Inflatispora, a novel lignicolous genus of *Pleosporales* from France

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A collection of dead wood of *Rhamnus alpina* from the forest floor in Aude, Belcaire, Clot de la Plaine, France, led to the discovery of a new pleosporalean genus. *Inflatispora pseudostromatica* gen. et sp. nov., is described and illustrated and compared with genera with similar characteristics. In addition, *nur*DNA and RPB2 gene sequences are analyzed phylogenetically to investigate its ordinal and/or familial status. Morphologically, *Inflatispora* resembles species of *Nodulosphaeria* in having ascospores with an enlarged supramedian cell. However, it is readily distinguished from *Nodulosphaeria* by the presence of a thick pseudostroma above half-immersed ascomata and a sheath surrounding the whole ascospore versus the terminal ascospore appendages in species of *Nodulosphaeria*. Both morphological and molecular data support the inclusion of *Inflatispora* in *Massarineae*. The familial placement is, however, unclear.

Keywords: *Massarineae*, new genus, phylogeny, taxonomy

Recent biodiversity studies in France have revealed several new taxa from woody substrata (Zhang et al. 2008a, b, 2009a, b, Réblová *et al*. 2010). During these studies, we also collected an interesting pleosporalean taxon that does not correspond to any previously described species. In particular, it is characterized by widely porated ascomata, bitunicate asci, cylindrical 3-septate hyaline ascospores with a swollen upper central cell and surrounded by a thin sheath, and narrowly cellular pseudoparaphyses that anastomose between and above the asci. This paper provides a detailed morphological description of this new fungus, and in addition, *nur*DNA and RPB2 gene sequences are ana-

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lyzed under different optimal criteria to infer its phylogenetic relationships with other pleosporalean taxa.

Materials and methods

Sample collection and specimen examination

The fresh specimen was collected in April 2007 from France by J. Fournier, and gently air-dried before being studied in the laboratory and isolated. Observations and photographs were prepared from material mounted in water, chlorazol black or Indian ink. Measurements followed the procotol previously outlined in Zhang *et al.* (2012). Cultures used in this study were obtained from the Centraalbureau voor Schimmelcultures in the Netherlands (CBS).

DNA extraction, amplification and sequencing

Fungal isolates were grown on potato-dextrose agar (PDA) and malt extract agar (MEA) and total genomic DNA was extracted from mycelia following the protocols as outlined by Zhang et al. (2009b).

18S *nu*-rDNA (SSU), 28S *nu*-rDNA (LSU) and RNA polymerase subunit 2 (RPB2) have been widely used in systematic studies of *Pleosporales* (Zhang *et al.* 2008a, 2009b, Jones *et al.* 2009, Shenoy *et al.* 2010). Here we are using these three loci to study the phylogenetic relationships among *Inflatispora* and other pleosporalean taxa. DNA amplification and sequencing were performed following the protocol of Zhang *et al.* (2009b).

Sequence alignment and phylogenetic analyses

Sequences generated from different primers were analyzed with other sequences obtained from the GenBank. A Blast search was performed to find the possible sister groups of the newly sequenced taxa. In addition, fungal members from different families of the *Pleosporales* were also included in the analyses. Multiple alignment and analyses were performed according to Zhang *et al.* (2009b). A combined 18S, 28S nurDNA and RPB2 dataset was analyzed respectively in this study. Maximum Parsimony (MP) was conducted using heuristic searches as implemented in PAUP, with the default options method. Analyses were conducted under different parameters of maximum parsimony criteria as outlined in Jeewon *et al.* (2004). Clade stability was assessed in a bootstrap analysis with 1000 replicates, random sequence additions with Maxtrees set to 1000 and other default parameters as implemented in PAUP. Trees were viewed in Treeview (Page 1996). The nucleotide sequences reported in this paper have been deposited in GenBank (Table 1).

Table 1. – Species and sequences database accession numbers used in this study (newly generated sequences are indicated in bold).

