Article

Occurrence and identification of *Nothophoma spiraeae* sp. nov. in China

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Abstract

Nothophoma as a phoma-related genus comprises plant pathogens, endophytes and saprobes with several hosts. In this study, three fresh strains were isolated from *Spiraea salicifolia* in Beijing, China. Both morphological observation and multi-locus phylogenetic analyses (ITS, LSU, *rpb2* and *tub2*) suggest the position of the new species in *Nothophoma*, which forms a monophyletic lineage with strong support. *Nothophoma spiraeae* sp. nov. is characterized by having dense and fluffy colony, producing abundant pycnidia with aseptate conidia, and differs from its relatives in sequence data and by host association.

Keywords: Didymellaceae; phylogeny; new species; *Nothophoma*; taxonomy

Introduction

Nothophoma was established by Chen *et al*. (2015) with five *Phoma* species clustered in a monophyletic clade in Didymellaceae. Most phoma-related genera include important plant pathogens, some of which are of quarantine concern (Aveskamp *et al*. 2010; Chen *et al*. 2015). *Nothophoma* includes 10 species, i.e. *N. anigozanthi*, *N. arachidis-hypogaeae*, *N. gossypiicola*, *N. infossa*, *N. macrospora*, *N. multilocularis*, *N. pruni*, *N. quercina*, *N. raii* and *N. variabilis* (Chen *et al*. 2015; Crous *et al*. 2016, 2017; Abdel-Wahab *et al*. 2017; Valenzuela-Lopez *et al*. 2018; Chethana *et al*. 2019). This genus is characterized by globose to elongated pycnidia, producing ovoid, oblong to ellipsoidal, hyaline but incidentally brown, aseptate conidia (Chen *et al*. 2015). Most species of *Nothophoma* have some overlapping morphological characteristics. Therefore, the reliable identification should be based on DNA sequence data contacted with morphology and ecology (Quaedvlieg *et al*. 2013; Verkley *et al*. 2014).

The host genus *Spiraea* belongs to the Rosaceae family, which is widely used in landscape greening as an ornamental shrub with pretty leaves and blossoms (Lu 1996). Furthermore, during the collecting trips of phytopathogens in China, some *S. salicifolia* trees were observed to suffer from dieback and stem canker caused by fungal pathogens (Zhu *et al*. 2018). In the current study, three *Nothophoma* specimens were collected from *Spiraea salicifolia* in Beijing, China. Morphology and phylogenetic analyses using combination of ITS, LSU, *rpb2* and *tub2* sequences indicated a new species in *Nothophoma*. *Nothophoma spiraeae* sp. nov. is introduced here with descriptions, illustrations and comparison with other species in the genus.

Materials and methods

Specimens collection and isolation
Fresh specimens of *Nothophoma* were collected from *Spiraea salicifolia* during collecting trips in Beijing, China. The mucoid spore mass from the conidia were suspended in a drop of sterile water. The spore suspension of each sample was then placed on the surface of 1.8 % potato dextrose agar (PDA) culture medium and cultured in a culture dish at 25 °C. After 24 h, the single germinated conidia were transferred to fresh PDA culture plate. Samples and isolates of the new species were deposited in the Museum of Beijing Forestry University (BJFC) and living cultures were deposited in the China Forestry Culture Collection Center (CFCC) as in Fan *et al*. (2020).

Morphology observation
Specimens were observed to record the structure and size of pycnidia and the size and shape of vesicles and spores. The spore masses were diluted and the suspension droplets were placed on the slide of the microscope. The macro-morphological characteristics were recorded by Leica stereomicroscope (M205FA), and the micro-morphological results were measured by differential interference contrast (DIC) Nikon compound microscope (Eclipse 80i). More than 20 pycnidia were sliced vertically and horizontally, and 50 conidia were randomly selected for length and width measurement. Cultural characteristics of isolates on PDA incubated in the dark at 25 °C were recorded, which included colony color (Rayner 1970) and pycnidial structure, at 3, 7 and 30-days. Adobe Bridge CS v. 6 and Adobe Photoshop CS v. 5 were used for the manual editing. Nomenclatural novelties and descriptions were deposited in MycoBank (Crous *et al*. 2004).

