus and concatenated gene trees were inferred from ITS, *TUB2* and *TEF1-α* (Table 1) using Bayesian and Maximum-likelihood analyses to help delimit species in *Nigrospora*. Sequences were aligned using an online version of MAFFT v. 7 (Katoh & Standley 2013). Ambiguous regions were excluded from the analyses and gaps were treated as missing data. A 70 % neighbour-joining (NJ) reciprocal bootstrap method with maximum-likelihood distance was applied to check the congruence of the individual loci in the multi-locus dataset (Mason-Gamer & Kellogg 1996).

The best nucleotide substitution model of each locus used for MrBayes v. 3.2.1 (Ronquist et al. 2012), was calculated with jModelTest v. 2.1.4 (Posada 2008). Posterior probabilities (PP) (Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) under the estimated model of evolution. Four simultaneous Markov chains were run for 10 M generations and trees were sampled every 1 000 generations. The run was stopped automatically when the average standard deviation of split frequencies fell below 0.01. The first 25 % of trees, which represented the burn-in phase of the analyses, were discarded and the remaining trees were used for calculating PP in the majority rule consensus tree. Maximum-likelihood analyses including 1 000 bootstrap replicates were conducted using RAxML v. 7.2.6 (Stamatakis & Alachiotis

Table 2 GenBank accession numbers of the sequences used for the LSU analyses of *Xylariales* and *Amphisphaeriales*.

Taxon name	Culture accession no.	GenBank accessions LSU	Taxon name	Culture accession no.	GenBank accessions LSU
Adisciso tricellulare	NBRC 32705	NG 042334	*Discostroma tostum*	NBRC 32626	AB 593727
Adisciso yakushimense	MAFF 242774	AB 593721	*Dyrithiopsis lakefuxianensis*	HKUCC 7303	AF 452047
Amphibambusa bambusicola	MFLUCC 11-0617	KP 744474	*Eutypa flavovirens*	MFLUCC 13-0625	KR 092774
Amphisphaeria sorbi	MFLUCC 13-0721	KP 744475	*Hyalotiella rubi*	MFLUCC 13-0660	KR 092775
Amphisphaeria umbrina	HKUCC 994	AF 452029	*Hyalotiella spartii*	MFLUCC 13-0397	KP 757752
Apiosordaria verruculosa	F152365	AY346258	*Hyponectria buxi*	UME 31430	AY 083834
Apiospora setosa	ATCC 58184	AY 346259	*Kretzschmaria deusta*	CBS 163.93	KT 281896
Apiospora tintinnabula	ICMP 6889-96	DQ 810217	*Lepteutypa cupressi*	IMI 052255	AF 382379
Arecophila bambusae	HKUCC 4794	AF 452038	*Lopadostoma americanum*	HV-2014h LG8	KC 774568
Arthrinium arundinis	CBS 106.12	KF 144927	*Lopadostoma dryophilum*	LG21	KC 774570
	CBS 114316	KF 144928	*Lopadostoma fagi*	HV-2014f LF1	KC 774575
Arthrinium aureum	CBS 244.83	KF 144935	*Lopadostoma quercicola*	HV-2014a LG27	KC 774610
Arthrinium hydei	CBS 114990	KF 144936	*Lopadostoma turgidum*	LT2	KC 774618
Arthrinium kogelbergense	CBS 113333	KF 144938	*Monochaetia kansensis*	PSHI2004Endo1032	DQ 534037
Arthrinium malaysianum	CBS 102053	KF 144942		PSHI2004Endo1030	DQ 534035
	CBS251.29	KF 144943	*Neopestalotiopsis aotearoa*	CBS 367.54	KM 116247
Arthrinium marii	CBS 497.90	KF 144947	*Neopestalotiopsis formicarum*	CBS 362.72	KM 116248
Arthrinium ovatum	CBS 115042	KF 144950	*Ophiodiaporthe cyatheae*	YMJ 1364	JX 570891
Arthrinium phaeospermum	CBS 114314	KF 144951	*Pestalotiopsis knightiae*	CBS 114138	KM 116227
	CBS 114318	KF 144954	*Pestalotiopsis malayana*	CBS 102220	KM 116238
Arthrinium phragmites	CPC 18900	KF 144956	*Phlogicylindrium eucalyptorum*	CBS 111689	KF 251708
Arthrinium pseudosinense	CPC 21546	KF 144957	*Phlogicylindrium uniforme*	CBS 131312	JQ 044445
Arthrinium pseudospegazzinii	CBS 102052	KF 144958	*Podosordaria tulasnei*	CBS 128.80	KT 281897
Arthrinium pterospermum	CPC 20193	KF 144960	*Poronia punctata*	CBS 656.78	KT 281900
Arthrinium rasikravindrii	CBS 337.61	KF 144961	*Pseudomassaria chondrospora*	MFLUCC 15-0545	KR 092779
Arthrinium sacchari	CBS 212.30	KF 144962		PC1	JF 44098
	CBS 372.67	KF 144964	*Pseudomassaria sepincoliformis*	CBS 129022	JF 440984
Arthrinium saccharicola	CBS 191.73	KF 144966	*Pseudopestalotiopsis cocos*	CBS 272.29	KM 116276
	CBS 463.83	KF 144968	*Pseudopestalotiopsis theae*	MFLUCC 12-0055	KM 116282
Arthrinium xenocordella	CBS 478.86	KF 144970	*Sarcostroma restionis*	CBS 118154	DQ 278924
	CBS595.66	KF 144971	*Sarcoxylon compunctum*	CBS 359.61	KT 281898
Atrotorquata spartii	MFLUCC 13-0444	KP 325443	*Seimatosporium cornii*	MFLUCC 14-0467	KR 559739
Bartalinia robillardoides	CBS 122705	KJ 710438	*Seimatosporium eucalypti*	CPC 156	JN 871209
	MFLUCC 12-0070	KR 559738	*Seimatosporium ficeae*	SGL002	KR 920686
Broomella vitalbae	MFLUCC 13-0798	KP 757749	*Seimatosporium hypericinum*	NBRC 32647	AB 593737
	MFLUCC 14-1000	KP 757750	*Seimatosporium rhombisporum*	MFLUCC 15-0543	KR 092780
Cainia anthoxanthis	MFLUCC 15-0539	KR 092777	*Seimatosporium rosae*	MFLUCC 14-0621	KT 198727
Cainia graminis	MFLUCC 15-0540	KR 092781	*Seiridium cardinale*	CBS 172.56	AF 382376
	CBS 136.62	AF 431949	*Seiridium papillatum*	CBS 340.97	DQ 414531
Ciferriascosea fluctamurum	MFLUCC 15-0541	KR 092778	*Seiridium phylicae*	CPC 19965	KC 005809
Ciferriascosea rectamurum	MFLUCC 15-0542	KR 092776	*Seynesia erumpens*	SMH 1291	AF 279410
Ciliochorella castaneae	HHUF 28799	AB 433277	*Sordaria fimicola*	HKUCC 3714	AF 132330
Clypeosphaeria uniseptata	–	AY 083830	*Truncatella angustata*	ICMP 7062	AF 382383
	HKUCC 6349	DQ 810219	*Truncatella hartigii*	CBS 118148	DQ 278928
Coniocessia maxima	Co117	GU 553344	*Truncatella laurocerasi*	ICMP 11214	AF 382385
Coniocessia nodulisporioides	CBS281.77	AJ 875224	*Truncatella restionacearum*	CMW 18755	DQ 278929
Creosphaeria sassafras	CM AT-018	DQ 840056	*Truncatella spartii*	MFLUCC 13-0397	KR 092782
Cryptodiaporthe aesculi	AFTOL-ID 1238	DQ 836905		MFLUCC 15-0573	KR 092783
Diatrype disciformis	MFLUCC 15–0538	KR 092784	*Vialaea mangifia*	MFLUCC 12-0808	KF 724975
Diatrype palmicola	MFLUCC 11-0018	KP 744481	*Vialaea minutella*	BRIP 56959	KC 181924
Discosia artocreas	NBRC 8975	AB 593705	*Xylaria polymorpha*	MUCL 49884	KT 281899
Discosia neofraxinea	MFLU 15-0375	KR 072672	*Xylaria obovata*	MFLUCC 13-0115	KR 049089
Discosia pini	MAFF 410149	AB 593708	*Zetiasplozna acaciae*	CPC 23421	KJ 869206
Discostroma fuscellum	MFLUCC 14-0052	KT 005514			

2010). A general time reversible model (GTR) was applied with a gamma-distributed rate variation. Novel sequences generated in this study were deposited in GenBank (Table 1), the final matrices used for phylogenetic analyses in TreeBASE (www.treebase.org; accession number S20829).

Fungus host distribution analysis

To better illustrate the distribution of *Nigrospora* species on different hosts, a heatmap was plotted using the 'pheatmap' package in R (R Development Core Team 2015), on the basis of data from this study and the USDA fungal database (Farr & Rossman 2017).

RESULTS

Phylogeny

The manually adjusted LSU alignment dataset contained 123 sequences from 110 taxa, in which 897 characters including alignment gaps (available in TreeBASE) were used in the phylogenetic analysis. According to the results of jModeltest v. 2.1.4, the GTR+I+G model was chosen for MrBayes. The phylogeny resulting from the analysis of LSU sequence data is shown in Fig. 1. All strains of *Nigrospora* formed a sister clade, with high statistical support to *Arthrinium*, indicating that *Nigrospora* belongs to *Apiosporaceae*, *Xylariales*. The two genera, however, are clearly phylogenetically distinct (Fig 1). The ex-type strain of *Nigrospora vietnamensis* (IMI 99670) is nested within *Arthrinium* and appeared conspecific to *A. malaysianum*.

The 70 % neighbour-joining (NJ) reciprocal bootstrap method with maximum-likelihood distance confirmed that single gene trees of *Nigrospora* inferred from ITS, *TUB2* and *TEF1-α* datasets were congruent (results not shown). The concatenated dataset of ITS, *TUB2* and *TEF1-α* contained 62 strains representing each clade of *Nigrospora* with reference to single locus trees, and *Arthrinium malaysianum* CBS 102053 as outgroup. A total of 1 581 characters including gaps (available in TreeBASE) were included in this dataset. For the Bayesian analyses, the best-fit models TIM1ef+I+G, TPM2uf+G, TrN+I+G were set for ITS, *TUB2* and *TEF1-α*, respectively. The concatenated gene tree (Fig. 2) is congruent with the single-locus gene trees (ITS, *TUB2* and *TEF1-α*) and comprises 18 species representing clades with high bootstrap and posterior probability supports values.

Fungus host distribution

Nigrospora species appear to be widely distributed on various hosts. Among which, *N. sphaerica*, *N. oryzae* and *N. chinensis* are the three most ubiquitous species. For example, *N. sphaerica*

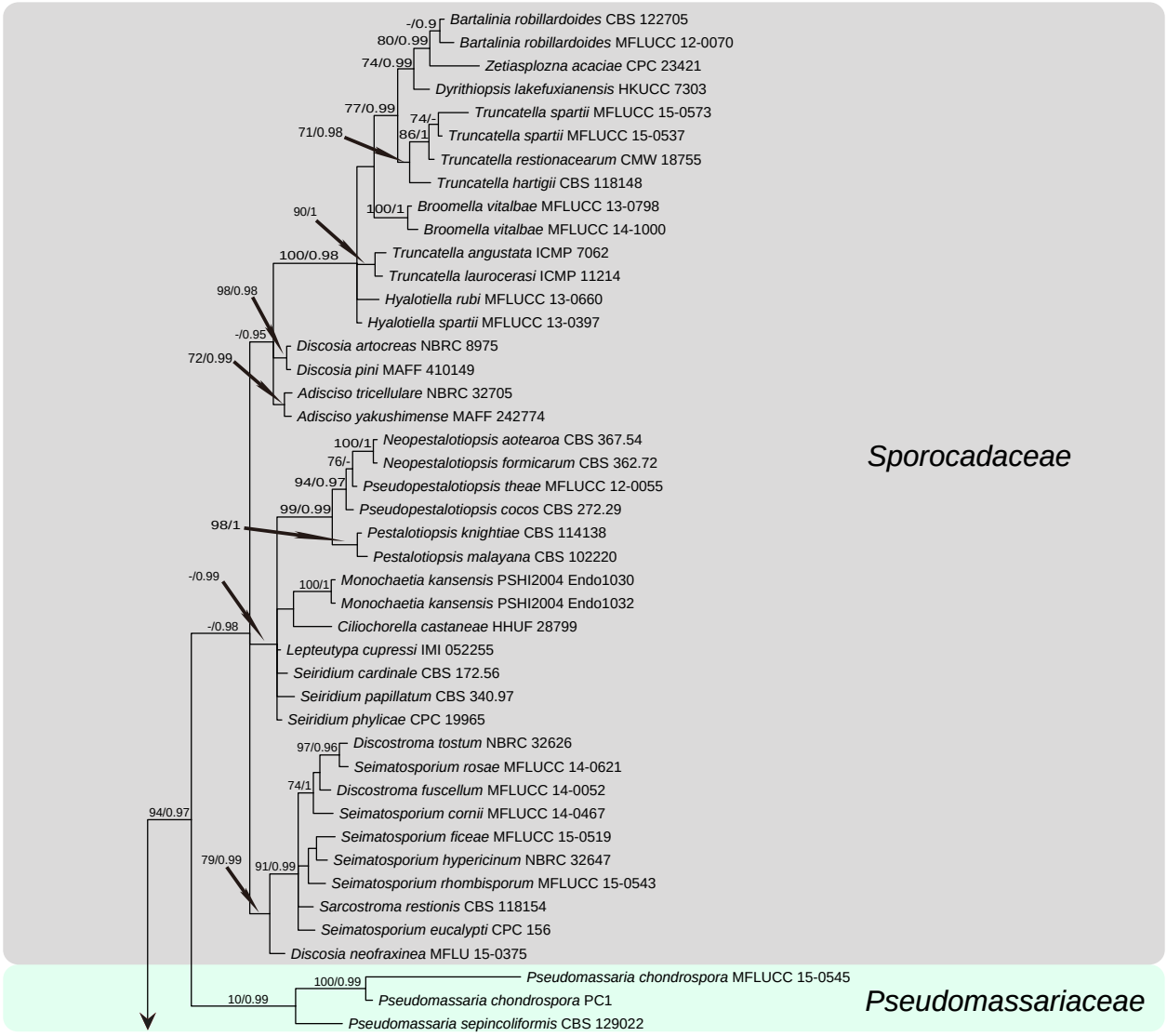
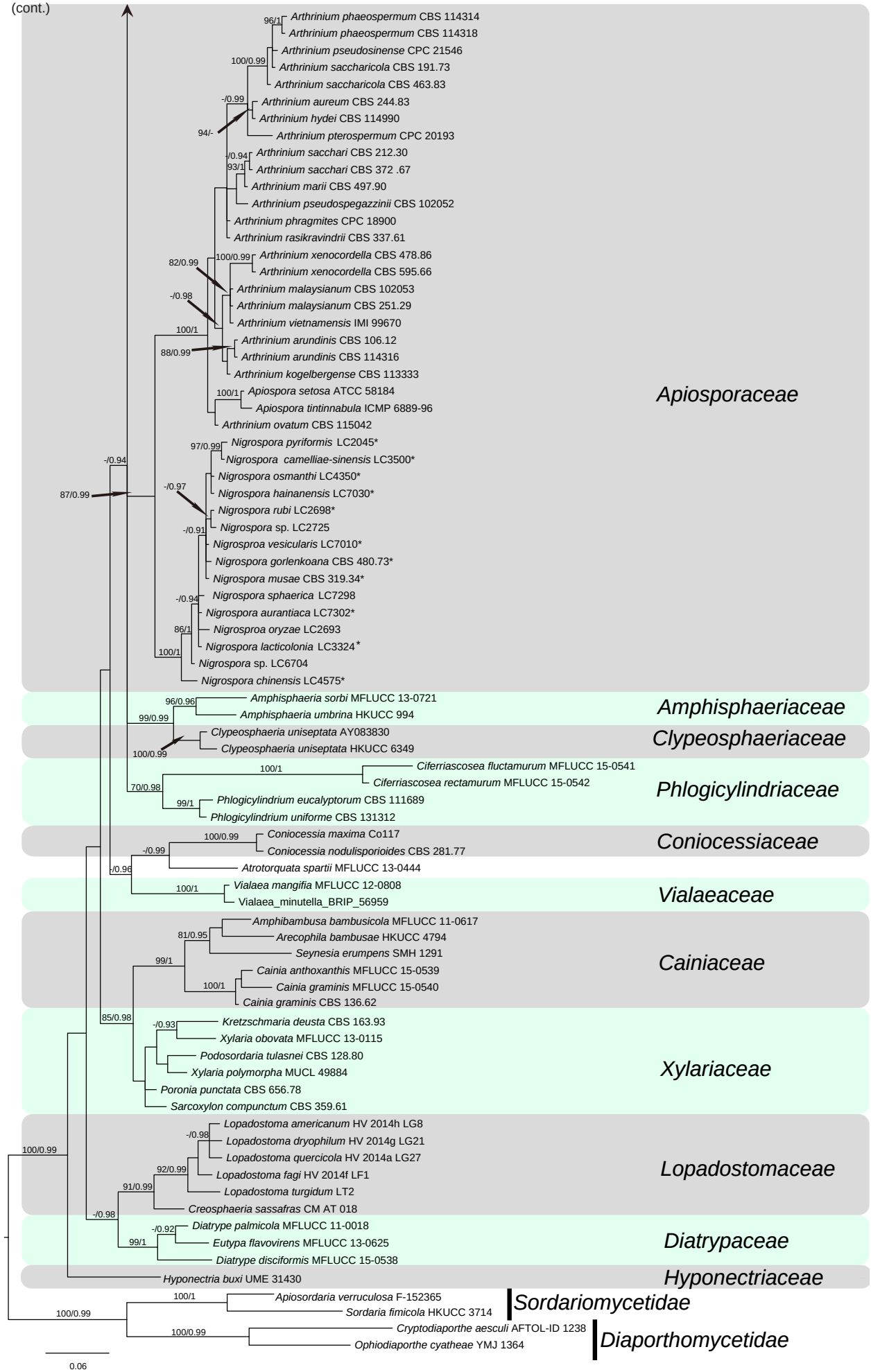


Fig. 1 Phylogenetic tree based on the LSU sequences generated from a Maximum likelihood phylogenetic analysis. Bootstrap support values (> 70 %) and posterior probabilities (> 0.9) are given at the nodes. The tree is rooted to *Sordariomycetidae* (*Apiosordaria verruculosa* F-152365 and *Sordaria fimicola* HKUCC 3714) and *Diaportheomycetidae* (*Cryptodiaporthe aesculi* AFTOL-ID 1238 and *Ophiodiaporthe cyatheae* YMJ 1364).

Fig. 1 (cont.)