Species	Source of sequences	GenBank accession no.		
		LSU	SSU	RPB2
Arthopyrenia salicis	CBS 368.94	AY538339	AY538333	NS
Bimuria novae-zelandiae	CBS 107.79	AY016356	AY016338	DQ470917
Byssothecium circinans	CBS 675.92	AY016357	AY016339	DQ767646
Cladosporium cladosporioides	CBS 170.54	DQ678057	DQ678004	DQ677952
Cochliobolus heterostrophus	CBS 134.39	AY544645	AY544727	DQ247790
Cochliobolus sativus	DAOM 226212	DQ678045	DQ677995	DQ677939
Cucurbitaria elongata	CBS 171.55	DQ678061	DQ678009	Dq677657
Curreya pityophila	CBS 149.32	DQ384102	DQ384072	NS
Davidiella macrospora	CBS 138.40	DQ008148	EU167591	NS
Davidiella tassiana	CBS 399.80	DQ678074	DQ678022	DQ677971
Dendryphiella arenaria	CBS 181.58	DQ470971	DQ471022	DQ470924
Diaporthe phaseolorum	FAU458	AY346279	AY779278	AY780175
Herpotrichia diffusa	CBS 250.62	DQ678071	DQ678019	DQ677968
Hydrocina chaetocladia	HME4375	AY789412	AY789411	NS
Inflatispora pseudostromatica	CBS 123110	**JN231131**	**JN231132**	**JN231133**
Lentithecium aquaticum	CBS 123099	FJ795434	FJ795477	FJ795455
Lentithecium arundinaceum	CBS 619.86	DQ813509	DQ813513	FJ795473
Lentithecium fluviatile	CBS 123090	FJ795450	FJ795492	FJ795467
Lentithecium fluviatile	CBS 122367	FJ795451	FJ795493	NS
Lentithecium lineare	IFRD 2008	FJ795435	FJ795478	NS
Lophiostoma arundinis	CBS 621.86	DQ782384	DQ782383	DQ782386
Lophiostoma caulium	CBS 623.86	FJ795436	FJ795479	FJ795456
Lophiostoma compressum	IFRD 2014	FJ795437	FJ795480	FJ795457
Lophiostoma crenatum	CBS 629.86	DQ678069	DQ678017	DQ677965
Lophiostoma fuckelii	CBS 101952	DQ399531	FJ795496	FJ795472
Lophiostoma glabrotunicatum	IFRD 2012	FJ795438	FJ795481	NS
Lophiostoma macrostomoides	CBS 123097	FJ795439	FJ795482	FJ795458
Lophiostoma macrostomum	Lundqvist 20504 (S)	DQ384094	NS	NS
Lophiostoma rugulosum	CBS 123093	FJ795440	FJ795483	FJ795459
Lophiostoma semiliberum	CBS 626.86	FJ795441	FJ795484	FJ795460
Lophiostoma viridarium	IFRDCC2081	FJ795443	FJ795486	NS
Lophiotrema brunneosporum	CBS 123095	FJ795444	FJ795487	NS
Lophiotrema lignicola	CBS 122364	FJ795445	FJ795488	FJ795462
Lophiotrema nucula	CBS 627.86	FJ795446	FJ795489	FJ795463
Lophiotrema vagabundum	CBS 628.86	FJ795442	FJ795485	FJ795461
Massaria platani	CBS 221.37	DQ678065	DQ678013	DQ677961
Massarina cisti	CBS 266.62	FJ795447	FJ795490	FJ795464
Massarina corticola	CBS 154.93	FJ795448	FJ795491	FJ795465

Species	**Source of sequences**	**GenBank accession no.**		
		LSU	**SSU**	**RPB2**
Massarina eburnea	CBS 473.64	FJ795449	AF164367	FJ795466
Massarina igniaria	CBS 845.96	FJ795452	FJ795494	FJ795469
Massarina phragmiticola	CBS 110446	DQ813510	DQ813512	NS
Massarina rubi	CBS 691.95	FJ795453	NS	FJ795470
Massarina walkeri	CBS 257.93	FJ795454	FJ795495	FJ795471
Melanomma pulvis-pyrius	IFRDCC 2044	FJ201984	FJ201985	FJ795474
Melanomma pulvis-pyrius	CBS 371.75	FJ201988	FJ201989	FJ795475
Neofabraea malicorticis	AFTOL-ID 149	AY544662	AY544706	DQ470885
Ophiosphaerella herpotricha	CBS 620.86	DQ678062	DQ678010	DQ677958
Ophiosphaerella herpotricha	CBS 240.31	DQ767656	DQ767650	DQ767645
Phaeodothis winteri	CBS 182.58	DQ678073	DQ678021	DQ677970
Phaeosphaeria avenaria	AFTOL-ID 280	AY544684	AY544725	DQ677941
Phaeosphaeria eustoma	CBS 573.86	DQ678063	DQ678011	DQ677959
Pleospora herbarum	CBS 714.68	DQ678049	DQ767648	DQ677943
Pleospora herbarum	CBS 191.86	DQ247804	DQ247812	DQ247794
Pleospora leptosphaerulinoides	CBS452.84	AY849956	NS	NS
Preussia terricola	AFTOL-ID 282	AY544686	AY544726	DQ470895
Sporidesmium australiense	HKUCC 10833	DQ408554	NS	DQ435080
Sporidesmium tengii	HKUCC 10837	DQ408559	NS	NS
Sporormiella minima	CBS 524.50	DQ678056	DQ678003	DQ677950
Trematosphaeria pertusa	CBS 122368	FJ201990	FJ201991	FJ795476
Westerdykella cylindrica	CBS 454.72	AY004343	AY016355	NS