DNA extraction, PCR amplification, and sequencing
Genomic DNA was extracted from fungal mycelia scraped from PDA cellophane by modified CTAB method (Doyle & Doyle 1990). The ITS region was amplified with the primers ITS1 and ITS4 (White *et al*. 1990); the LSU region was amplified using the primers LR0R and LR7 (Vilgalys & Hester 1990); the *rpb2* region with RPB2-5F and fRPB2-7cR (Liu *et al*. 1999) and the partial *tub2* region with Bt-2a and Bt-2b (Glass & Donaldson 1995). The amplified products of PCR were conveniently identified by 2 % agarose gel electrophoresis. The PCR products were sequenced in two directions using the PCR primers and the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequences were joined and their quality was checked by using Seqman v.7.1.0 in the DNASTAR lasergene core suite software (DNASTAR Inc.; Madison, WI).

DNA sequence analysis
The analysis using the combined dataset (ITS, LSU, *rpb2* and *tub2*) was performed to compare *Nothophoma* species from the current study with other strains in GenBank. Sequences were aligned using MAFFT v.6 (Katoh & Standley 2013) and edited manually using MEGA v.6.0 (Tamura *et al*. 2013). Some characters were excluded from both ends of the alignments so that the size of the sequence is unified to those contained in the dataset.

MP analysis was carried out using PAUP v.4.0b10 with a heuristic search option of 1,000 random-addition sequences with a tree bisection and reconnection (TBR) as the branch swapping algorithm (Swofford 2003). Zero length branches were collapsed, nevertheless, all equally parsimonious trees were saved. The stability of clade was evaluated by 1,000 repeated bootstrap analyses (Hillis & Bull 1993). Other measures calculated parsimony scores were consistency index (CI), rescaled consistency (RC), tree length (TL) and retention index (RI). ML analysis was carried out by RAxML v.7.2.8 with a GTR+G+I model of site substitution, which includes estimation of gamma-distributed rate heterogeneity and a proportion of invariant sites (Stamatakis 2006). Branch supports from MP and ML analyses were evaluated by 1,000 repeated bootstrapping methods (Hillis & Bull 1993).

Bayesian analysis (BI) employing a Markov Chain Monte Carlo (MCMC) algorithm was performed using in MrBayes v.3.1.2 with the inverse gamma rates (GTR+I+G) nucleotide substitution model, selected based on the AIC criterion, using MrModeltest v.2.3 (Posada & Crandall 1998; Ronquist & Huelsenbeck 2003). Two MCMC chains were run from random trees for 1,000,000 generations, and trees were sampled by each 100th generation, resulting in 10,000 total trees. The first 25 % of trees were discarded as the burn-in phase of each analysis and the Bayesian posterior probabilities (BPP) were calculated to assess the remaining 7,500 trees (Rannala & Yang 1996). Phylograms were examined in Figtree v.1.3.1 (Rambaut & Drummond 2010). Novel sequences generated in the current study were deposited in GenBank (Table 1) and the aligned matrices used for phylogenetic analyses and the resulting trees in TreeBASE (www.treebase.org; accession number: S25432).

## Results

The combined ITS, LSU, *rpb2* and *tub2* dataset from 16 ingroup strains representing 11 species of *Nothophoma* (sequences of three strains from this study and sequences of 13 strains available in GenBank). The alignment including gaps comprised 2,719 characters of which 2,447 characters were constant, 100 variable characters were parsimony-uninformative, and 172 were parsimony informative. MP analyses generated 200 parsimonious trees, one of which is presented in Fig. 1 (CI = 0.705, RI = 0.719, RC = 0.507). The topologies of ML and BI analyses were similar to the MP tree. The isolates CFCC 53928, CFCC 53929 and CFCC 53930 represented a monophyletic clade with high support values (MP/ML/BI = 97/100/0.99) in genus *Nothophoma*.