Arthrinium phaeospermum CBS 114314
Arthrinium phaeospermum CBS 114318
Arthrinium pseudosinense CPC 21546
Arthrinium saccharicola CBS 191.73
Arthrinium saccharicola CBS 463.83
Arthrinium aureum CBS 244.83
Arthrinium hydei CBS 114990
Arthrinium pterospermum CPC 20193
Arthrinium sacchari CBS 212.30
Arthrinium sacchari CBS 372 .67
Arthrinium marii CBS 497.90
Arthrinium pseudospegazzinii CBS 102052
Arthrinium phragmites CPC 18900
Arthrinium rasikravindrii CBS 337.61
Arthrinium xenocordella CBS 478.86
Arthrinium xenocordella CBS 595.66
Arthrinium malaysianum CBS 102053
Arthrinium malaysianum CBS 251.29
Arthrinium vietnamensis IMI 99670
Arthrinium arundinis CBS 106.12
Arthrinium arundinis CBS 114316
Arthrinium kogelbergense CBS 113333
Apiospora setosa ATCC 58184
Apiospora tintinnabula ICMP 6889-96
Arthrinium ovatum CBS 115042
Nigrospora pyriformis LC2045*
Nigrospora camelliae-sinensis LC3500*
Nigrospora osmanthi LC4350*
Nigrospora hainanensis LC7030*
Nigrospora rubi LC2698*
Nigrospora sp. LC2725
Nigrosproa vesicularis LC7010*
Nigrospora gorlenkoana CBS 480.73*
Nigrospora musae CBS 319.34*
Nigrospora sphaerica LC7298
Nigrospora aurantiaca LC7302*
Nigrosproa oryzae LC2693
Nigrospora lacticolonia LC3324*
Nigrospora sp. LC6704
Nigrospora chinensis LC4575*
Apiosporaceae
Amphisphaeria sorbi MFLUCC 13-0721
Amphisphaeria umbrina HKUCC 994
Amphisphaeriaceae
Clypeosphaeria uniseptata AY083830
Clypeosphaeria uniseptata HKUCC 6349
Clypeosphaeriaceae
Ciferriascosea fluctamurum MFLUCC 15-0541
Ciferriascosea rectamurum MFLUCC 15-0542
Phlogicylindrium eucalyptorum CBS 111689
Phlogicylindrium uniforme CBS 131312
Phlogicylindriaceae
Coniocessia maxima Co117
Coniocessia nodulisporioides CBS 281.77
Atrotorquata spartii MFLUCC 13-0444
Coniocessiaceae
Vialaea mangifia MFLUCC 12-0808
Vialaea_minutella_BRIP_56959
Vialaeaceae
Amphibambusa bambusicola MFLUCC 11-0617
Arecophila bambusae HKUCC 4794
Seynesia erumpens SMH 1291
Cainia anthoxanthis MFLUCC 15-0539
Cainia graminis MFLUCC 15-0540
Cainia graminis CBS 136.62
Cainiaceae
Kretzschmaria deusta CBS 163.93
Xylaria obovata MFLUCC 13-0115
Podosordaria tulasnei CBS 128.80
Xylaria polymorpha MUCL 49884
Poronia punctata CBS 656.78
Sarcoxylon compunctum CBS 359.61
Xylariaceae
Lopadostoma americanum HV 2014h LG8
Lopadostoma dryophilum HV 2014g LG21
Lopadostoma quercicola HV 2014a LG27
Lopadostoma fagi HV 2014f LF1
Lopadostoma turgidum LT2
Creosphaeria sassafras CM AT 018
Lopadostomaceae
Diatrype palmicola MFLUCC 11-0018
Eutypa flavovirens MFLUCC 13-0625
Diatrype disciformis MFLUCC 15-0538
Diatrypaceae
Hyponectria buxi UME 31430
Hyponectriaceae
Apiosordaria verruculosa F-152365
Sordaria fimicola HKUCC 3714
Sordariomycetidae
Cryptodiaporthe aesculi AFTOL-ID 1238
Ophiodiaporthe cyatheae YMJ 1364
Diaporthomycetidae
0.06

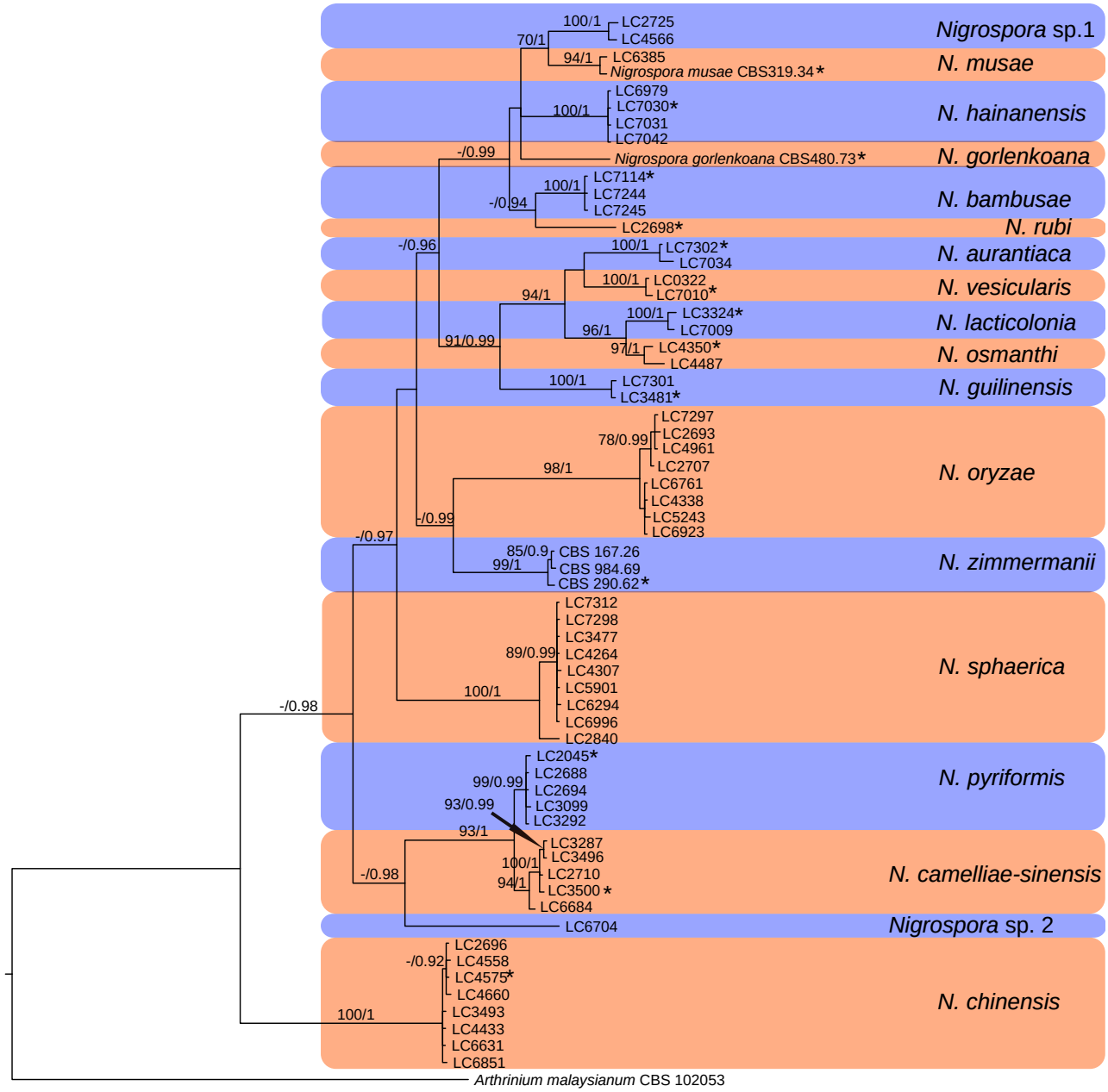


Fig. 2 Multilocus phylogenetic tree based on the combined ITS, *TEF1-α* and *TUB2* sequences alignment generated from a Maximum likelihood phylogenetic analysis. Bootstrap support values (> 70 %) and posterior probabilities (> 0.9) are given at the nodes (MLB/PP). The tree is rooted with *Arthrinium malaysianum*. The novel species are highlighted (* indicates the ex-type cultures).

has been reported from 40 different host genera including *Zea*, *Andropogon* and *Cymbopogon*, while *N. oryzae* has been reported from 20 different genera including *Oryza*, *Zea* and *Phyllostachys*. Host genera such as *Musa* and *Camellia* appear to be amongst the most preferred hosts for *Nigrospora*, having 10 and 8 *Nigrospora* species reported on each respective host genus. Eight species, i.e., *N. arundinacea*, *N. canescens*, *N. gorlenkoana*, *N. gossypii*, *N. javanica*, *N. maydis*, *N. padwickii*, and *N. panici*, are hitherto only known from one host genus each.

TAXONOMY

Nigrospora Zimm., Centralbl. Bakteriol. Parasitenk., 1. Abth. 8: 220. 1902

Type species. *Nigrospora panici* Zimm., Centralbl. Bakteriol. Parasitenk., 1. Abth. 8: 220. 1902.

Synonym. *Khuskia* H.J. Huds., Trans. Brit. Mycol. Soc. 46: 358. 1963.

Type species. *Khuskia oryzae* H.J. Huds., Trans. Brit. Mycol. Soc. 46: 358. 1963.

Classification — *Apiosporaceae*, *Xylariales*, *Sordariomycetes.*

Colonies on PDA at first white with small, shiny black conidia easily visible under a low-power dissecting microscope due to its large size, later becoming brown or black when sporulation is abundant. *Mycelia* immersed or partly superficial. *Stroma* absent. *Hyphopodia* absent. *Setae* rarely observed. *Conidiophores* micronematous or semi-macronematous, branched, flexuous, hyaline to brown, smooth, usually reduced to conidiogenous cells. *Conidiogenous cells* monoblastic, discrete, solitary, determinate, subspherical, doliiform, ampulliform, subcylindrical to clavate, hyaline. *Conidia* solitary, acrogenous, with an equatorial hyaline line or a germ slit in some species, simple, spherical or broadly ellipsoidal or pyriform, compressed dorsiventrally, black, shiny, smooth, aseptate, rarely with a violent discharge mechanism. *Ascomata* perithecial, formed in clusters

of 1–7, uniseriate or in irregular rows, subepidermal, erumpent, globose obovoid, with papillate ostioles; surrounded by blackened host tissue. *Asci* short-stalked, unitunicate, clavate, with eight biseriate ascospores. *Paraphyses* thin-walled, septate. *Ascospores* hyaline, granular, curved, inequilateral, tapering towards base with rounded ends, initially aseptate, at times with a single transverse septum.

Notes — The conidiophores of most species of *Nigrospora* are reduced to conidiogenous cells, each of which normally produces a single conidium. The conidia of *Nigrospora* are deeply pigmented, with germ slits present in some species. Mason (1927, 1933) revised the taxonomy of *Nigrospora*, and pointed out that numerous species apparently differ only in spore size, and so far traditional classification has been mainly based on conidial dimensions. In this study, morphological characters were re-evaluated and combined DNA sequence data were analysed to investigate the phylogenetic relationships of *Nigrospora* species. Furthermore, additional distinguishable characters were employed for distinguishing species, such as conidiogenous cell dimensions, and the presence/absence of vesicles and setae. Sterile cells are often observed in *Nigrospora* species (Mason 1927, Minter 1985). They are similar to conidia in being deeply pigmented, but are much larger than conidia in dimensions. In addition, sterile cells are formed directly from the hyphae, rather than borne from the conidiogenous cells. Setae are also deeply pigmented and borne from the hyphae, but differ from sterile cells in being longer and narrower, and 1–2-septate.

Nigrospora arundinacea (Cooke & Massee) Potl., Microbiologia, Moscow 21: 224. 1952 — Fig. 3

Type. England, from *Arundo conspicua*, 1887, *Cooke & Massee* (holotype K(M) 203264).

Basionym. *Hadrotrichum arundinaceum* Cooke & Massee, Grevillea 16, no.77: 11. 1887.

Hyphae dark brown, smooth, branched, septate. *Conidiophores* usually reduced to conidiogenous cells. *Conidiogenous cells* monoblastic, discrete, solitary, determinate, pale brown, smooth, subglobose or ampulliform. *Conidia* globose or subglobose, solitary, black, shiny, smooth, aseptate, 17–21 µm diam (av. = 19.24 ± 0.83).

Notes — The conidial size of *N. arundinacea* was described as 30 µm diam (Cooke 1887). However, we did not find any conidia matching these dimensions on the type specimen loaned from K. Conidia of *N. arundinacea* were globose or subglobose, 17–21 µm diam and resembled those of *N. sphaerica*. We failed to find sufficiently distinguishable morphological characters between the two species solely based on the morphology of their type specimens. DNA extraction from the type specimen of *N. arundinacea* from K was not permitted, thus the relationship between *N. arundinacea* and *N. sphaerica* remains unsolved pending further collections and typification.

Nigrospora aurantiaca Mei Wang & L. Cai, *sp. nov.* — MycoBank MB820730; Fig. 4

Etymology. Named after the orange colony colour on PDA.

Type. China, Jiangxi Province, Jiangxi Agricultural University, on *Nelumbo* sp., 21 Sept. 2015, *M.F. Hu* (HMAS 247065 holotype, ex-type living culture CGMCC3.18130 = LC7302 = JAUCC0677).

Hyphae pale brown, smooth, branched, septate, 1.5–5 µm diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* dispersed on hyphae, pale brown, monoblastic, discrete, solitary, determinate, doliiform, ovoid or ampulliform, 7.5–13 × 6–8.5 µm (av. = 9.76 ± 1.34 × 7.06 ± 0.56). *Conidia* solitary, mostly ellipsoidal, black, shiny, smooth, 12–16.5 × 9–15.5 µm (av. = 14.82 ± 0.79 × 11.78 ± 1.07).

Culture characteristics — On PDA, colonies flat, edge entire, floccose at the centre with grey aerial mycelia, initially orange, becoming black with age in the centre. Colonies reaching 9 cm diam after 7 d at 25 °C. On SNA, colonies flat, spreading, with abundant aerial mycelia, surface dirty white to greyish and reverse light pink with olivaceous grey patches.

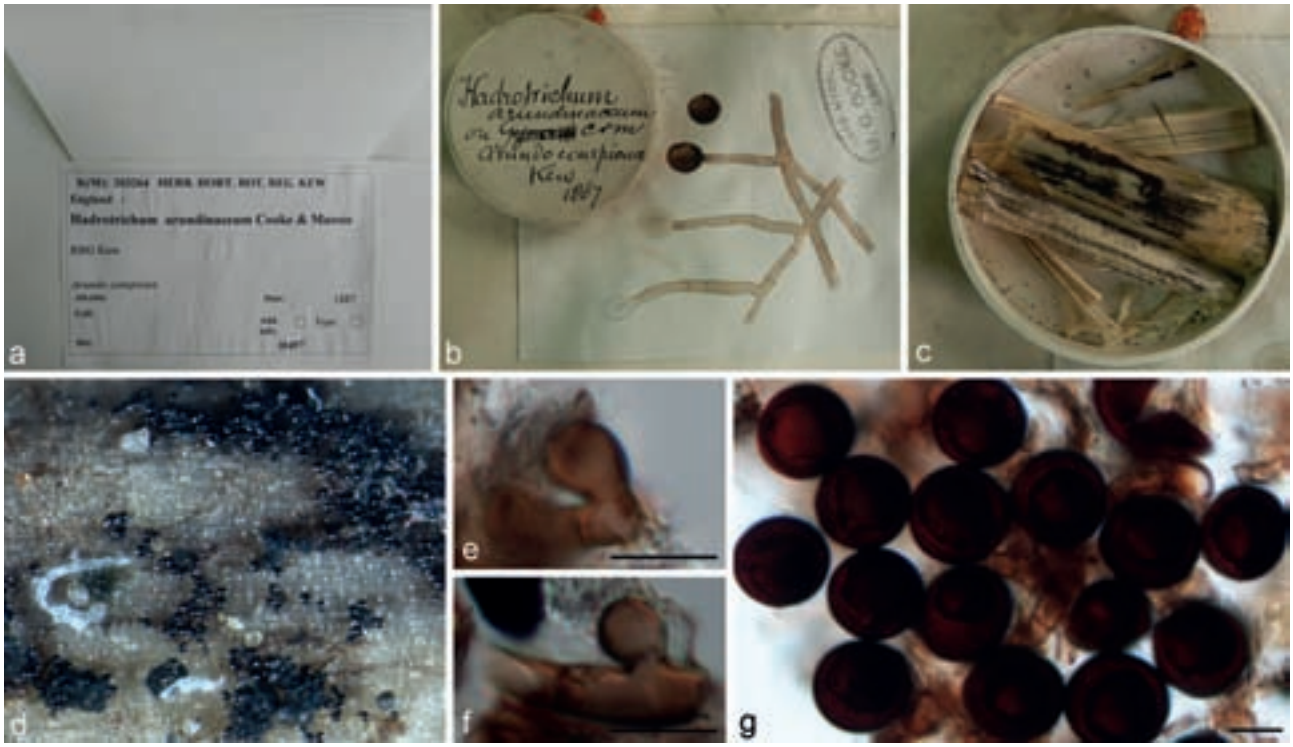


Fig. 3 *Nigrospora arundinacea* (from holotype K(M) 203264). a–c. Overview of the type specimen; d. conidia on *Arundo conspicua*; e–f. conidiogenous cells; g. conidia. — Scale bars = 10 µm.

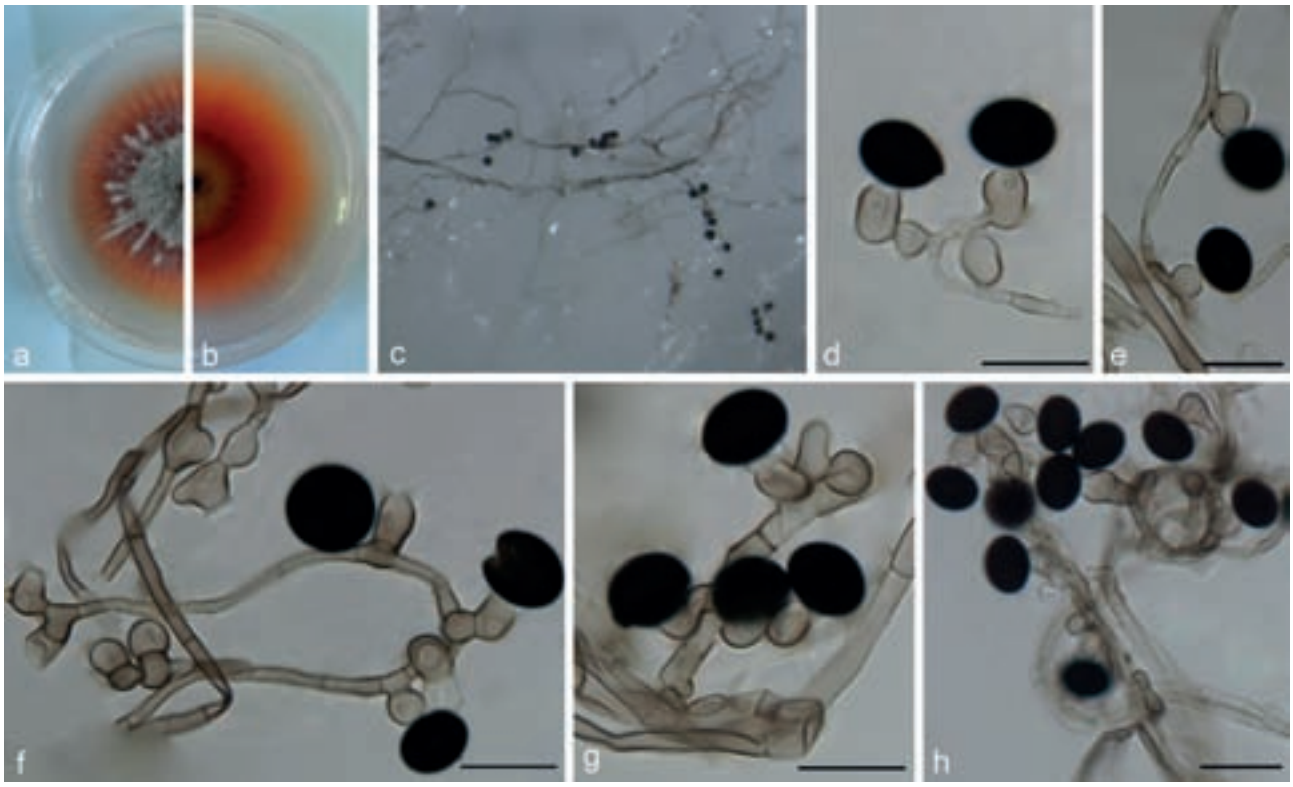


Fig. 4 *Nigrospora aurantiaca* (from ex-type strain CGMCC3.18130). a–b. Upper surface and reverse overview of culture 5 d after inoculation on PDA medium; c. colony on SNA; d–h. conidiogenous cells giving rise to conidia. — Scale bars = 10 µm.

Additional specimen examined. China, Hainan Province, Chengmai, on leaves of *Musa paradisiaca*, 25 Dec. 2015, *F.J. Liu*, living culture LC7034 = WM268.

Notes — Two strains representing *N. aurantiaca* clustered in a well-supported clade (Fig. 2), and closely related to *N. vesicularis* (99 % identity in ITS; 89 % in *TEF1-α*; 96 % in *TUB2*), *N. lacticolonia* (99 % in ITS; 87 % in *TEF1-α*; 93 % in *TUB2*) and *N. osmanthi* (99 % in ITS; 88 % in *TEF1-α*; 94 % in *TUB2*). *Nigrospora aurantiaca* differs from *N. vesicularis* in the absence of vesicles surrounding the septum between its conidiogenous cells and conidia, from *N. lacticolonia* in the colour of the culture (initially orange, becoming black in *N. aurantiaca* vs remaining creamy white in *N. lacticolonia*), from *N. osmanthi* in the larger *conidiogenous cells* (av. = 9.76 ± 1.34 × 7.06 ± 0.56 in *N. aurantiaca* vs av. = 8.02 ± 1.5 × 6.04 ± 1.16 in *N. osmanthi*). In addition, *N. aurantiaca* is a morphologically distinct species of *Nigrospora* that produces orange pigment in culture.

Nigrospora bambusae Mei Wang & L. Cai, *sp. nov.* — MycoBank MB820800; Fig. 5

Etymology. Named after the host from which all strains were isolated, bamboo.

Type. China, Guangdong Province, on bamboo leaves, 10 July 2016, *D.W. Xiao* (HMAS 246696 holotype, ex-type living culture CGMCC3.18327 = LC7114).

Hyphae smooth, hyaline to pale brown, branched, septate, 2.5–7 µm diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* aggregated in clusters on hyphae, pale brown, globose to subglobose to ampulliform, 5.5–12.5 × 3–9.5 µm (av. = 7.85 ± 1.41 × 6.27 ± 1.31). *Conidia* solitary, globose or subglobose, black, shiny, smooth, aseptate, 13.5–17.5 × 10–17 µm (av. = 15.99 ± 0.94 × 14.23 ± 1.84).

Culture characteristics — On PDA, colonies floccose, edge entire, initially white, becoming grey to black with age, reaching 9 cm diam after 7 d at 25 °C, reverse smoke-grey with black patches. On SNA, colonies flat, with some mycelia immersed, surface olivaceous grey and reverse olivaceous grey with black patches due to sporulation.

Additional specimens examined. China, Jiangxi Province, on bamboo leaves, 19 July 2016, *J.E. Huang*, living culture, LC7244 = WM478; ibid., living culture LC7245 = WM479.

Notes — Three strains representing *N. bambusae* clustered in a well-supported clade and related to *N. rubi* (99 % identity in ITS; 93 % in *TEF1-α*; 94 % in *TUB2*). *Nigrospora bambusae* differs from *N. rubi* (Fig. 17) in producing slightly larger conidia (13.5–17.5 × 10–17 µm vs 11.5–16.5 µm). In addition, *N. bambusae* sporulates easier than *N. rubi* (5 d vs 1 mo on SNA). *Nigrospora bambusae* occurs on bamboo (*Poaceae*) while *N. rubi* occurs on *Rubus* sp. (*Rosaceae*).

Nigrospora camelliae-sinensis Mei Wang & L. Cai, *sp. nov.* — MycoBank MB820731; Fig. 6

Etymology. Named after the epithet of *Camellia sinensis*, the host from which most strains were collected in this study.

Type. China, Guangxi Province, on *Camellia sinensis*, Sept. 2013, *T.W. Hou* (HMAS 247068 holotype, ex-type living culture CGMCC3.18125 = LC3500).

Hyphae smooth, hyaline, branched, septate, 1.5–4 µm diam. *Conidiophores* mostly reduced to conidiogenous cells and aggregated in clusters on hyphae. *Conidiogenous cells* hyaline to pale brown, globose to ampulliform or clavate (ear-shaped), sometimes appearing as the bulge directly from the mycelia without septa, 6–11 × 4.5–8.5 µm (av. = 7.85 ± 1.43 × 5.95 ± 0.78). *Conidia* solitary, spherical or slightly ellipsoidal, black, shiny, smooth, aseptate, spherical, 13–18 µm diam (av. = 15.57 ± 1.19), ellipsoidal, 12–18 × 9–14.5 µm (av. = 14.24 ± 1.43 × 10.84 ± 1.21).

Culture characteristics — On PDA, colonies flat, edge entire. Colonies initially white, becoming grey due to sporulation, reaching 9 cm diam in 8 d at 25 °C. On SNA, colonies flat,

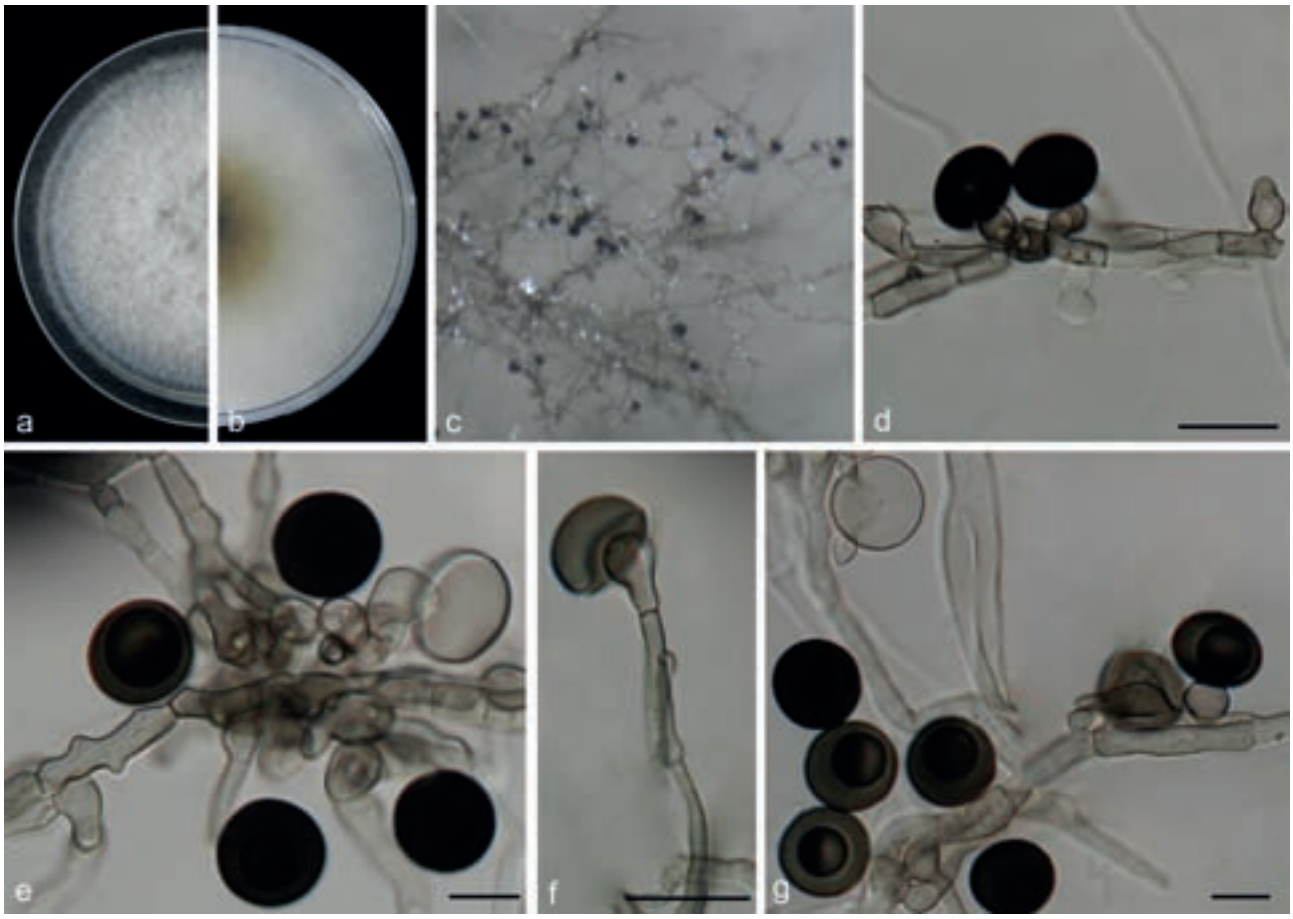


Fig. 5 *Nigrospora bambusae* (from ex-type strain LC7114). a–b. Upper surface and reverse overview of culture 5 d after inoculation on PDA medium; c. colony on SNA; d–f. conidiogenous cells giving rise to conidia; g. conidia. — Scale bars: d–g = 10 µm.

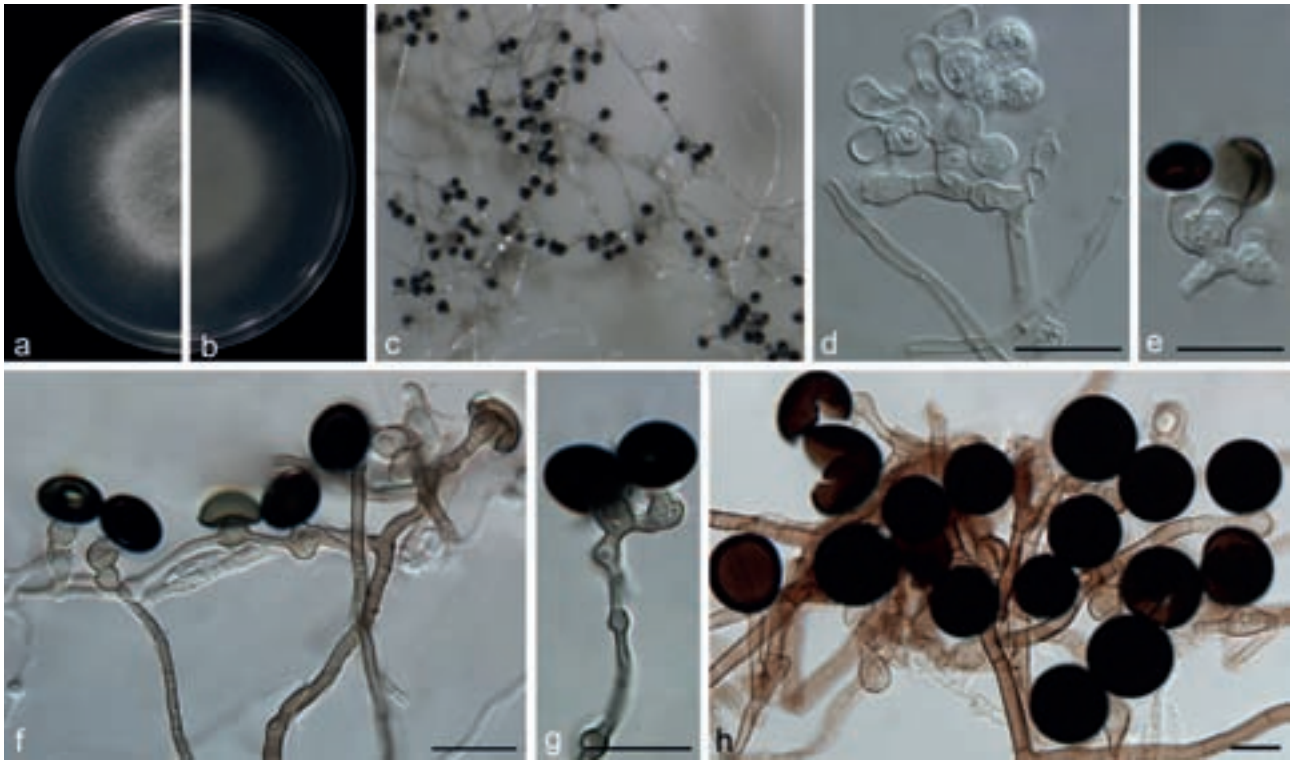


Fig. 6 *Nigrospora camelliae-sinensis* (from ex-type strain CGMCC3.18125). a–b. Upper surface and reverse overview of culture 4 d after inoculation on PDA medium; c. colony on SNA; d–g. conidiophores and conidiogenous cells giving rise to conidia; h. conidia. — Scale bars = 10 µm.

growing slowly, spreading, mycelia partially immersed, surface white to greyish and reverse grey olivaceous without patches, sporulating profusely.

Additional specimens examined. China, Guangxi Province, Guilin, on *Camellia sinensis*, Sept. 2013, *T.W. Hou*, living culture LC3496; Hainan Province, on the leaf of *Musa paradisiaca*, 21 Sept. 2015, *F.J. Liu*, living culture LC6984 = WM218; ibid., LC6989 = WM223; ibid., LC6992 = WM226; ibid., LC7018 = WM252; ibid., LC7044 = WM278; Jiangxi Province, on *Camellia sinensis*, 24 Apr. 2013, *F. Liu*, living culture LC3287; on *Castanopsis* sp., 6 Sept. 2013, *N. Zhou*, living culture LC2710; ibid., LC4460; Yunnan Province, on *Camellia sinensis*, 21 April 2015, *F. Liu*, living culture LC6304 = LF1311.

Notes — Five strains representing *N. camelliae-sinensis* clustered in a well-supported clade (Fig. 2), sister to *N. pyriformis* (99 % identity in ITS; 99 % identity in *TUB2*; 96 % identity in *TEF1-α*). Morphologically, *N. camelliae-sinensis* differs from *N. pyriformis* (Fig. 16) in its smaller conidiogenous cells (6–11 × 4.5–8.5 µm vs 7.5–26 × 3.5–8.5 µm) and conidial shape. *Nigrospora camelliae-sinensis* is comparable to *N. lacticolonia* (Fig. 11) in conidial size, but its conidiogenous cells are scattered rather than aggregated as in *N. lacticolonia*.

Nigrospora chinensis Mei Wang & L. Cai, *sp. nov.* — MycoBank MB820732; Fig. 7

Etymology. Named after the country where this species was first collected, China.

Type. China, Jiangxi Province, on *Machilus breviflora*, 5 Sept. 2013, *Y.H. Gao* (HMAS 247069 holotype, ex-type living culture CGMCC3.18127 = LC4575).

Hyphae hyaline, smooth, branched, septate, 2–5 µm diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* monoblastic, discrete, solitary, determinate, ampulliform, or subspherical, hyaline, 5–9.5 × 4–7 µm (av. = 7.59 ± 1.29 × 5.7 ± 0.72). *Sterile cells* terminal on hyphae, pale to dark brown, elongated ellipsoidal to clavate, 23–40.5 × 5.5–12.5 µm, or somewhat curved or irregularly angled or lobed. *Conidia* solitary, globose or subglobose, black, shiny, smooth, aseptate, 10–14 µm diam (av. = 12.19 ± 1.07); ellipsoidal, 10–14.5 × 7.5–11 µm (av. = 11.78 ± 0.75 × 9.18 ± 0.61).

Culture characteristics — On PDA, colonies floccose, undulate. Colonies growing quickly, initially white, becoming black with age, reaching 9 cm diam in 6 d at 25 °C. On SNA, with sparse aerial mycelia, surface dirty white to greyish, and reverse iron-grey with dark patches but sporulating poorly.

Additional specimens examined. China, Guangxi Province, on *Camellia sinensis*, 7 Sept. 2013, *Y. Zhang*, living culture LC3441; ibid., living culture LC3493; Hainan Province, on *Musa paradisiaca*, 25 Dec. 2015, *F.J. Liu*, living culture LC 6972 = WM206; Jiangxi Province, on *Lindera aggregate*, 6 Sept. 2013, *N. Zhou*, living culture LC2696; on *Camellia sinensis*, 24 Apr. 2013, *F. Liu*, living culture LC3085; ibid., living culture LC3175; ibid., living culture LC3275; ibid., living culture LC3286; ibid., living culture LC3293; ibid., living culture LC3400; on *Aucuba japonica*, 5 Sept. 2013, *Y.H. Gao*, living culture LC4364; on *Castanopsis* sp., 5 Sept. 2013, *Y.H. Gao*, living culture LC4433; on *Itea* sp., 5 Sept. 2013, *Y.H. Gao*, living culture LC4565; on *Machilus duthiei*, 5 Sept. 2013, *Y.H. Gao*, living culture LC4593; on *Osmanthus* sp., 5 Sept. 2013, *Y.H. Gao*, living culture LC4619; on *Quercus* sp., 5 Sept. 2013, *Y.H. Gao*, living culture LC4660; on *Smilax ocreata*, 5 Sept. 2013, *Y.H. Gao*, living culture LC4673; Yunnan Province, on *Camellia sinensis*, 19 Apr. 2015, *F. Liu*, living culture LC6631 = LF1276; Tibet, 14 June 2015, *Q. Chen*, living culture LC6851 = WM085.

Notes — Strains of *N. chinensis* constitutes a distinct clade on concatenated gene trees with a high support value and basal to all other *Nigrospora* species (Fig. 2). Morphologically it is similar to *N. gallarum*, reported from dead larvae of *Lipara lucens* from France (Mason 1927). However, *N. chinensis* differs from *N. gallarum* in producing longer sterile cells (23–40.5 µm vs max. 18 µm).

Nigrospora gorlenkoana Novobr., Novosti Sist. Nizsh. Rast. 9: 180. 1972 — Fig. 8

Type. Kazakhstan, Alma-Ata region, from *Vitis vinifera*, leaf and fruit, 1972, *T.I. Novobranova* (isotype CBS H-7430, ex-isotype living culture CBS 480.73 = ATCC 24718 = IMI 174726 = VKMF-1761).