TABLE 1. Strains of *Nothophoma* spp. used in the molecular analyses in this study.

Species[1]	Strain	Host	Location	ITS	LSU	rpb2	tub2	References
N. anigozanthi	CBS 381.91[ET]	*Anigozanthus maugleisii*	The Netherlands	GU237852	GU238039	KT389655	GU237580	Chen *et al.* 2015
N. arachidis-hypogaeae	CBS 125.93	*Arachis hypogaea*	India	GU237771	GU238043	KT389656	GU237583	Chen *et al.* 2015
N. gossypiicola	CBS 377.67	*Gossypium* sp.	USA	GU237845	GU238079	KT389658	GU237611	Chen *et al.* 2015
N. infossa	CBS 123395[NT]	*Fraxinus pennsylvanica*	Argentina	FJ427025	GU238089	KT389659	FJ427135	Chen *et al.* 2015
N. macrospora	UTHSC DI09-853 = CBS 140674[T]	Human respiratory tract	USA	LN880536	LN880537	LT593073	LN880539	Crous *et al.* 2016
N. multilocularis	AUMC-12003[T]	*Rhazya stricta*	Saudi Arabia	KY996744	NA	NA	NA	Abdel-Wahab *et al.* 2017
N. pruni	MFLUCC 18-1600	*Prunus avium*	Beijing, China	MH827007	MH827028	MH853664	MH853671	Chethana *et al.* 2019
N. pruni	JZB380015	*Prunus avium*	Beijing, China	MH827004	MH827025	MH853661	MH853668	Chethana *et al.* 2019
N. pruni	MFLUCC 18-1601[T]	*Prunus avium*	Beijing, China	MH827005	MH827026	MH853662	MH853669	Chethana *et al.* 2019
N. pruni	JZB380017	*Prunus avium*	Beijing, China	MH827006	MH827027	MH853663	MH853670	Chethana *et al.* 2019
N. quercina	CBS 633.92 = ATCC 36786	*Microsphaera alphitoides*	Ukraine	GU237900	EU754127	KT389657	GU237609	Chen *et al.* 2015
N. raii	MCC 1082[T]	Soil	India	MF664467	NA	NA	MF664468	Crous *et al.* 2017
N. spiraeae	**CFCC 53928[T]**	**Spiraea salicifolia**	**Beijing, China**	MN737833	MN737828	MN879292	MN879295	In this study
N. spiraeae	**CFCC 53929**	**Spiraea salicifolia**	**Beijing, China**	MN737834	MN737829	MN879293	MN879296	In this study
N. spiraeae	**CFCC 53930**	**Spiraea salicifolia**	**Beijing, China**	MN737832	MN737830	MN879294	MN879297	In this study
N. variabilis	UTHSC DI16-285 = CBS 142457[T]	Human respiratory tract	USA	LT592939	LN907428	LT593078	LT593008	Valenzuela-Lopez *et al.* 2018
Phoma herbarum	CBS 615.75	*Rosa multiflora* cv. *Cathayensis*	the Netherlands	FJ427022	KF251715	KP330420	KF252703	Valenzuela-Lopez *et al.* 2018
Vacuiphoma bulgarica	CBS 357.84	*Trachystemon orientale*	Bulgaria	GU237837	GU238050	LT623256	GU237589	Valenzuela-Lopez *et al.* 2018