Hyphae smooth, hyaline, branched, septate, 1.5–4.5 µm diam. *Conidiophores* micronematous or semi-macronematous, flexuous or straight, pale brown, smooth, 2–6 µm thick. *Conidiogenous cells* pale brown, monoblastic, discrete, solitary, determinate, doliiform to ampulliform, 7–13.5 × 4–9 µm (av. = 10.09 ± 1.94 × 5.98 ± 1.11). *Conidia* sparse, solitary, globose or sub-

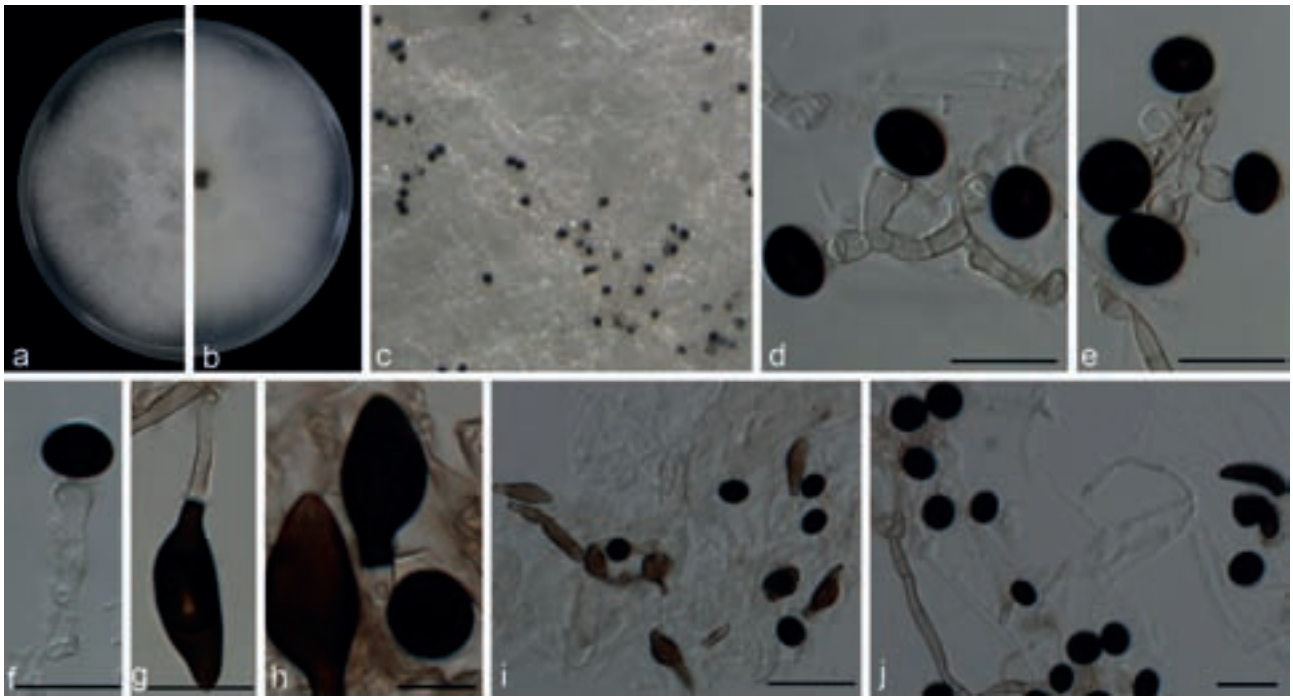


Fig. 7 *Nigrospora chinensis* (from ex-type strain CGMCC3.18127). a–b. Upper surface and reverse overview of culture 5 d after inoculation on PDA medium culture; c. colony on SNA; d–f. conidiogenous cells giving rise to conidia; g–h. sterile cells; i–j. conidia. — Scale bars: d–h = 10 µm; i–j = 20 µm.

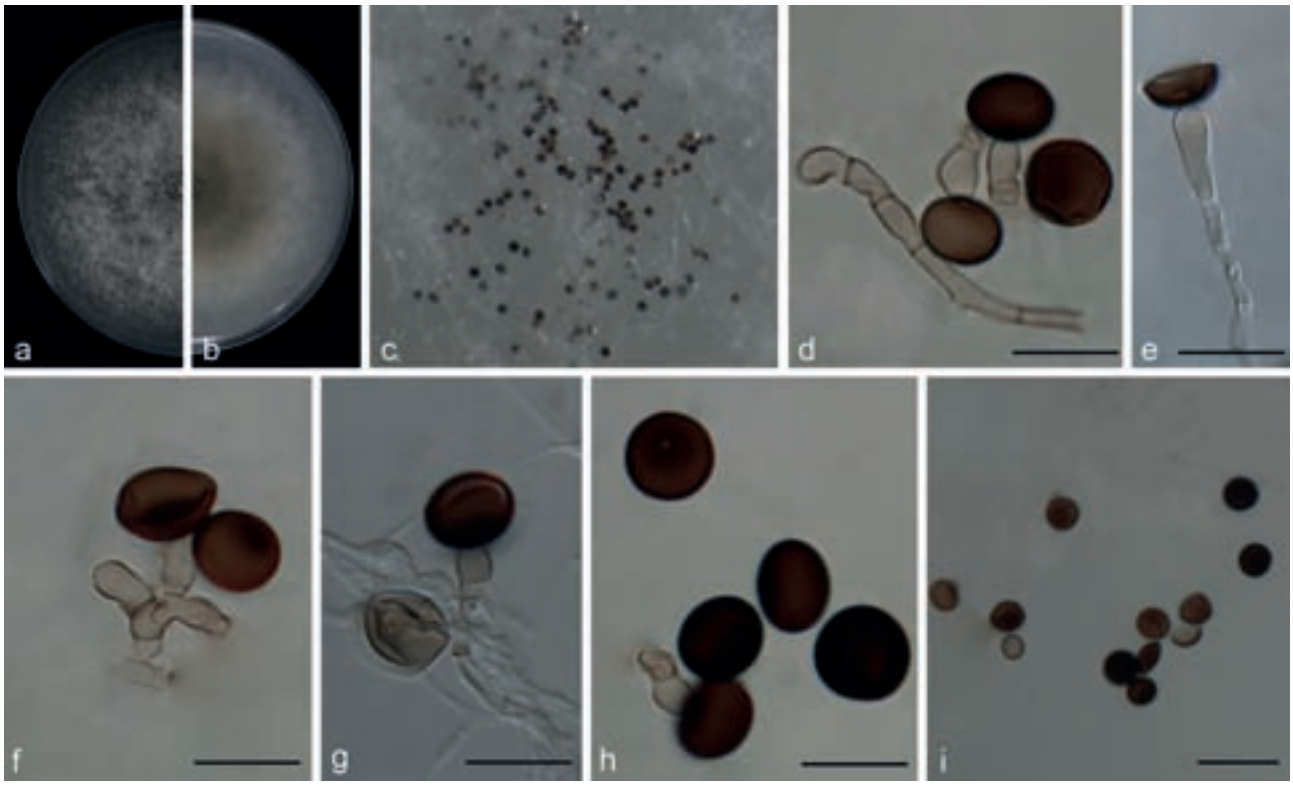


Fig. 8 *Nigrospora gorlenkoana* (from ex-isotype strain CBS 480.73). a–b. Upper surface and reverse overview of culture 6 d after inoculation on PDA medium; c. colony on SNA; d–g. conidiogenous cells giving rise to conidia; h–i. conidia. — Scale bars: d–h = 10 µm; i = 20 µm.

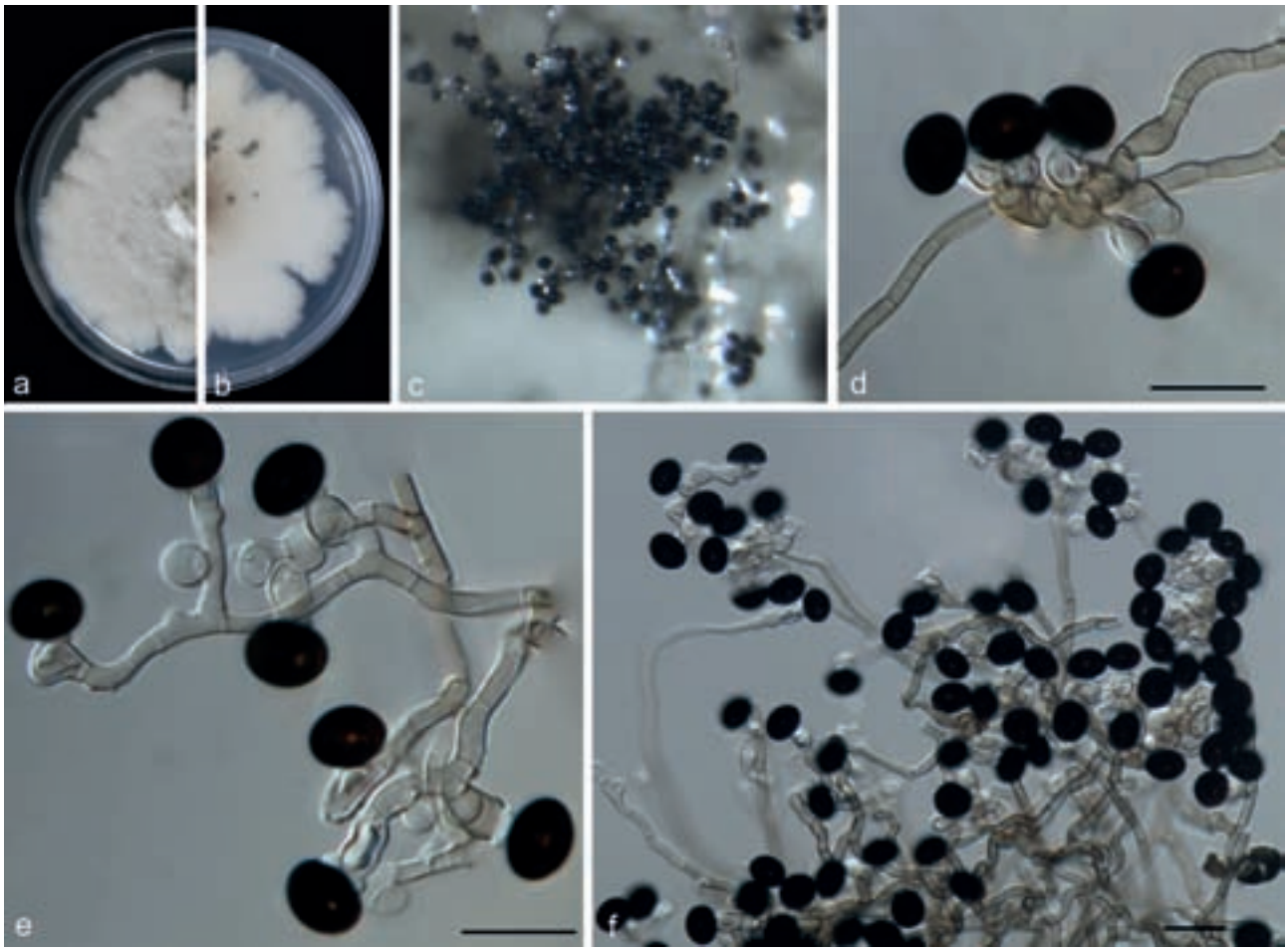


Fig. 9 *Nigrospora guilinensis* (from ex-type strain CGMCC3.18124). a–b. Upper surface and reverse overview of culture 9 d after inoculation on PDA medium; c. colony on SNA; d–e. conidiogenous cells giving rise to conidia; f. conidia. — Scale bars: d–e = 10 µm; f = 20 µm.

globose, pale brown to black, discrete on aerial mycelia, 11.5–17 µm diam (av. = 14.79 ± 1.21), shiny, smooth, with equatorial slit.

Culture characteristics — On PDA, colonies flat, woolly, spreading, initially white, becoming greyish with age. Colonies reaching 9 cm in 6 d at 25 °C. On SNA, colonies flat, with sparse mycelia and growing poorly, reverse with no patches, sporulating poorly.

Notes — *Nigrospora gorlenkoana* is currently listed as a synonym of *N. oryzae* in MycoBank. However, we could not find any literature in support of this synonymy. Our multi-locus molecular phylogeny herein also depicts that these two species cannot be considered as conspecific. These two species are also morphologically distinct. The conidiophores of *N. gorlenkoana* are discrete, solitary, rather than aggregated in clusters as in *N. oryzae*, and the conidial colour of *N. gorlenkoana* is paler brown than that of *N. oryzae*. Equatorial slits are present in some conidia of *N. gorlenkoana*, but absent in *N. oryzae*. However, the affinities of the ex-type of *N. gorlenkoana* are still unresolved as it is an independent taxon and its relationships to *N. hainanensis* as well as *N. musae* lack support (Fig. 2).

Nigrospora guilinensis Mei Wang & L. Cai, *sp. nov.* — MycoBank MB820733; Fig. 9

Etymology. Referring to the location where the holotype was collected, Guilin.

Type. China, Guangxi Province, on *Camellia sinensis*, 7 Sept. 2013, *T.W. Hou* (HMAS 247072 holotype, ex-type living culture CGMCC3.18124 = LC3481).

Hyphae smooth, hyaline to pale brown, branched, septate, 1.5–4 µm diam. *Conidiophores* usually reduced to conidiogenous cells, aggregated in clusters on hyphae. *Conidiogenous cells* monoblastic, determinate, hyaline, smooth, doliiform to clavate to ampulliform, in clusters on aerial mycelia, 6–11 × 4–7.5 µm (av. = 8.73 ± 1.33 × 6.01 ± 0.64). *Conidia* solitary, black, shiny, smooth, aseptate, spherical, 11.5–15 µm diam (av. = 12.91 ± 0.7), ellipsoidal, 10.5–14 × 8–12 µm (av. = 12.46 ± 0.62 × 9.69 ± 0.71).

Culture characteristics — On PDA, colonies woolly, cottony, margin irregular. Colonies growing slowly, dirty white to greyish and producing red pigment after 3 wk. Colonies reaching 9 cm after 14 d at 25 °C. Reverse dirty white to light pink with black patches due to pigment. On SNA, colonies flat, growing slowly, mycelia partially immersed in the medium, surface dirty white to grey and reverse pale brown with black patches.

Additional specimen examined. China, Jiangxi Province, on the stem of *Nelumbo* sp., 21 Sept. 2015, *M.F. Hu*, culture LC7301 = JAUCC0673.

Notes — Two strains representing *N. guilinensis* clustered in a well-supported clade (Fig. 2), which appeared closely related to *N. vesicularis* (98 % identity in ITS; 84 % in *TEF1-α*; 92 % in *TUB2*), *N. aurantiaca* (98 % in ITS; 83 % in *TEF1-α*; 91 % in *TUB2*), *N. osmanthi* (98 % in ITS; 86 % in *TEF1-α*; 91% in *TUB2*) and *N. lacticolonia* (98 % in ITS; 84 % in *TEF1-α*; 91 % in *TUB2*). *Nigrospora guilinensis* is morphologically distinct from these four species. It differs from *N. vesicularis* (Fig. 20) in the absence of a vesicle, from *N. aurantiaca* (Fig. 4) in producing different pigment in culture (red pigment in *N. guilinensis* vs orange pigment in *N. aurantiaca*), from *N. osmanthi* (Fig. 15) in the arrangement of conidiogenous cells (aggregated in clusters in *N. guilinensis* vs scattered in *N. osmanthi*) and from *N. lacticolonia* (Fig. 11) in the smaller ellipsoidal conidia (10.5–14 × 8–12 µm vs 13.5–17.5 × 10.5–13.5 µm).

Nigrospora hainanensis Mei Wang & L. Cai, *sp. nov.* — MycoBank MB820734; Fig. 10

Etymology. Named after the province in China where the type was collected, Hainan.

Type. China, Hainan Province, on a leaf of *Musa paradisiaca*, 21 Sept. 2015, *F.J. Liu* (HMAS 247064 holotype, ex-type living culture CGMCC3.18129 = LC7030).

Hyphae smooth, hyaline to pale brown, branched, septate, 2–6 µm diam. *Conidiophores* usually reduced to conidiogenous cells, which are dispersed on hyphae. *Conidiogenous cells* monoblastic, discrete, solitary, determinate, hyaline, smooth, globose or ampulliform, 6.5–12.5 × 4.5–9.5 µm (av. = 8.89 ± 1.28 × 6.85 ± 0.94). *Conidia* sphaerical or ellipsoidal, solitary, black, shiny, smooth, aseptate, spherical, 12.5–17.5 µm diam (av. = 15.39 ± 1.04), ellipsoidal 13.5–19 × 9–16.5 µm (av. = 15.98 ± 0.98 × 12.11 ± 1.25). *Setae* straight to irregularly curved, black, smooth, subcylindrical, tapering in apical cell to subobtuse or obtuse apex, base truncate, up to 60 µm long, 5–12 µm diam.

Culture characteristics — On PDA, colonies floccose, margin circular, growing rapidly, initially white, becoming black with age, reaching 9 cm diam in 5 d at 25 °C. On SNA, colonies spreading, not flat, mycelia partially immersed in the medium, cottony, surface grey to black and reverse with dark grey patches at the edge and black in the middle due to abundant sporulation.

Additional specimens examined. China, Hainan Province, on the leaf of *Musa paradisiaca*, 21 Sept. 2015, *F.J. Liu*, living culture, LC6979 = WM213; ibid., living culture LC7031 = WM265; ibid., living culture LC7042 = WM276.

Notes — All strains of *N. hainanensis* were isolated from *Musa paradisiaca* from Hainan, China, and they clustered in a well-supported clade (Fig. 2), appearing closely related to *N. gorlenkoana* (99 % identity in ITS; 84 % in *TEF1-α*; 95 % in *TUB2*). These two species could be morphologically differentiated from each other based on conidial colour, which is darker in *N. hainanensis*. Morphologically, *N. hainanensis* also resembles *N. guilinensis* (Fig. 9), but differs in the arrangement of conidiogenous cells (dispersed on aerial mycelia, discrete, solitary, unbranched in *N. hainanensis* vs clustered on aerial mycelia, or forming black sporodochial conidiomata in *N. guilinensis*) and setae (present in *N. hainanensis* vs absent in *N. guilinensis*). Another two *Nigrospora* species, i.e., *N. musae* and *N. canescens* have also been reported from *Musa* spp. *Nigrospora hainanensis* differs in producing smaller conidia (spherical, 12.5–17.5 µm diam in *N. hainanensis* vs globose or subglobose, 15–19.5 µm in *N. musae*, and subglobose, 19 × 17 µm in *N. canescens*) and the presence of setae.

Nigrospora lacticolonia Mei Wang & L. Cai, *sp. nov*. — MycoBank MB820735; Fig. 11

Etymology. Named after the creamy white colony colour on PDA and SNA.

Type. China, Jiangxi Province, on *Camellia sinensis*, 24 Apr. 2013, *F. Liu* (HMAS 247070 holotype, ex-type living culture CGMCC 3.18123 = LC3324).

Hyphae smooth, hyaline, branched, septate, 1.5–4 µm diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* aggregated in clusters on hyphae, pale brown, finely verruculose, globose to clavate to doliiform, 6.5–11.5 × 5.5–9 µm (av. = 8.29 ± 1.11 × 6.82 ± 0.73). *Conidia* solitary, spherical or slightly ellipsoidal, black, shiny, smooth, aseptate, spherical 11.5–16.5 µm diam (av. = 14.36 ± 1.04), ellipsoidal 13.5–17.5 × 10.5–13.5 µm (av. = 15.21 ± 0.75 × 11.72 ± 0.66).