Notes: AUMC: Assuit University Mycology Center, Assuit, Egypt; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CFCC: China Forestry Culture Collection Center, Beijing, China; JZB: Beijing Academy of Agriculture and Forestry Sciences culture collection, Beijing, China; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; UTHSC: Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, Texas, USA. The new strains from the current study are in bold. Ex-type, ex-epitype and ex-neotype strains are marked with a T, ET and NT, respectively.
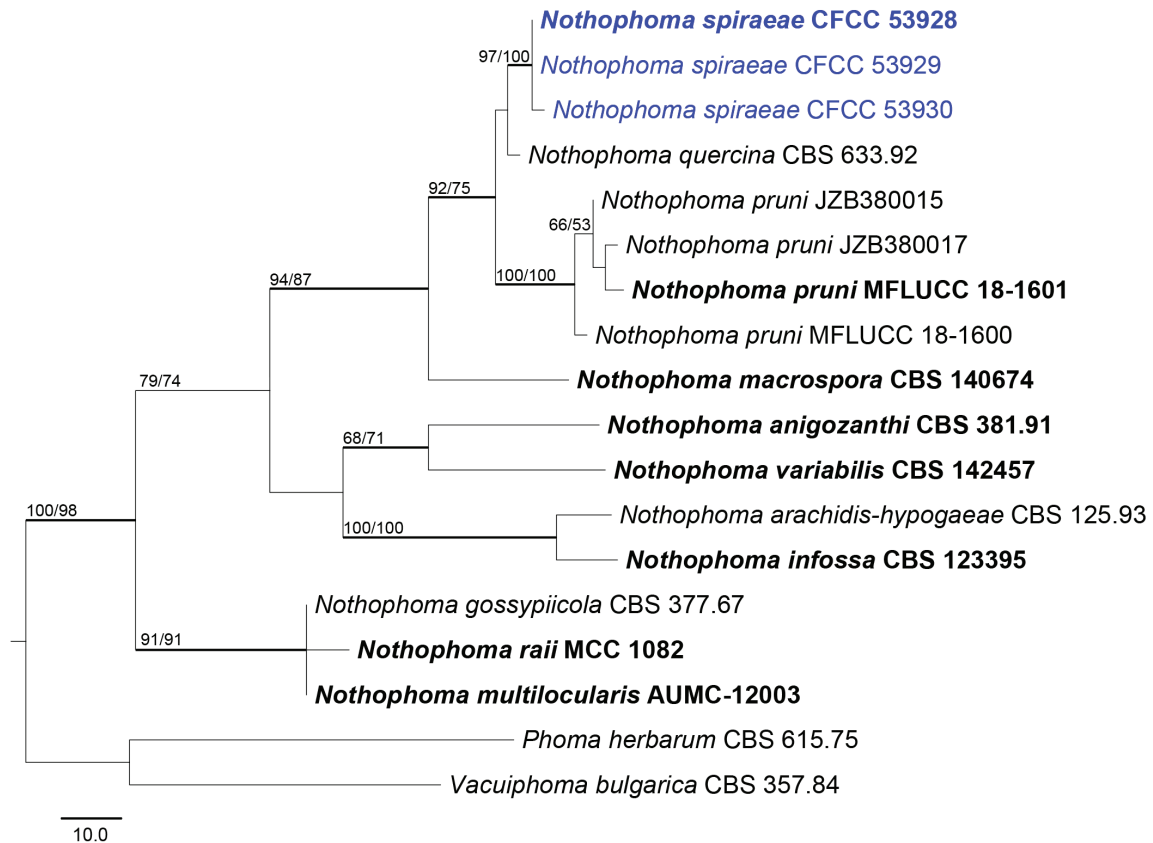
FIGURE 1. Phylogram of *Nothophoma* based on combined ITS, LSU, *rpb2* and *tub2* genes. MP and ML bootstrap support values above 50% are shown at the first and second position. Thickened branches represent posterior probabilities above 0.95 from BI. Ex-type, ex-epitype and ex-neotype strains are in bold. Strains in current study are in blue.