Culture characteristics — On PDA, colonies floccose, entire edge, surface and reverse creamy white, with dark brown patches in the reverse, reaching 9 cm diam in 6 d at 25 °C. On

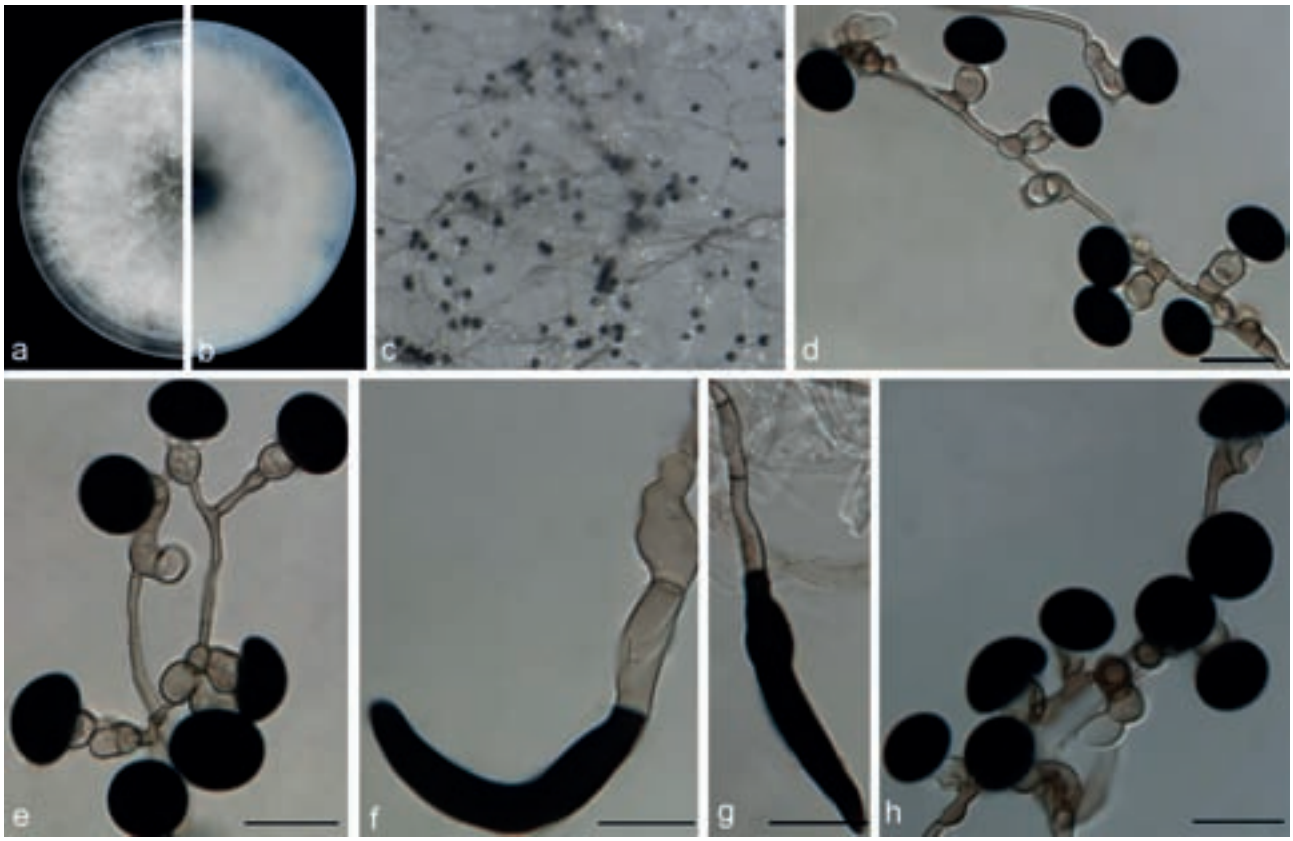


Fig. 10 *Nigrospora hainanensis* (from ex-type strain CGMCC3.18129). a–b. Upper surface and reverse overview of culture 5 d after inoculation on PDA medium; c. colony on SNA; d–e. conidiogenous cells giving rise to conidia; f–g. setae; h. conidia. — Scale bars = 10 µm.

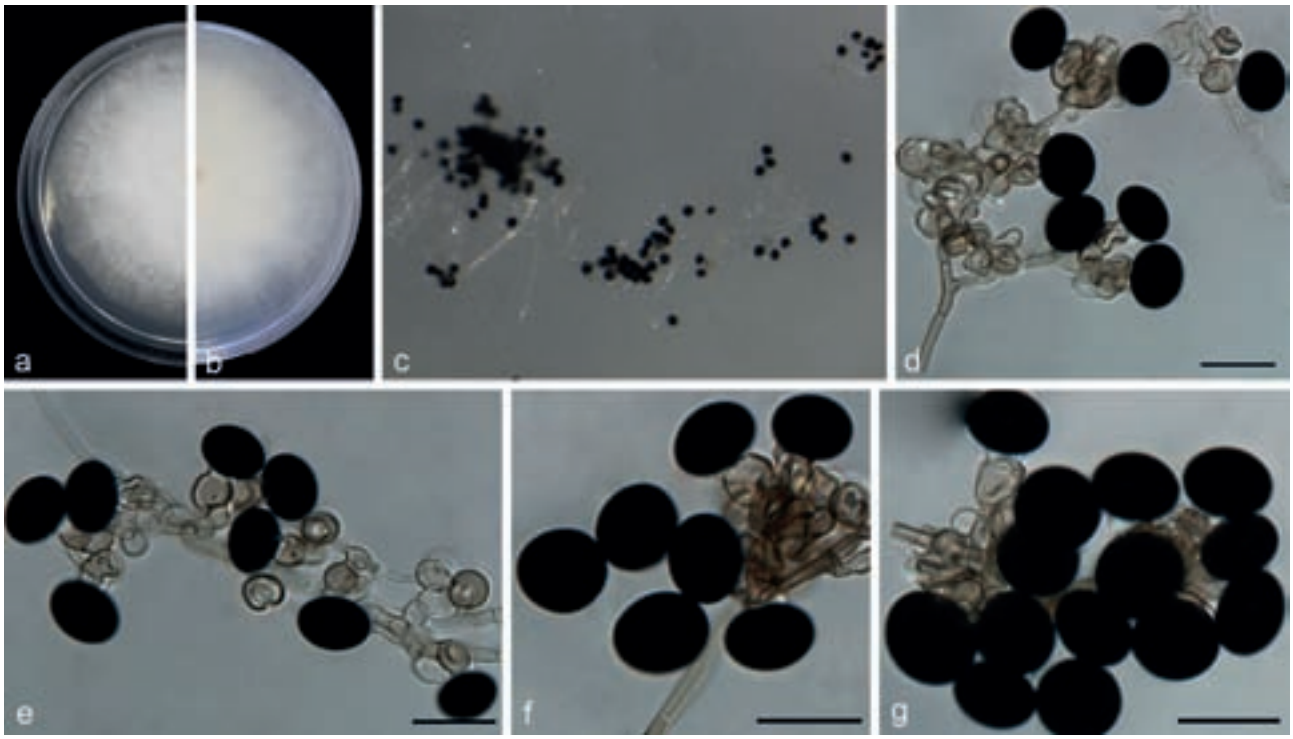


Fig. 11 *Nigrospora lacticolonia* (from ex-type strain CGMCC3.18123). a–b. Upper surface and reverse overview of culture 6 d after inoculation on PDA medium; c. colony on SNA; d–f. conidiogenous cells giving rise to conidia; g. conidia. — Scale bars = 10 µm.

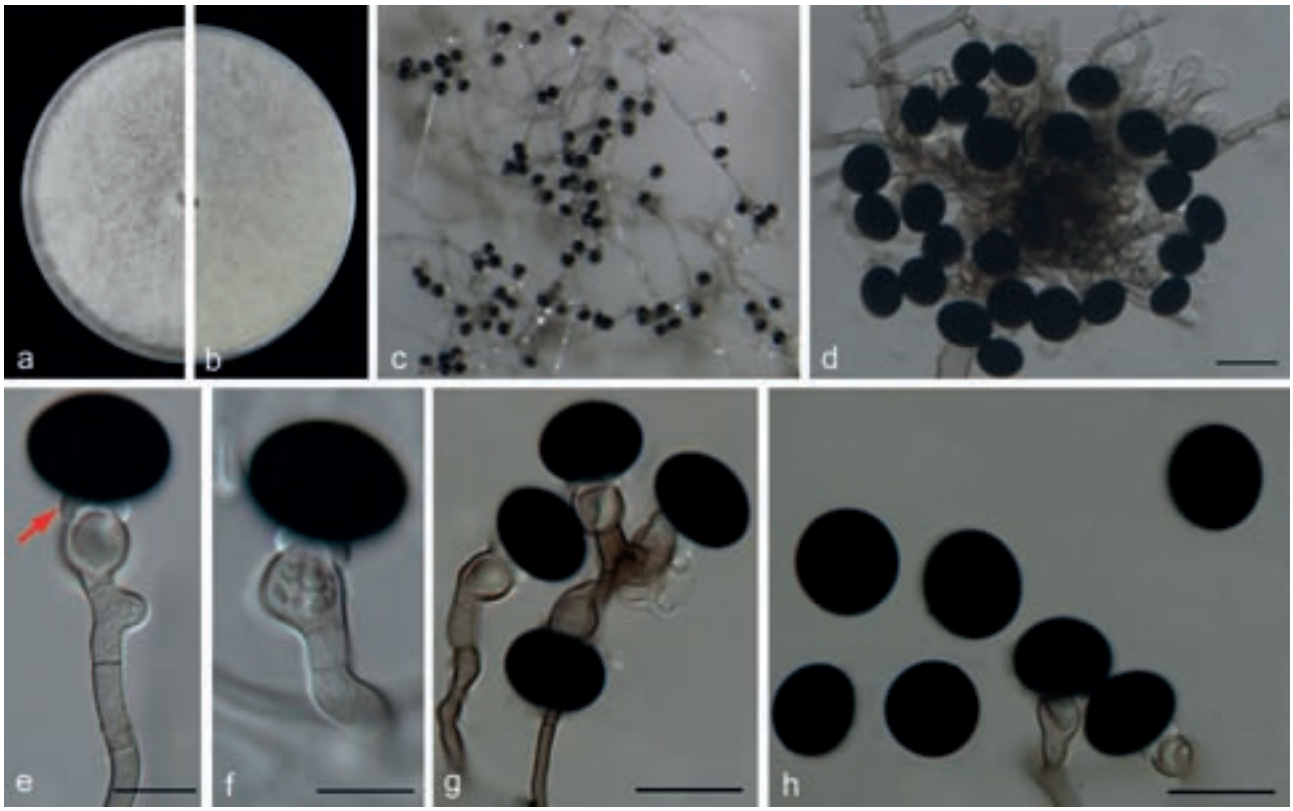


Fig. 12 *Nigrospora musae* (from ex-type strain CBS 319.34). a–b. Upper surface and reverse overview of culture 7 d after inoculation on PDA medium; c. colony on SNA; d. conidiophores; e–g. conidiogenous cells giving rise to conidia; h. conidia. — Scale bars: d = 20 µm; e–h = 10 µm.

SNA, colonies flat, surface and reverse remains white and black patches in the reverse, with moderate aerial mycelia, growing very quickly, but sporulating after 2 wk.

Additional specimen examined. China, Hainan Province, on *Musa paradisiaca*, 25 Dec. 2015, *F.J. Liu*, living culture LC7009 = WM243.

Notes — Two strains representing *N. lacticolonia* clustered in a well-supported clade which is closely related to *N. osmanthi* (100 % identity in ITS; 91 % in *TEF1-α*; 98 % in *TUB2*), but they could be distinguished from one another based on the morphology of their conidiogenous cells (Fig. 11, 17).

Nigrospora musae McLennan & Hoëtte, Aust. Inst. Sci. Industr. Res. Bull. 75: 15. 1933 — Fig. 12

Type. Australia, from the fruit of *Musa sapientum*, 1933, *E. McLennan* (ex-type culture CBS 319.34 = MUCL 8368).

Hyphae pale brown, smooth, branched, septate, 2–6 µm diam. *Conidiophores* aggregated in black sporodochia, micronematous or semi-macronematous, flexuous or straight, pale brown, smooth, much branched, 3.5–8 µm diam, some conidiophores reduced to conidiogenous cells. *Conidiogenous cells* aggregated, pale brown, monoblastic, subglobose to ampulliform, 6.5–14 × 6–9 µm (av. = 9.16 ± 1.49 × 7.45 ± 0.74); *hyaline vesicles* (Fig. 12, arrowed) delimiting the conidia from conidiogenous cells. *Conidia* sparse, solitary, globose or subglobose, black, shiny, smooth, 15–19.5 (mostly 16–18 µm) (av. = 17.01 ± 0.84).

Culture characteristics — On PDA, colonies woolly, margin circular. Colonies initially white, becoming dark grey with age, most mycelia immersed, and the reverse were olive-citrine, reaching 9 cm diam in 7 d at 25 °C. On SNA, colonies flat, the aerial mycelia growing sparsely, most mycelia immersed, reverse olivaceous grey with black patches, with abundantly sporulation.

Additional specimen examined. China, Guizhou Province, on *Camellia sinensis*, 21 July 2014, *Z.F. Zhang*, living culture, LC6385 = LF1013.

Notes — *Nigrospora musae* was originally described from fruit of *Musa sapientum* in Australia (McLennan & Hoëtte 1933), but the ex-type strain (CBS 319.34) appeared to be sterile. We therefore re-described it based on a freshly collected strain LC6385 (similarity: 99 % identity in ITS; 99 % in *TEF1-α*; 99 % in *TUB2*) from *Camellia sinensis*. The description of *N. musae* was emended in this study, adding the presence of hyaline vesicles. *Nigrospora canescens*, originally reported from leaves of *Musa sapientum* (McLennan & Hoëtte 1933), was never isolated from the fruits and was endophytic in banana. Moreover, *N. canescens* sporulates more quickly and abundantly than *N. musae* in culture.

Nigrospora oryzae (Berk. & Broome) Petch, J. Indian Bot. Soc. 4: 24. 1924 — Fig. 13–14

Basionym. *Monotospora oryzae* Berk. & Broome, J. Linn. Soc., Bot. 14: 99. 1873.

Synonym. *Khuskia oryzae* H.J. Huds., Trans. Brit. Mycol. Soc. 46: 358. 1963.

Type. Sri Lanka, Jaffra, H.S.O. Russell, Esq. Government Agent of the Northern Provinces, from rice leaves, 1873, *Berk. & Broome* (IMI 99832, slide of holotype).

Hyphae branched, septate, smooth, hyaline, 2–6 µm diam, becoming brown closer to the conidiogenous region. *Conidiophores* aggregated in black sporodochia, micronematous or semi-macronematous, multiseptate, extensively branched, flexuous or straight, pale brown, smooth, 3–7 µm diam; sometimes reduced to conidiogenous cells. *Conidiogenous cells* aggregating in clusters on hyphae, monoblastic, determinate, ampulliform or subspherical, hyaline, 4–13 × 3–8.5 µm (av. = 8.26 ± 1.03 × 6.45 ± 0.76). *Conidia* formed abundantly, solitary, globose or subglobose, black, shiny, smooth, aseptate, 12.5–16 (mostly 12–14) µm diam (av. = 14.26 ± 0.79, n = 50). *Perithecia* formed in clusters of 1–7, uniseriate or in irregular rows, up to 2 mm long, subepidermal, erumpent, globose, obovoid,

up to 250 µm diam, with papillate ostioles; perithecial clusters surrounded by a blackened area of host tissue. *Asci* 8-spored, biseriate, short-stalked, unitunicate, clavate, 55–75 × 8.5–12 µm. *Paraphyses* thin-walled, septate. *Ascospores* hyaline, granular, curved, inequilateral, 16–21 × 5–7 µm, tapering to the base with rounded ends, initially aseptate but on discharge from the ascus and on germination ascospores may develop a single transverse septum.

Culture characteristics — On PDA, colonies woolly, floccose, margin circular, growing rapidly, white to grey to black with age, reaching 9 cm diam in 5 d at 25 °C. On SNA, colonies flat, with abundant aerial mycelia, surface and reverse dark brown to black without patches, sporulating quickly and abundantly.

Additional specimens examined. China, Fujiang Province, on *Pittosporum illicioides*, 8 Nov. 2012, *Q. Chen*, living culture LC4961; Hubei Province, on *Citrus reticulate*, 25 Sept. 2015, *X. Zhou*, living culture LC 6893 = WM127; on *Oryza sativa*, 25 Sept. 2015, *X. Zhou*, living culture LC 6923 = WM157; ibid., living culture LC 6955 = WM189; ibid., living culture LC 6957 = WM191; ibid., living culture LC 6759 = HBN3-18; ibid., living culture LC 6760 = HBN4-11; ibid., living culture LC 6763 = HBN4-23; ibid., living culture LC 6766 = JXN1-4; Jiangxi Province, on *Nelumbo* sp., 21 Sept. 2014, *M.F. Hu*, living

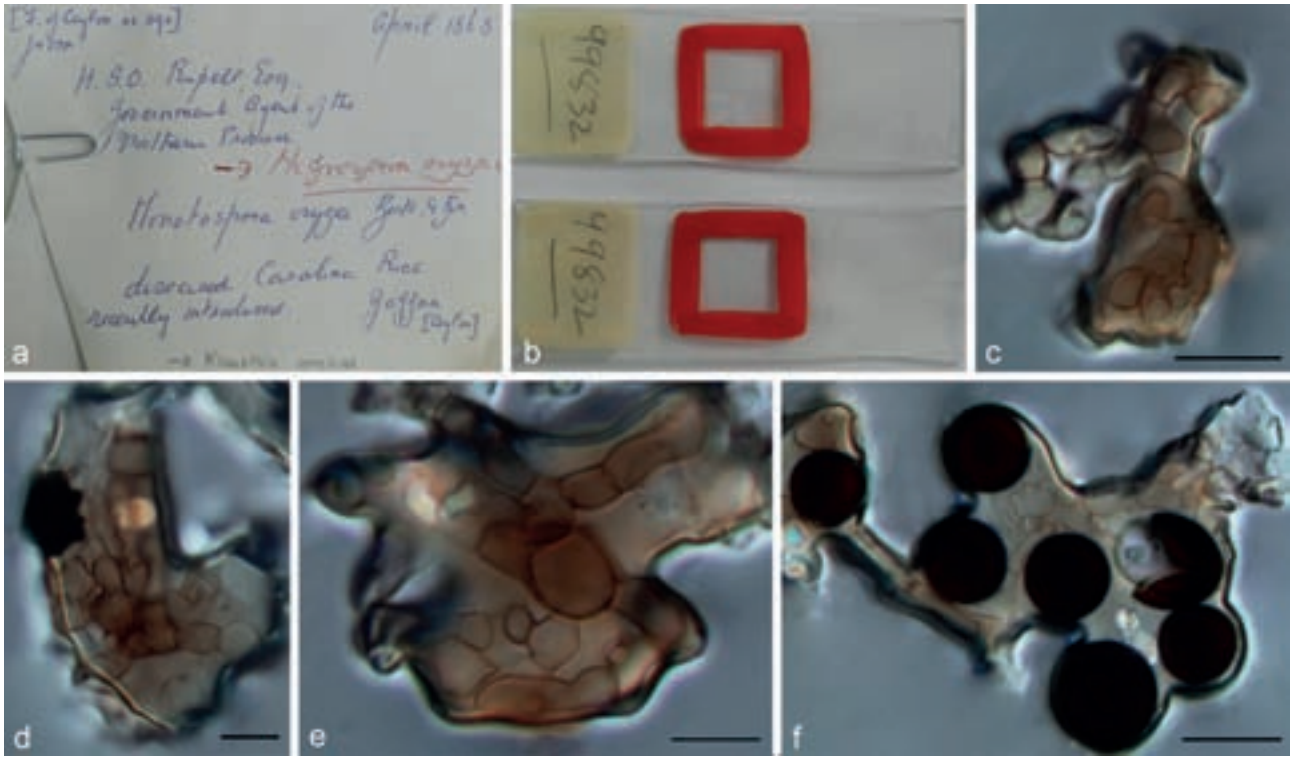


Fig. 13 *Nigrospora oryzae* (from slide of holotype K(M) 99832). a–b. Overview of the type specimen; c–e. conidiophores; f. conidia. — Scale bars = 10 µm.