Taxonomy

Nothophoma spiraeae L.X. Zhang & X.L. Fan *sp. nov.* (Fig. 2)
Mycobank MB 833556

Holotype:—**China, Beijing City,** Huairou District, Labagoumen Primeval Forest, from branches of *Spiraea salicifolia*, June 2018, X.L. Fan, deposited by X.L. Fan, holotype BJFC CF20186815, ex-type living culture CFCC 53928.
    Etymology:—Named after the host genus on which it was collected, *Spiraea salicifolia*.
   *Descriptions*:—Asexual morph: Pycnidia solitary or aggregated, globose to subglobose, glabrous, olivaceous buff, superficial on or semi-immersed in agar, (65–)70–130 μm diam; pycnidia with age becoming black, broadly globose to irregular, with some white hyphal outgrows and a clear elongated neck around ostioles, (145–)155–280(–300) × (120–)140–230(–250) μm. Ostioles 1–4(–6) μm, on a distinctly elongated neck (up to 170 μm). Pycnidial wall pseudoparenchymatous, 3–6-layered, 16–41 μm thick, composed of isodiametric cells, outer wall 2–3-layered, pigmented. Conidiogenous cells phialidic, hyaline, smooth, ampulliform to doliiform, 5–9 × 4.5–7.5 μm. Conidia hyaline but incidentally olivaceous buff, ovoid, oblong to ellipsoidal, smooth, thin-walled, aseptate, 5–6.5(–7) × (3–)3.5–4 μm (av. = 5.7 ± 0.6 × 3.6 ± 0.3 μm, n = 50), sometimes with several very small guttules. Sexual morph: not observed.
    Culture characteristics:—on PDA, cultures were hazel, flat, with a thick texture in the middle and thin texture surrounding at 3 days, and darkened gradually after 7–10 days. Colony were dense and fluffy, with abundant pycnidia, irregular distribution on the surface of the medium, producing creamy white conidial matrix drop.
    Material examined:—**China, Beijing City,** Huairou District, Labagoumen Primeval Forest, from branches of *Spiraea salicifolia*, June 2018, X.L. Fan, deposited by X.L. Fan, BJFC CF20186816, living culture CFCC 53929; *ibid.* BJFC CF20186817, living culture CFCC 53930.

Notes:—*Nothophoma spiraeae* is associated with canker disease of *Spiraea salicifolia*, representing the first *Nothophoma* species isolated from this host. The phylogenetic inferences resolved this species as an individual clade (MP/ML/BI = 97/100/0.99) in phylogram, which was closed to *Nothophoma quercina* (Fig. 1). Morphologically, conidia of them are similar ($5–6.5 \times 3.5–4$ vs. $5.5–7.5 \times 3–4.5$ μm). However, the pycnidia of *N. spiraeae* are longer than *N. quercina*, especially in width ($155–280 \times 140–230$ vs. $95–200 \times 65–130$ μm). Conidia of *N. spiraeae* are olivaceous, while conidia of *N. quercina* are brown (Aveskamp *et al*. 2010) (Fig. 2). Considering the clearly distinction between these two species based on sequence data and the host affiliation, *Nothophoma spiraeae* is thus described as a new species.
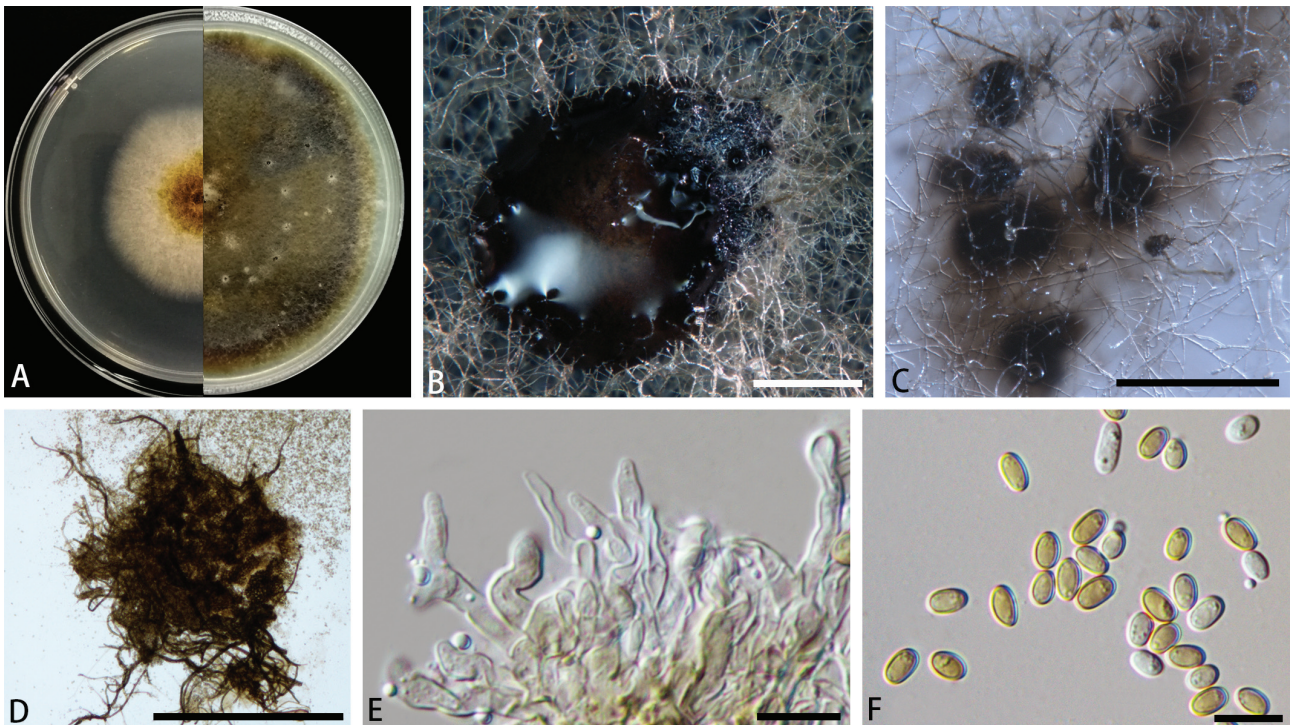
FIGURE 2. Morphology of *Nothophoma spiraeae* (BJFC CF20186815). A: Colonies on PDA after 3 d and 14 d. B, C: Pycnidia. D, E: Conidiophores and conidiogenous cells. F: Conidia. Scale bars: B–D = 500 μm; E, F = 10 μm.