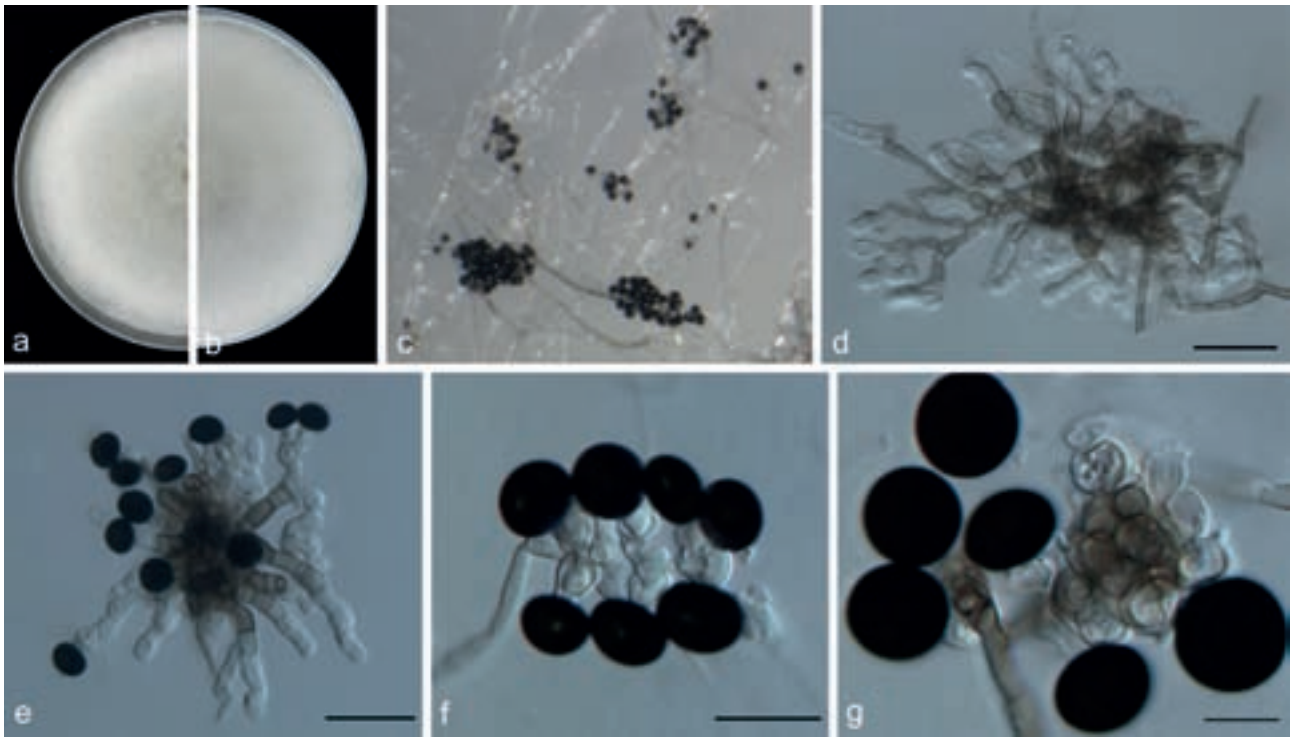


Fig. 14 *Nigrospora oryzae* (LC7293). a–b. Upper surface and reverse overview of culture 7 d after inoculation on PDA medium; c. colony on SNA; d. conidiophores; e–f. conidiogenous cells giving rise to conidia; g. conidia. — Scale bars: d–e = 20 µm; f–g = 10 µm.

culture, LC6029; ibid., living culture LC7299 = JAUCC0669; ibid., living culture LC7300 = JAUCC0672; ibid., living culture LC7305 = JAUCC0708; ibid., living culture LC7306 = JAUCC0709; ibid., living culture LC7308 = JAUCC0713; ibid., living culture LC7309 = JAUCC0757; ibid., living culture LC7310 = JAUCC0758; ibid., living culture LC7311 = JAUCC0767; 15 Sept. 2014, *X.X. Zhan*, living culture LC7293 = JAUCC0004; ibid., living culture LC7297 = JAUCC00027; on *Rhododendron* sp., 5 Sept. 2013, *Y.H. Gao*, living culture, LC4260; on *Osmanthus* sp., 5 Sept. 2013, *Y.H. Gao*, living culture, LC4679; ibid., living culture LC2689; on *Cephalotaxus sinensis*, 5 Sept. 2013, *Y.H. Gao*, living culture LC4273; on *Rhododendron* sp., 5 Sept. 2013, *Y.H. Gao*, living culture LC4275; on submerged wood, 21 Sept. 2014, *M.F. Hu*, living culture LC5964; ibid., living culture LC5982; ibid., living culture LC5999; on *Neolitsea* sp., 6 Sept. 2013, *N. Zhou*, living culture LC2693; on *Rubus reflexus*, 6 Sept. 2013, *N. Zhou*, living culture LC2695; on *Hamamelis mollis*, 3 Sept. 2013, *N. Zhou*, living culture LC2699; on *Rubus* sp., 2 Sept. 2013, *N. Zhou*, living culture LC2702; on *Rhododendron* sp., 2 Sept. 2013, *N. Zhou*, living culture LC2704; ibid., living culture LC2706; ibid., living culture LC2707; ibid., living culture LC2708; ibid., living culture LC2709; on *Castanopsis* sp., 6 Sept. 2013, *N. Zhou*, living culture LC2712; on *Ternstroemia* sp., 3 Sept. 2013, *N. Zhou*, living culture LC2749; on *Osmanthus* sp., 4 Sept. 2013, *N. Zhou*, living culture LC2752; on *Symplocos zizyphoides*, 2 Sept. 2013, *N. Zhou*, living culture LC3690; on *Daphniphyllum macropodum*, 5 Sept. 2013, *Y.H. Gao*, living culture LC4294; ibid., living culture LC4295; on *Daphniphyllum oldhamii*, 5 Sept. 2013, *Y.H. Gao*, living culture LC4320, on *Camellia* sp., living culture LC4327; ibid., living culture LC4345; ibid., living culture LC4680; Qinghai Province, on *Pentactina rupicola*, 2 Sept. 2013, *Q. Chen*, living culture LC5181; Sichuan Province, on *Tutcheria microcarpa*, 5 Oct. 2012, *D.M. Hu*, living culture LC2972, on *Cleyera japonica*, 5 Oct. 2012, *F. Liu*, living culture LC2991.

Notes — The type of *N. oryzae* is preserved in K as two slides, and only partial morphological characters could be observed (Fig. 13), e.g., branched conidiophores, black and globose/subglobose conidia, 12.5–16 µm diam. Although we could not obtain sequences from the type specimen or culture for comparisons, all strains from the original host (rice) isolated in this study clustered in one single clade, sister to *N. zimmermanii* (Fig. 2), and their morphological characteristics were in good accordance with *N. oryzae.* Therefore, we regard this clade as *N. oryzae.*

Nigrospora osmanthi Mei Wang & L. Cai, *sp. nov.* — MycoBank MB820736; Fig. 15

Etymology. Named after the host genus from which the holotype was collected, *Osmanthus.*

Type. China, Jiangxi Province, on *Osmanthus* sp., 5 Sept. 2013, *Y.H. Gao* (HMAS 247066 holotype, ex-type living culture CGMCC3.18126 = LC4350).

Hyphae branched, septate, hyaline to pale brown, 2.5–4.5 µm diam. *Conidiophores* mostly reduced to conidiogenous cells. *Conidiogenous cells* monoblastic, discrete, solitary, determinate, at first hyaline, subspherical, then turning to pale brown, ampulliform to cylindrical, 5.5–12 × 4–8.5 µm (av. = 8.02 ± 1.5 × 6.04 ± 1.16). *Conidia* solitary, globose or subglobose, black, shiny, smooth, sometimes formed directly from the mycelia, aseptate, 13.5–16.5 µm diam (av. = 14.87 ± 0.63).

Culture characteristics — On PDA, colonies flat, floccose, lobate. Colonies growing slowly, initially white, becoming black with age, reaching 9 cm diam in 10 d at 25 °C. On SNA, colonies flat, surface greyish to grey olivaceous with dark grey patches and reverse dark brown with black patches, mycelia sparse on the surface with delayed sporulation.

Additional specimen examined. China, Jiangxi Province, on *Hedera nepalensis*, 5 Sept. 2013, *Y.H. Gao*, living culture LC4487.

Notes — Two strains representing *N. osmanthi* clustered in a well-supported clade which is closely related to *N. lacticolonia* (Fig. 2), but they could be distinguished from one another based on the morphology of their conidiogenous cells (Fig. 11, 17). Morphologically, *N. osmanthi* also resembles *N. oryzae* in its conidial size, but differs in its conidiophores that are reduced to conidiogenous cells in *N. osmanthi*, but branched and clustered in *N. oryzae*. *Nigrospora osmanthi* differs from another morphologically similar species, *N. gallarum*, by the absence of sterile cells.

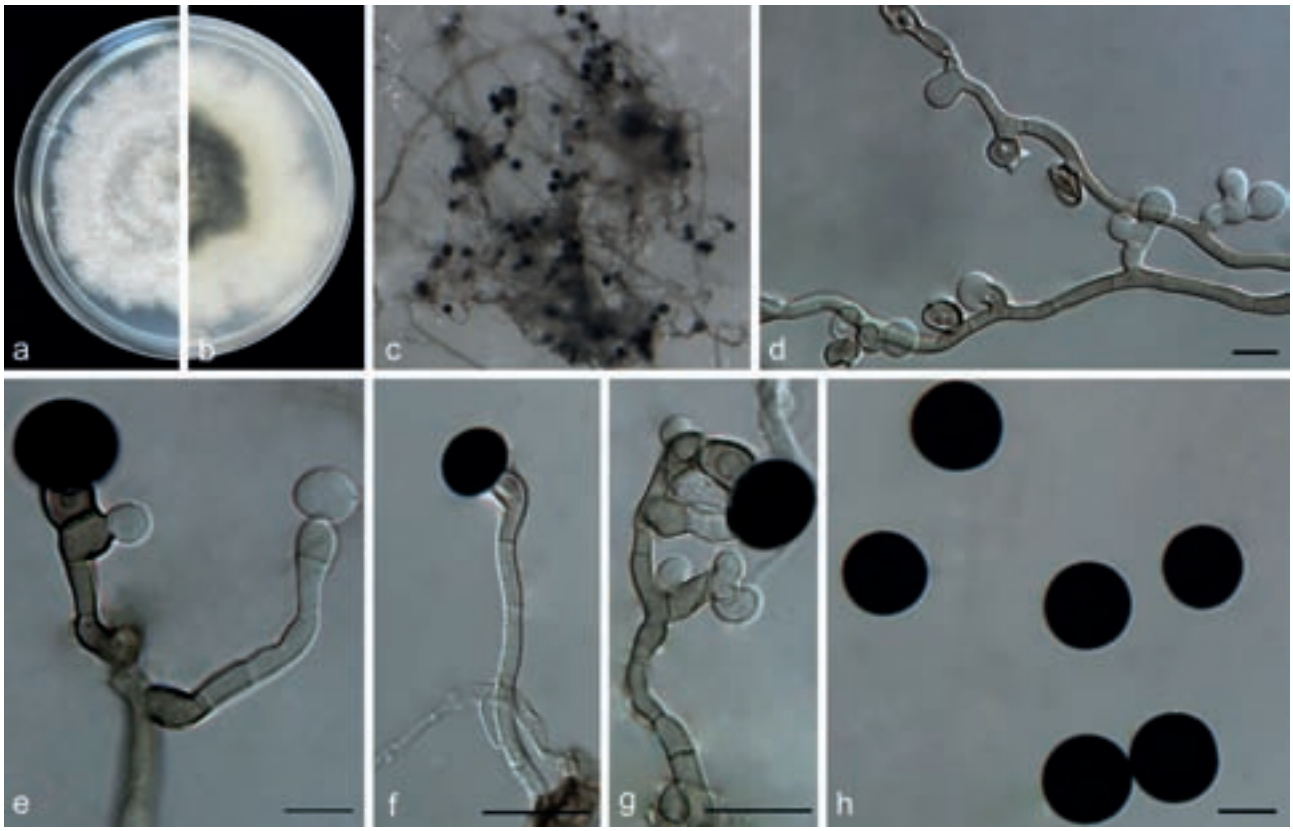


Fig. 15 *Nigrospora osmanthi* (from ex-type strain CGMCC3.18126). a–b. Upper surface and reverse overview of culture 8 d after inoculation on PDA medium; c. colony on SNA; d–g. conidiogenous cells giving rise to conidia; h. conidia. — Scale bars = 10 µm.

Nigrospora pyriformis Mei Wang & L. Cai, *sp. nov.* — MycoBank MB820737; Fig. 16

Etymology. Named after the presence of pyriform conidia.

Type. China, Jiangxi Province, *Citrus reticulata*, 11 Mar. 2012, *X.M. Tan* (HMAS 247067 holotype, ex-type culture CGMCC3.18122 = LC2045).

Hyphae smooth, hyaline, branched, septate, 2–6 µm diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* monoblastic, discrete, solitary, determinate, ampulliform or subcylindrical, pale brown, 7.5–26 × 3.5–8.5 µm (av. = 13.38 ± 4.81 × 6.31 ± 1.46). *Conidia* initially pale brown, become black with age, dimorphic, globose to subglobose, black, shiny, smooth, aseptate, 12.5–16.5 µm diam (av. = 15.41 ± 0.77); or pyriform, black, shiny, smooth, aseptate, 17.5–27.5 × 10–18.5 µm (av. = 19.97 ± 4.95 × 11.77 ± 2.53).

Culture characteristics — On PDA, colonies woolly, floccose, margin circular. Colonies initially white, becoming black with age, reaching 9 cm diam in 7 d at 25 °C. On SNA, colonies flat, spreading, with moderate aerial mycelia, reverse black due to sporulation.

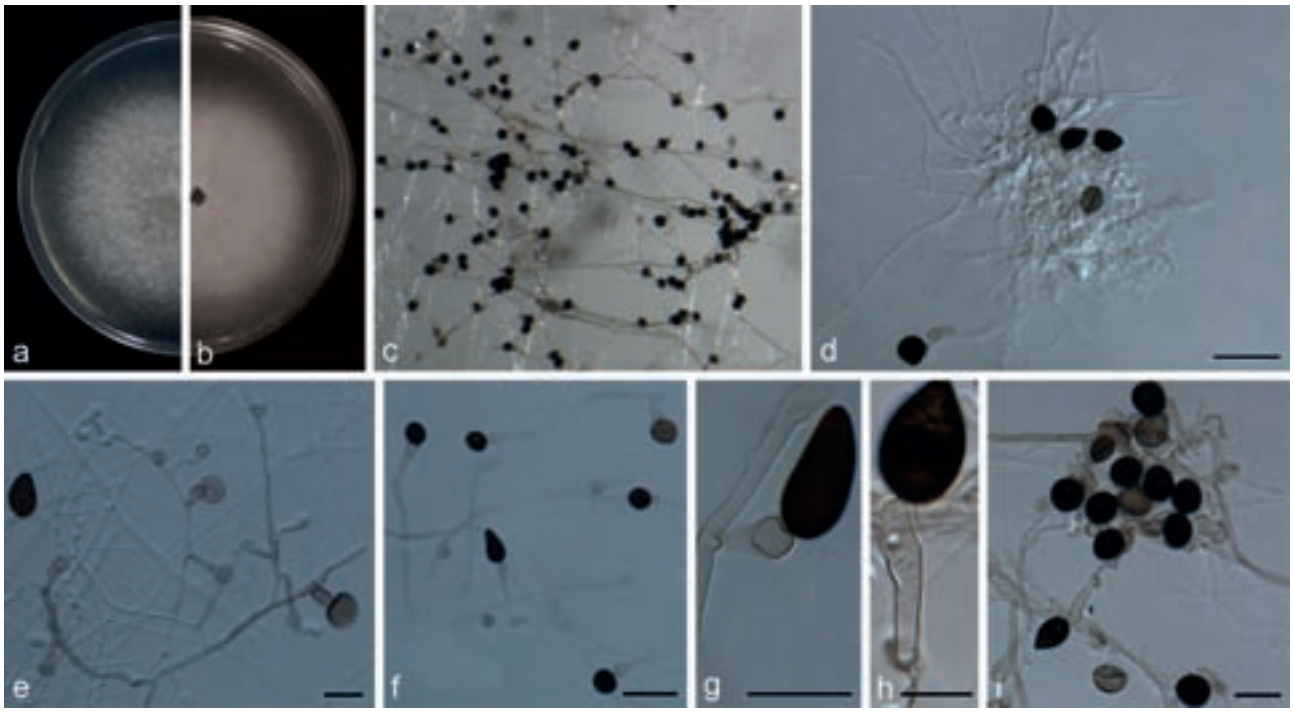


Fig. 16 *Nigrospora pyriformis* (from ex-type strain CGMCC3.18122). a–b. Upper surface and reverse overview of culture 5 d after inoculation on PDA medium; c. colony on SNA; d–f. conidiophores and conidiogenous cells giving rise to conidia; g–i. conidia. — Scale bars: d–f, i = 20 µm; g–h = 10 µm.

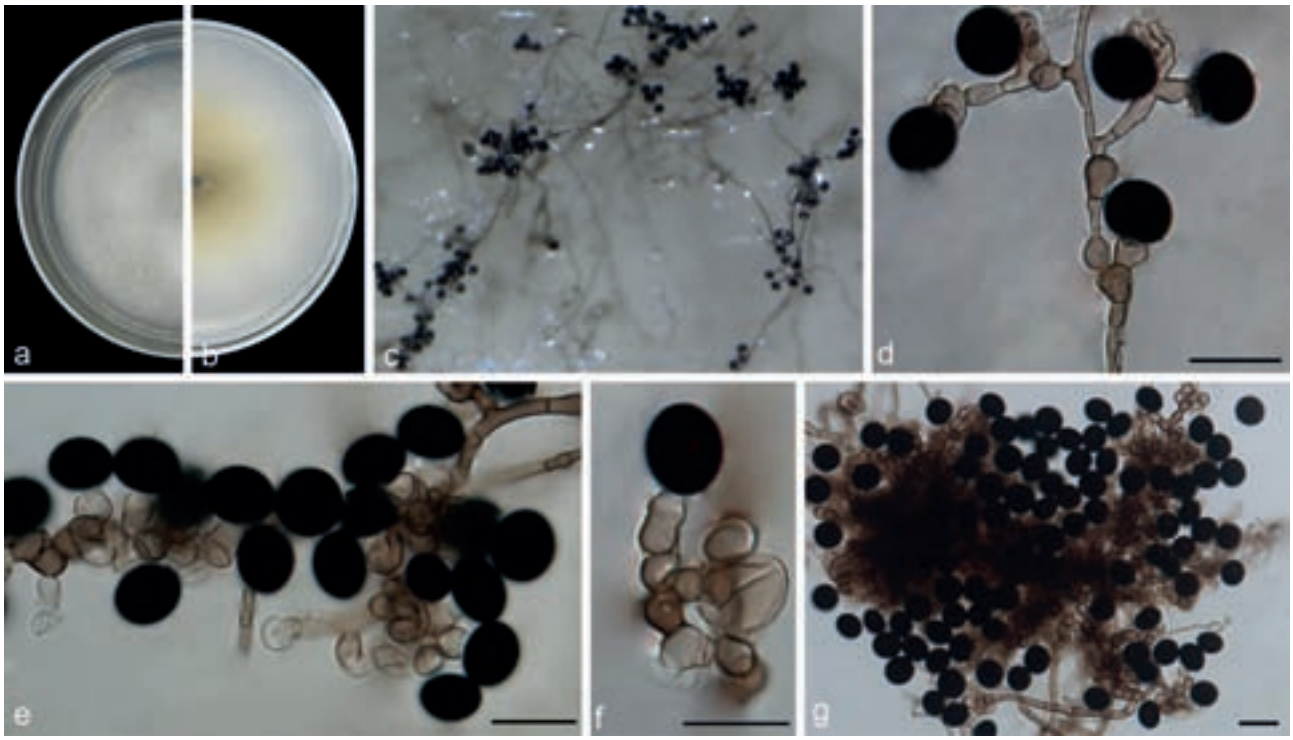


Fig. 17 *Nigrospora rubi* (from ex-type strain LC2698). a–b. Upper surface and reverse overview of culture 5 d after inoculation on PDA medium; c. colony on SNA; d–f. conidiogenous cells giving rise to conidia; g. conidia. — Scale bars: d–f = 10 µm; g = 20 µm.

Additional specimens examined. China, Hainan Province, on leaves of *Musa paradisiaca*, 21 Sept. 2015, *F.J. Liu*, living culture LC6985 = WM219; ibid., LC6988 = WM222; Jiangxi Province, on *Camellia sinensis*, 24 Apr. 2013, *F. Liu*, living culture LC3099; ibid., LC3292; on *Lindera aggregata*, 6 Sept. 2013, *N. Zhou*, living culture LC2688; on *Rubus reflexus*, 6 Sept. 2013, *N. Zhou*, living culture LC2694; on *Castanopsis* sp., 5 Sept. 2013, *Y.H. Gao*, living culture LC4669; on *Rosa* sp., 2 Sept. 2013, *N. Zhou*, living culture LC2690.

Notes — Five strains representing *N. pyriformis* clustered in a well-supported clade (Fig. 2), and appeared as a sister clade to *N. camelliae-sinensis* (99 % identity in ITS; 96 % in *TEF1-α*; 99 % in *TUB2*). Morphologically, *N. pyriformis* is unique in *Nigrospora* by producing pyriform conidia.

Nigrospora rubi Mei Wang & L. Cai, *sp. nov.* — MycoBank MB820801; Fig. 17

Etymology. Named after the host genus on which the holotype was collected, *Rubus*.

Type. China, Jiangxi Province, on *Rubus* sp., 6 Sept. 2013, *N. Zhou* (HMAS 246699 holotype, ex-type living culture CGMCC3.18326 = LC2698).

Hyphae smooth, hyaline, branched, septate, 2–6 µm diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* aggregated in clusters on hyphae, pale brown, subglobose to ampulliform to lageniform, 6.5–14 × 5–9 µm (av. = 9.94 ± 1.71 × 7.16 ± 0.8). *Conidia* solitary, spherical or subglobose, black, shiny, smooth, aseptate, (11.5–)13–15(–16.5) µm diam (av. = 14.23 ± 0.97).

Culture characteristics — On PDA, colonies floccose, entire edge, initially white, becoming black with age, reaching 9 cm diam in 6 d at 25 °C, reverse smoke-grey in patches. On SNA, colonies flat, with moderate aerial mycelia, surface dirty white, growing very quickly, but with delayed sporulation. Surface and reverse pale luteous to sienna with greyish patches.

Notes — See notes of *N. bambusae*.

Nigrospora sphaerica (Sacc.) E.W. Mason, Trans. Brit. Mycol. Soc. 12: 158. 1927 — Fig. 18–19

Type. USA, Newfield, N.J., from *Zea mays*, 1822, *P.A. Saccardo* (slide of holotype, IMI 103253).

Basionym. *Trichosporum sphaericum* Sacc., Michelia 2 (no. 8): 579. 1882.

Hyphae smooth, hyaline, branched, septate, 3–8 µm diam. *Conidiophores* micronematous or semi-macronematous, multiseptate, extensively branched, flexuous or straight, hyaline to

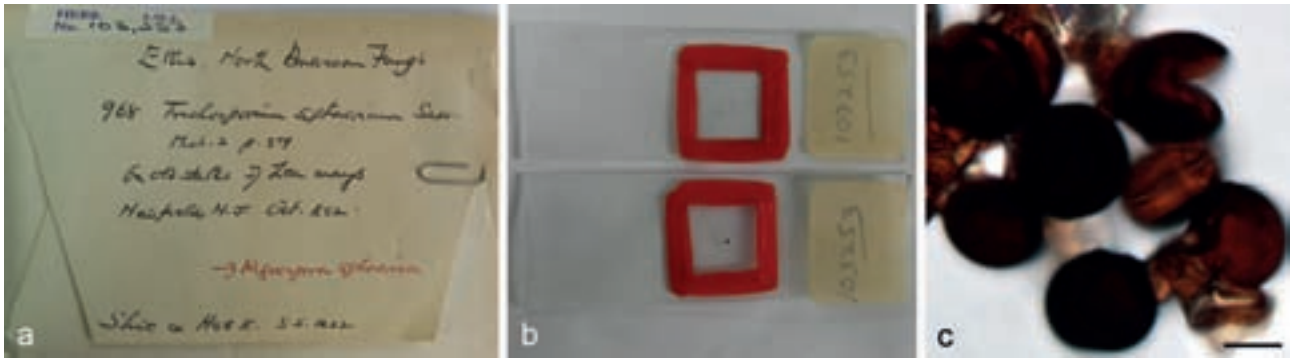


Fig. 18 *Nigrospora sphaerica* (from slide of holotype K(M) 103253). a–b. Overview of the type specimen; c. conidia. — Scale bars = 10 µm.

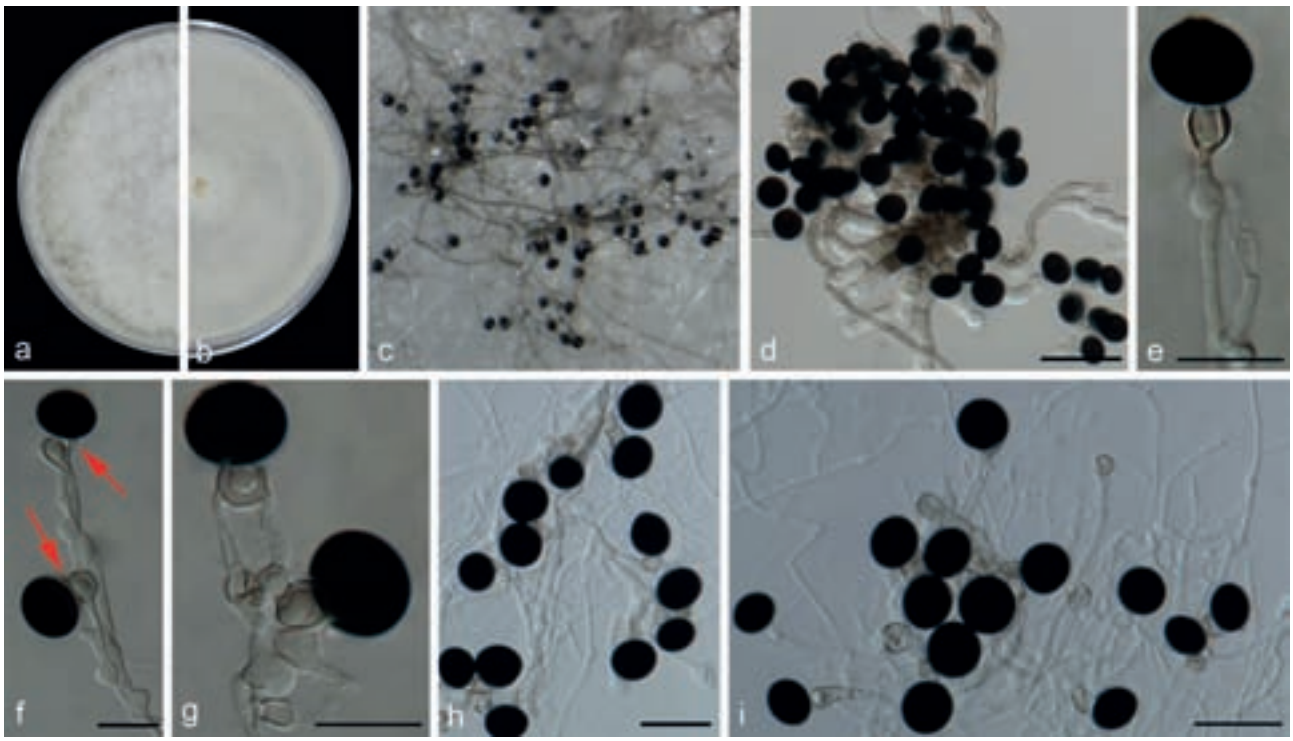


Fig. 19 *Nigrospora sphaerica* (from strain LC2840). a–b. Upper surface and reverse overview of culture 6 d after inoculation on PDA medium; c. colony on SNA; d–g. conidiogenous cells giving rise to conidia; h–i. conidia. — Scale bars: d, h–i = 20 µm; e–g = 10 µm.

pale brown, smooth, 4–8 µm thick; *hyaline vesicles* (Fig. 19, arrowed) usually surrounding the septum to delimit the conidia and their conidiogenous cells. *Conidiogenous cells* pale brown, monoblastic, determinate, subspherical, 6–12 µm diam (av. = 7.97 ± 0.99). *Conidia* are formed abundantly, solitary, globose or subglobose, black, shiny, smooth, aseptate, 16–21 (mostly 16–19) µm diam (av. = 18.22 ± 1.0).

Culture characteristics — On PDA, colonies floccose, margin circular. Colonies initially white, becoming black with age, reaching 9 cm diam in 6 d at 25 °C. On SNA, colonies flat, spreading, with abundant aerial mycelia, surface greyish and reverse olivaceous grey without patches, sporulating profusely.

Additional specimens examined. China, Hainan Province, on *Musa paradisiacal*, 24 Dec. 2015, *F.J. Liu*, living culture LC7026 = WM260; ibid., living culture LC6969 = WM 203; ibid., living culture LC6996 = WM 230; ibid., living culture LC 6998 = WM 232; Jiangxi Province, on *Nelumbo* sp., 25 Feb. 2014, *X.X. Zhan*, living culture LC7294 = JAUCC0005; ibid., living culture LC7295 = JAUCC0006; ibid., living culture LC7296 = JAUCC0007; ibid., living culture LC7312 = JAUCC0009; ibid., living culture LC7298 = JAUCC00030; on *Nelumbo* sp., 21 Sept. 2015, *M.F. Hu*, living culture JAUCC0705; ibid., living culture LC7304 = JAUCC0706; on submerged wood, 24 Aug. 2014, *X.T. Wu*, living culture LC5944, ibid., living culture LC5966; ibid., living culture LC5901; ibid., living culture LC5932; on *Rosa* sp., 2 Sept. 2013, *N. Zhou*, living culture LC2705; on *Camellia sinensis*, 7 Sept. 2013, *Y. Zhang*, living culture LC 3420; ibid., living culture LC3477; on *Rhododendron* sp., 5 Sept. 2014, *Y.H. Gao*, living culture LC4174; ibid., living culture LC4263; ibid., living culture LC4264; ibid., living culture LC4274; ibid., living culture LC4278; ibid., living culture LC4291; ibid., living culture LC4303; ibid., living culture LC4307; ibid., living culture LC4372; on *Daphniphyllum macropodum*, 5 Sept. 2013, *Y.H. Gao*, living culture LC4293; on *Deutzia* sp., 5 Sept. 2013, *Y.H. Gao*, living culture LC4241; unknown host, 5 Sept. 2013, *Y.H. Gao*, living culture LC4447; Sichuang Province, on *Cleyera japonica*, 5 Oct. 2012, *F. Liu*, living culture LC2958; on *Camellia* sp., 5 Oct. 2013, *F. Liu*, living culture LC2983; Yunnan Province, on *Camellia sinensis*, 16 Apr. 2015, *F. Liu*, living culture LC6294 = LF1301; on *Harpullia longipetala*, 15 Sept. 2011, *F. Liu*, living culture LC2839; ibid., living culture LC2840.

Notes — We examined the type specimen of *N. sphaerica* from K, and the conidia were revealed to be globose or subglobose, 16–19(–21) µm diam. The conidial size of all strains in the clade of *N. sphaerica* (Fig. 2) are consistent with that of the type specimen, and the vesicle structures presented in all of these strains. Although no sequence data were available from the type specimen for comparison, we concluded that these strains represented *N. sphaerica*. In addition, the ITS sequence of *N. sphaerica* isolate QY-6 (KP731976) causing leaf blight on *Camellia sinensis*, also clustered in this clade (Liu et al. 2015). However, this isolate was not added into the multi-locus phylogenetic analysis in this study as all loci could not be successfully amplified.

Nigrospora vesicularis Mei Wang & L. Cai, *sp. nov.* — MycoBank MB820738; Fig. 20

Etymology. *Vesicularis*, referring to the vesicle, a structure surrounding the septum, delimiting the conidium from its conidiogenous cell.

Type. China, Hainan province, on *Musa paradisiaca*, 25 Dec. 2015, *F.J. Liu* (HMAS 247071 holotype, ex-type living culture CGMCC 3.18128 = LC7010 = WM244).

Hyphae smooth, hyaline, branched, septate, 1.5–4 µm diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* monoblastic, discrete, solitary, determinate, scattered, hyaline to pale brown, smooth, doliiform to ampulliform, 7–12.5 × 6–9 µm (av. = 9.13 ± 1.12 × 7.04 ± 0.75). *Hyaline vesicles* (Fig. 20, arrowed) usually surrounding the septum to delimit the conidia from their conidiogenous cells. *Conidia* sparse, solitary, globose, subglobose, black, shiny, smooth, aseptate, 12.5–16.5 µm diam (av. = 14.8 ± 0.76, n = 50); or ellipsoidal, 12.5–16.5 × 9–15 µm (av. = 14.7 ± 0.7 × 11.63 ± 1.09).

Culture characteristics — On PDA, colonies floccose, entire edge. Colony surface white to greyish and pale luteous reverse, reaching 9 cm diam in 6 d at 25 °C. On SNA, colonies flat, surface dirty white and reverse dirty white to greyish without patches, with abundant aerial mycelia, but with delayed and sparse sporulation.

Additional specimen examined. Thailand, Chiang Rai, endophyte of unknown host plant, s.d., *D.S. Manamgoda*, living culture LC0322.

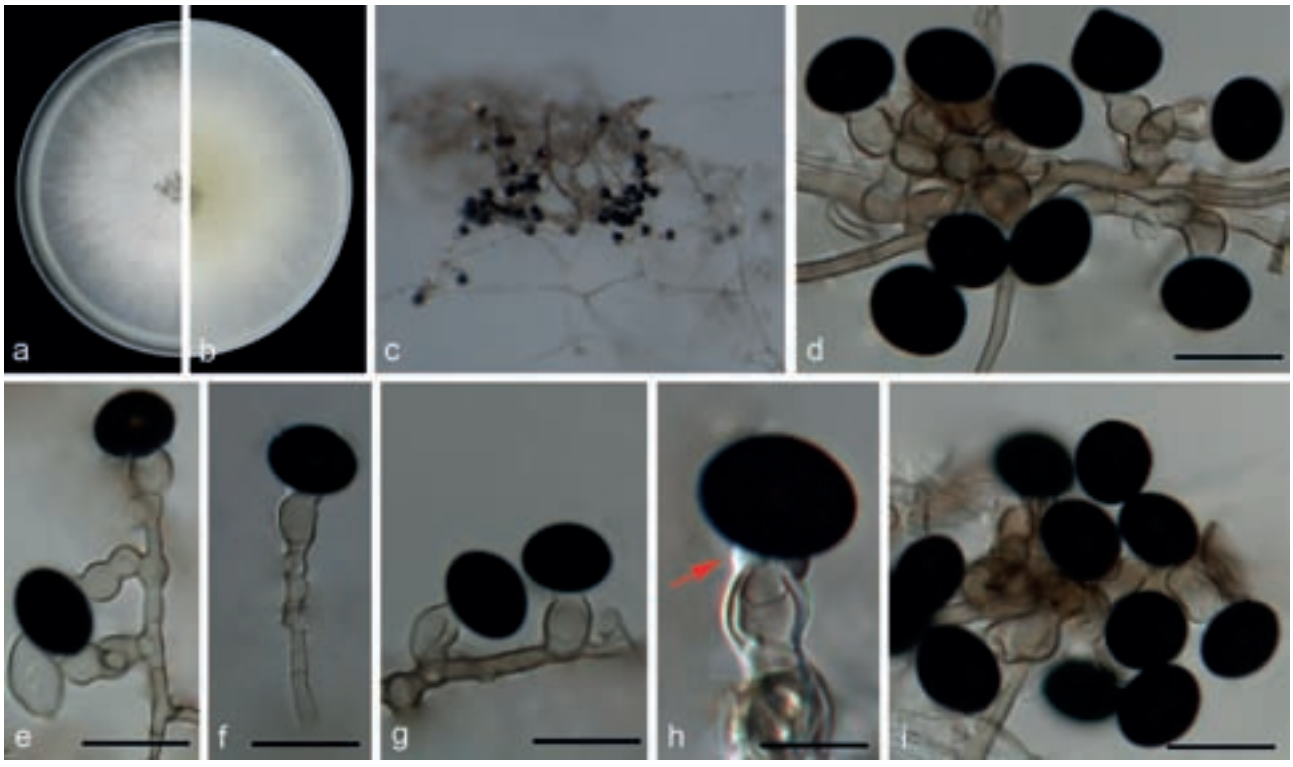


Fig. 20 *Nigrospora vesicularis* (from ex-type strain CGMCC3.18128). a–b. Upper surface and reverse overview of culture 5 d after inoculation on PDA medium; c. colony on SNA; d–h. conidiogenous cells giving rise to conidia; i. conidia. — Scale bars = 10 µm.