Key to species of *Nothophoma*

1. Conidia below 10 μm in length 2
1. Conidia over 10 μm in length 5
2. Pycnidia below 250 μm in length 3
2. Pycnidia over 250 μm in length...4
3. Parasitic on *Arachis hypogaea*; pycnidia globose to bottle-shaped, 80–200 μm; conidia $3.2–5.2 \times 1.8–2.4$ μm ....
.... *N. arachidis-hypogaeae*
3. Parasitic on *Fraxinus pennsylvanica*; pycnidia subglobose to elongated $190–250 \times 140–180$ μm; conidia $4.5–6 \times 2.5–3.5$ μm ......
....*N. infossa*
3. Parasitic on *Microsphaera alphitoides*; pycnidia globose to subglobose, $95–200 \times 65–130$ μm; conidia $5.5–7.5 \times 3–4.5$ μm..........
....*N. quercina*
4. Parasitic on *Anigozanthus* spp.; pycnidia olivaceous buff and turn black with age, $155–280 \times 140–230$ μm; conidia $3.5–5 \times 1.5–2.5$ μm...*N. anigozanthi*
4. Parasitic on *Prunus avium*; pycnidia black, 220–430 μm; Conidia $4.8–8.5 \times 2.7–3.9$ μm.. *N. pruni*
4. Isolated from human respiratory tract; pycnidia brown, $150–350 \times 130–270$ μm; conidia $4–7 \times 3–3.5$ μm ....
....*N. variabilis*
4. Parasitic on *Spiraea salicifolia*; pycnidia olivaceous buff, $155–280 \times 140–230$ μm; conidia $5–6.5 \times 3.5–4$ μm ....
.... *N. spiraeae*
5. Parasitic on *Gossypium* spp.; pycnidia honey turn to olivaceous black with age, 100–250 μm; conidia$10–12.5 \times 2.5–3.5$ μm ....
....*N. gossypiicola*
5. Isolated from clinical human; pycnidia dark-brown, 100–300 μm; conidia $10–15 \times 2.5–3$ μm ....
....*N. macrospora*
5. Endophyte of *Rhazya stricta*; pycnidia black, 175–1500 μm; conidia $9–20 \times 3–4$ μm ....
....*N. multilocularis*
5. Isolated from soil; pycnidia olivaceous, $195–315 \times 195–410$ μm; conidia $11–14.5 \times 1.5–2.5$ μm.. *N. raii*

TABLE 2. Comparison of species in *Nothophoma*.

Species	Pycnidia	Conidiogenous cells	Conidia	Chlamydospores	Host	References
N. anigozanthi	Pycnidia 70–130 μm diam, solitary or aggregated, olivaceous buff, turn black with age, 155–280 × 140–230 μm. Ostioles 1–4 on long neck. Wall made up of 3–6 layers 16–41 μm thick.	Phialidic, hyaline, ampulliform to doliiform, 5–9 × 4.5–7.5 μm.	Ellipsoidal, aseptate, hyaline, with several minute guttules, 3.5–5 × 1.5–2.5 μm.	NA	Parasitic on *Anigozanthus* spp.	Chen *et al.* 2015
N. arachidis-hypogaeae	Pycnidia 80–200 μm in diam, globose to bottle-shaped, solitary or in raws, not confluent, papillate, citrine-honey then olivaceous to black. Wall made up of 3–5 layers, outer layers pigmented.	Globose to bottleshaped, 3–8 × 3–7 μm.	Oblong to ellipsoidal, aseptate, hyaline, without or with two minute polar guttules, 3.2–5.2 × 1.8–2.4 μm.	NA	Parasitic on *Arachis hypogaea.*	Chen *et al.* 2015
N. gossypiicola	Pycnidia 100–250 μm in diam, globose to subglobose, solitary or confluent, without or with one nonpapillate ostiole, honey, later olivaceous to black Walls made up of 3–10 layers of cells.	Globose to bottleshaped, 5–8 μm diam.	Ellipsoidal, aseptate, hyaline, with several minute guttules, 10–12.5 × 2.5–3.5 μm.	Globose to elongate, usually in chains, olivaceous with greenish guttules, 8–12 μm diam.	Parasitic on *Gossypium* spp.	Chen *et al.* 2015
N. infossa	Pycnidia 190–250 × 140–180 μm, mostly solitary, subglobose to elongated. Ostioles mostly single 40–75 μm diam. Wall 5–9 layers, 28.5–55 μm thick.	Phialidic, hyaline, simple, smooth, flaskshaped, 5.5–8 × 5–5.5 μm.	Ovoid, aseptate, hyaline, 4.5–6 × 2.5–3.5 μm.	Honey to cinnamon, dictyosporous or phragmosporous, solitary or forming long chains, 18–32 × 11.5–17 μm.	Parasitic on *Fraxinus pennsylvanica.*	Chen *et al.* 2015
N. macrospora	Pycnidia 100–300 μm in diam pyriform, dark-brown, 2–3 necks. Wall 3–5 layers, 15–25 μm.	Enteroblastic, phialidic, globose to flask-shaped, hyaline, 5–10 μm diam.	Ellipsoidal or clavate, septate, hyaline, guttulate, 10–15 × 2.53 μm.	NA	Isolated from human clinical specimen.	Crous *et al.* 2016

TABLE 2. (Continued)