Notes — Two strains representing *N. vesicularis* clustered in a well-supported clade, and appeared closely related to *N. aurantiaca* (99 % identity in ITS; 89 % in *TEF1-α*; 96 % in *TUB2*), *N. lacticolonia* (99 % in ITS; 87 % in *TEF1-α*; 93 % in *TUB2*) and *N. osmanthi* (99 % in ITS; 88 % in *TEF1-α*; 93 % in *TUB2*). *Nigrospora vesicularis* differs from *N. aurantiaca*, *N. lacticolonia* and *N. osmanthi* by the presence of vesicles surrounding the septum between its conidiogenous cells and conidia. In addition, conidia of *N. vesicularis* (globose, 12.5–16.5 µm; ellipsoidal, 12.5–16.5 × 9–15 µm) are much smaller than those of other *Nigrospora* species which produce vesicles, e.g., *N. panici* (25–30 × 22–25 µm), *N. sphaerica* (16–21 µm diam) and *N. musae* (15–19.5 µm diam).

Nigrospora zimmermanii Crous, *sp. nov.* — MycoBank MB820739; Fig. 21

Etymology. Named for Albrecht Wilhelm Phillip Zimmerman (1860–1931), who introduced the genus *Nigrospora*.

Type. Ecuador, Ingenio Valdez, on leaf of *Saccharum officinarum*, 1962, *J.L. Bezerra* (CBS H-23018 holotype, ex-type living culture CBS 290.62 = IMI 129007).

Hyphae hyaline, smooth, branched, septate, 2–3.5 µm diam. *Conidiophores* solitary or aggregated in sporodochia, subcylindrical, hyaline to pale brown, smooth, 0–2-septate, branched or not, with terminal conidiogenous cells, 10–50 × 3–7 µm. *Conidiogenous cells* monoblastic, discrete, determinate, smooth, hyaline to pale brown, ampulliform, 10–20 × 5–7 µm. *Conidia* solitary, spherical or ellipsoid, dark brown, granular, smooth, (11–)14–16(–18) × (14–)15–16(–18) µm (av. = 15 × 15.5).

Culture characteristics — Colonies on PDA floccose, margin circular, regular, reaching 9 cm diam in 7 d at 25 °C, surface and reverse olivaceous grey. On SNA, spreading, flat, with immersed mycelia and sparse aerial hyphae.

Additional specimens examined. Brazil, Salvador, Bahia, on leaf of *Saccharum officinarum*, Oct. 1969, *C. Ram*, CBS H-15168, living culture CBS 984.69 = DSM 3392. – Unknown location, *C. van Overeem*, living culture CBS 167.26.

Notes — Three strains representing *N. zimmermanii* clustered in a well-supported clade, and closely to *N. oryzae* (97 % identity in ITS; 82 % in *TEF1-α*; 89 % in *TUB2*). *Nigrospora zimmermanii* differs from *N. oryzae* by its larger conidiogenous cells (10–20 × 5–7 µm vs 4.0–13.0 × 3.0–8.5 µm) and different shaped, larger conidia ((11–)14–16(–18) × (14–)15–16(–18) µm vs 12–14(–16) µm diam).

SPECIES EXCLUDED FROM NIGROSPORA

Arthrinium vietnamensis (Hol.-Jech.), Mei Wang & L. Cai, *comb. nov*. — MycoBank MB820740; Fig. 22

Basionym. *Nigrospora vietnamensis* Hol.-Jech., Česká Mykol. 17: 19. 1963.

Synonym. *Arthrinium malaysianum* Crous in Crous &J.Z. Groenew., IMA Fungus 144: 2013.

Descriptions — See Jechová (1963) and Crous et al. (2013).

Specimen examined. Vietnam, on decayed fruit of *Citrus sinensis*, 1960, deposited in CABI in 1963, *V. Jechova*, ex-type living culture IMI 99670.

Notes — *Arthrinium* is morphologically similar to the genus *Nigrospora* in many aspects, such as the deeply pigmented conidia with a germ slit, presence of setae, abnormal conidia and violent spore discharge mechanism (Minter 1985, Webster 1952, 1966). The most peculiar difference between these two genera is that only one conidium is produced on each conidiogenous cell in *Nigrospora*, while the conidia of *Arthrinium* are usually produced in clusters, and two or more conidia are produced on each conidiogenous cell (Minter 1985, Crous et al. 2013).

The ex-type strain of *N. vietnamensis* (IMI 99670) produces aggregated, brown and globose conidia, about 5–6 µm diam in surface view, 3–4 µm diam in side view, and the conidia are much smaller and paler-coloured than that of other species of *Nigrospora*. In the LSU tree (Fig. 1), strain IMI 99670 is nested within the *Arthrinium* clade, and cluster together with *A. malaysianum*. Other analyses based on ITS phylogeny (results not shown) also demonstrated that the ex-type strain of *N. vietnamensis* and *A. malaysianum* are conspecific. Morphological data available herein also support that these two species should be conspecific. Therefore, a new combination *Arthrinium vietnamensis* is proposed because *Nigrospora vietnamensis* is the older name.

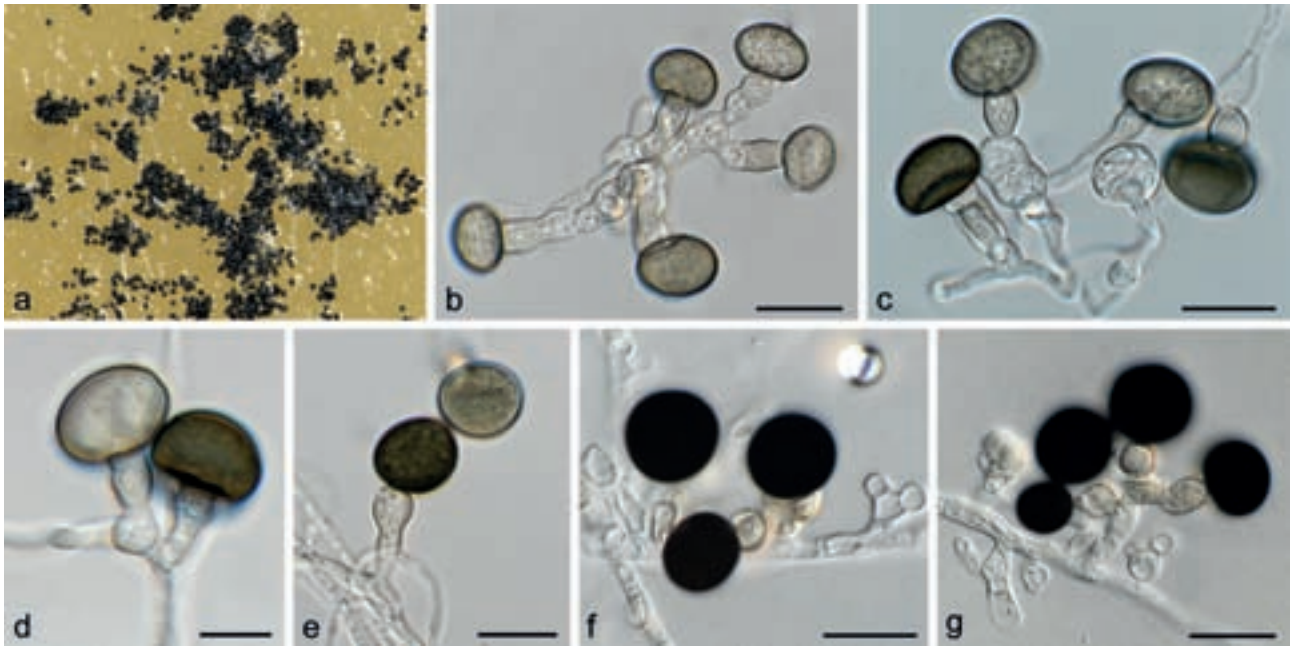


Fig. 21 *Nigrospora zimmermanii* (from ex-type strain CBS 290.62). a. Sporulating colony on MEA medium; b–g. conidiogenous cells giving rise to conidia on SNA. — Scale bars = 10 µm.

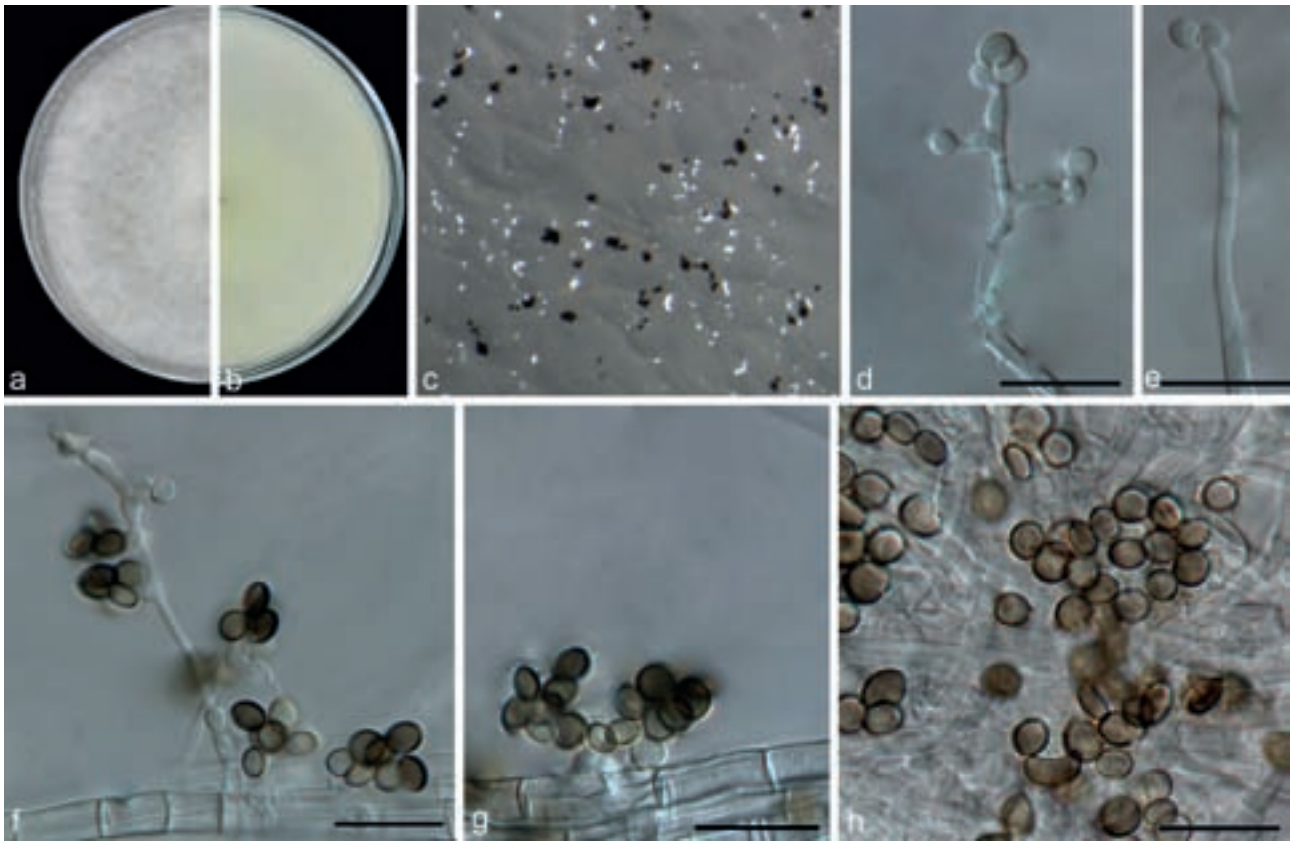


Fig. 22 *Arthrinium vietnamensis* (from ex-type strain IMI 99670). a–b. Upper surface and reverse overview of culture 6 d after inoculation on PDA medium; c. colony on SNA; d–g. conidiogenous cells giving rise to conidia; h. globose conidia in surface view. — Scale bars = 10 µm.

DISCUSSION

In this study *Nigrospora* was confirmed as belonging to the family *Apiosporaceae* (*Xylariales*, *Sordariomycetes*). Based on the newly accepted unitary nomenclature (Hawksworth et al. 2011), the sexual morph, *Khusia*, is accepted as synonym of *Nigrospora*. In previous studies, species of *Nigrospora* were primarily delimited via a comparison of morphological characters, especially conidial dimensions (Mason 1927, 1933). However, as we have shown here, conidial sizes frequently overlap among morphologically similar, but phylogenetically distinct species of *Nigrospora*. For instance, conidia of *N. musae* (15–)16–18(–19.5 µm) and *N. sphaerica* 16–19(–21) µm are similar, but the two species are phylogenetically distinct (Fig. 2). Overlapping morphologies are commonly observed among *Nigrospora* species, such as *N. osmanthi* and *N. camelliae-sinensis*, as well as *N. lacticolonia* and *N. vesicularis*, resulting in ambiguity in the traditional taxonomic treatments of this genus. The phylogenetic investigations among *Nigrospora* species in this study significantly stabilise the taxonomy of the genus, as well as provide a classification framework for future species discovery. It also underlines the fact that in future studies species of *Nigrospora* would best be distinguished based on a combination of morphological and molecular data, rather than one without the other.

This study contributed to an increase in the number of known species in *Nigrospora* from 15 to 27, with the descriptions of five previously known species (i.e., *N. arundinacea*, *N. gorlenkoana*, *N. musae*, *N. oryzae* and *N. sphaerica*) emended with additional characters (conidiogenous cells, sterile cells and the presence of vesicles and setae) through careful examination of type specimens or fresh collections. New species were characterised employing morphological and molecular characters, as well as information of host associations and ecological distributions. Another two distinct clades (Fig. 2) representing two distinct phylogenetic species are not named and described because they remained sterile in culture in spite of all attempts to induce sporulation.

Type specimens of a few known species in *Nigrospora* have not been available for molecular study, which to some extent impeded the full resolution of species relationships. For example, the conidial dimensions of *N. gossypii* (12–13.6 µm diam) was inseparable from that of *N. oryzae* (12.5–16 µm diam). Jaczewski (1929), however, treated them as distinct species based on the fact that the latter had only been recorded on monocotyledons, and was not known from Russia and Central Asia. The type of the genus, *N. panici*, was reported from *Panicum amphibium* from Java (Zimmerman 1902) and its holotype has been lost. Unfortunately, to date we have been unable to find a suitable specimen to neotypify this species. Nevertheless, *Nigrospora* (= *Khusia*) has been shown to be a monophyletic genus in the *Apiosporaceae*.

Overall the data presented here revealed that, for the most part, species of *Nigrospora* do not display evidence of host or geographical limitation (Palmateer et al. 2003, Wu et al. 2014, Eken et al. 2016). Comparing the heatmap (Fig. 23) with the phylogeny (Fig. 1–2), it is noteworthy that the top three most ubiquitous *Nigrospora* species (i.e., *N. sphaerica*, *N. oryzae*, *N. chinensis*), all belong to early divergent species in the genus (Fig. 2). On the other hand, species hitherto only known from a single host genus include *N. arundinacea*, *N. bambusae*, *N. canescens*, *N. gorlenkoana*, *N. gossypii*, *N. hainanensis*, *N. javanica*, *N. padwickii* and *N. rubi* (Fig. 23). Among these, *N. bambusae*, *N. gorlenkoana, N. hainanensis* and *N. rubi* have available DNA sequences and thus have been analysed for their phylogenetic relationships. Interestingly, these four species clustered in the upper part of the tree (Fig. 2), which unambiguously belong to the recently evolved taxa in the genus. This is a strong indication that the general evolutionary trend in *Nigrospora* is from species with a wide to a narrow host range.

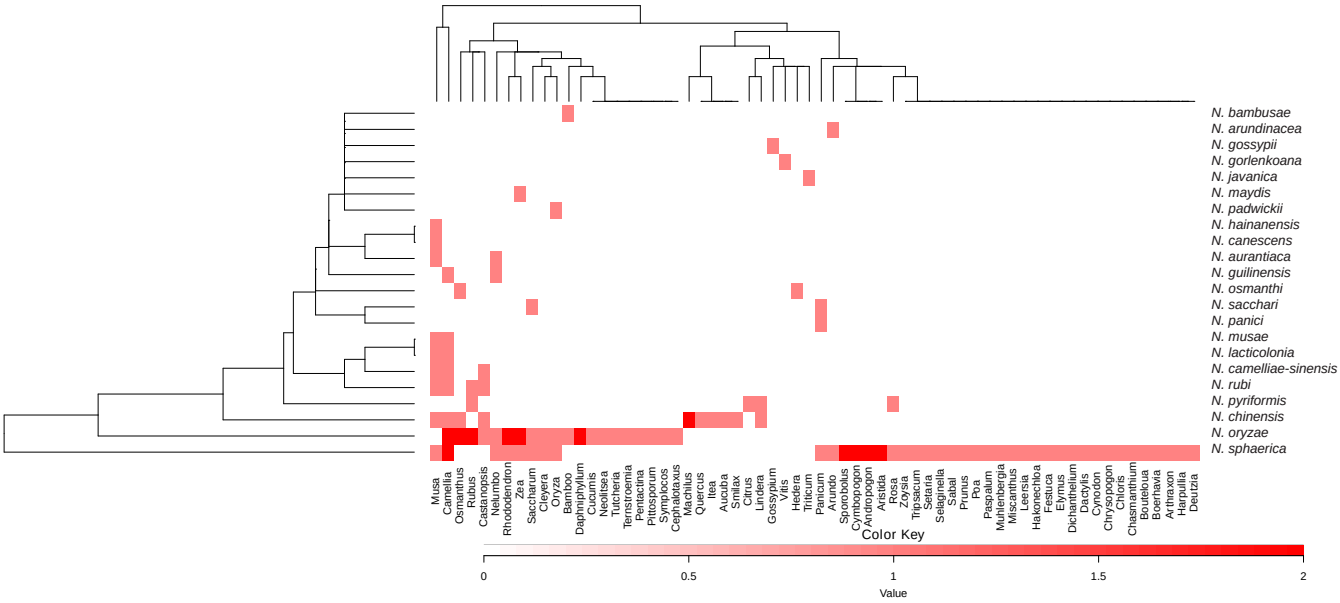


Fig. 23 Heat-map showing the fungal distribution on host (genus level).

The latter generally refers to species that are considered to be plant pathogens, and that are more important to agriculture and forestry management.

Acknowledgements We thank Qian Chen, Yu Zhang, Yahui Gao, Nan Zhou, Jiarui Jiang, Xin Zhou, Xiaoling Zhang and Dianming Hu for their help in data analysis and providing strains. We kindly acknowledge the curator of the Kew herbarium for providing access to type specimens. This work was financially supported by the NSFC 31400017, and Key Research and Development Program of Guangxi Province (2016AB07288). Mei Wang acknowledges the CAS grant QYZDB-SSW-SMC044 for supporting her post graduate studentship. Mieke Starink and Ewald Groenewald are sincerely thanked for providing access to DNA sequence data of CBS strains.

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