Species	Pycnidia	Conidiogenous cells	Conidia	Chlamydospores	Host	References
N. multilocularis	Pycnidia globose, uniloculate to multiloculate or confluent with up to 6 long necks diam. Wall 38–80 μm thick, 8–18 cell layers.	Phialidic, flask-shaped or polygonal, hyaline to yellow-brown 11–17 × 9–18 μm.	Ellipsoidal or clavate aseptate, hyaline, with a few minute polar guttules, 9–20 × 3–4 μm.	globose, subglobose to polygonal, brown to darkbrown, 10–16 μm diam.	endophytic of *Rhazya stricta*.	Abdel-Wahab *et al.* 2017
N. pruni	NA	phialidic, hyaline, simple, doliiform to ampulliform, variable in size.	Ellipsoidal to obovoid or oblong, aseptate, hyaline, 4.8–8.5 × 2.7–3.9 μm.	NA	Parasitic on diseased leaves of *Prunus avium*.	Chethana *et al.* 2019
N. quercina	Pycnidia 65–130 × 95–200 μm solitary, globose to subglobose with single non-papillate ostiole. Wall 8.5–14.5 μm.	Phialidic, hyaline, smooth, doliiform to ampulliform, 3.5–5 × 3–4 μm.	Ellipsoidal to oval or obtuse, aseptate, hyaline, 5.5–7.5 × 3–4 μm.	NA	Parasitic on *Quercus* sp.	Chen *et al.* 2015
N. raii	Pycnidia 194–315 × 195–411 μm, solitary or confluent, globose to subglobose.	Hyaline, thin-walled, bottle-shaped.	Ellipsoidal, aseptate, hyaline, with several small, scattered guttules, 11–14.5 × 1.5–2.5 μm.	Elongated barrel-shaped, olivaceous brown, 11–21.5 μm × 4.5–8.5 μm.	Isolated from soil.	Crous *et al.* 2017
N. spiraeae*	Pycnidia 155–280 × 140–230 μm, broadly globose to irregular, black, with some white hyphal outgrows and a clear elongated neck around the ostioles.	Phialidic, hyaline, smooth, ampulliform to doliiform, 5–9 × 4.5–7.5 μm.	Oblong to ellipsoidal, aseptate, hyaline, 5–6.5 × 3.5–4 μm.	NA	Parasitic on *Spiraea salicifolia*.	This study
N. variabilis	Pycnidia 150–350 × 130–270 μm, confluent, superficial, glabrous, subglobose, brown, with a single papillate ostiolar neck.	Phialidic, hyaline, smoothwalled, ampulliform, 6 × 5 μm.	Ellipsoidal to cylindrical or irregularly shaped, aseptate, hyaline, guttulate, 4–7 × 3–3.5 μm.	NA	Isolated from human respiratory tract.	Valenzuela-Lopez *et al.* 2018

Notes: the new species in this study is marked by an asterisk (*); NA: not applicable.

Discussion

The phoma-related species are widespread and species-rich, with species occurring on a various range of substrates, from air to soil, plants to animals, and even humans (Aveskamp *et al.* 2010). However, as most other anamorph genera, *Phoma* has largely been regarded as a form genus, instead of a phylogenetic entity (Aveskamp *et al.* 2010). Several phoma-related taxa have been re-evaluated according to phylogenetic and morphological data (Chen *et al.* 2015). *Nothophoma* was introduced as a new genus based on phylogenetic differences (ITS, LSU, *rpb2* and *tub2*) (Chen *et al.* 2015).

Nothophoma species were previously identified by host association. *Nothophoma infossa* is often associated with ash trees (*Fraxinus* sp.), *Nothophoma gossypiicola* is reported only on cotton plants (Aveskamp *et al.* 2010), *Nothophoma raii* comes from soil and *Nothophoma macrospora* is found from respiratory secretion of a patient with pneumonia (Crous *et al.* 2016). Nevertheless, not all fungal-host associations are clearly defined in Didymellaceae. The strains used in previous studies were mainly from Europe and the USA, and the number of cultures per species was still limited (Chen *et al.* 2015). In this study, *Nothophoma spiraeae* sp. nov. is described from *Spiraea salicifolia*. The morphological comparison (Table 2) and the phylogram of *Nothophoma* (Fig. 1) indicated *N. spiraeae* is a separate species with highly supported values (MP/ML/BI = 97/100/0.99).

In future studies, more extensive fresh materials should be collected to help clarify confused species concepts of phoma-like fungi, and the taxonomy requires collections from wide geographical ranges.

Acknowledgements

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