NS: no sequence available in GenBank.

Taxonomy

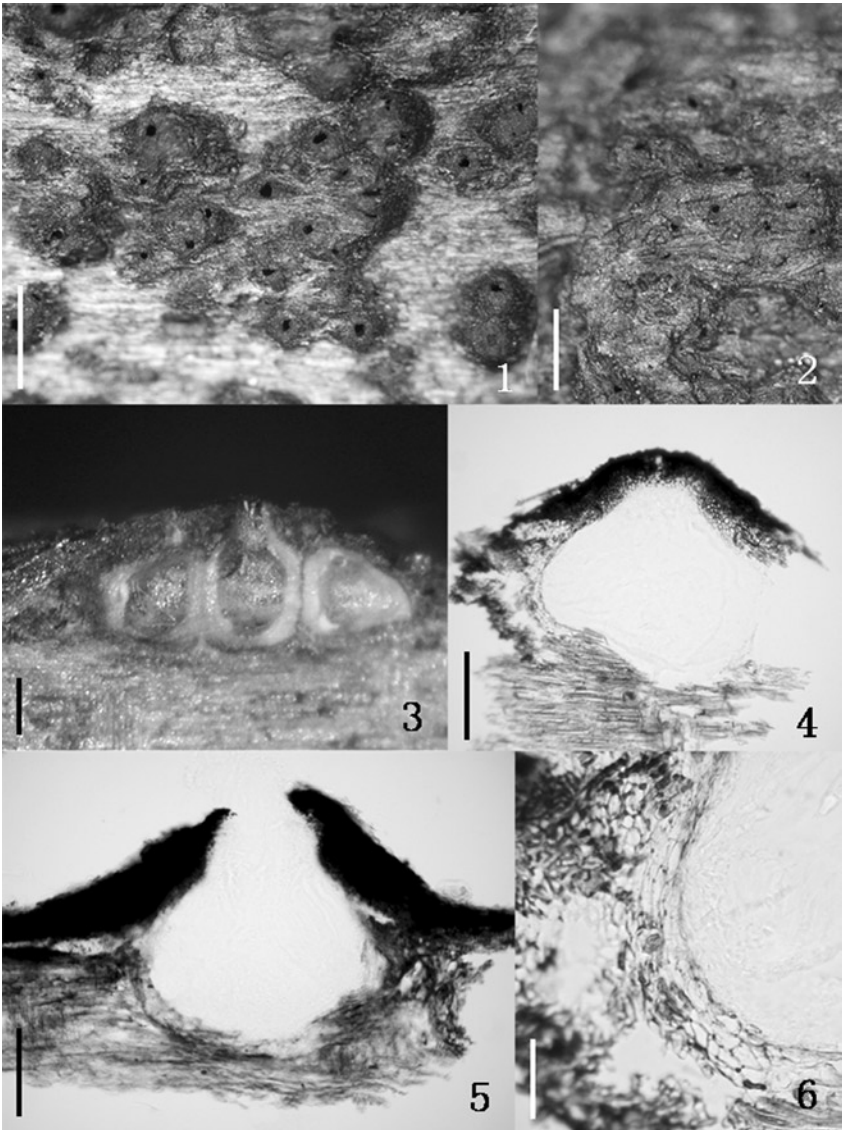
Inflatispora Yin. Zhang, J. Fourn. & K.D. Hyde, **gen. nov.**
MycoBank no.: MB 561844.

Saprophytum, lignincola. *Ascomata* globosa vel subglobosa, semi-immersa, sub pseudostroma obtecta, disperci vel gregariculi, nigra. *Asci* 8-spori, clavati, bitunicati. *Ascosporae* supra-medius cella amplifica, hyalinae.

Etymology – from the Latin "*inflati*" and "*spora*", in reference to the "swollen" centre of the slender ascospores.

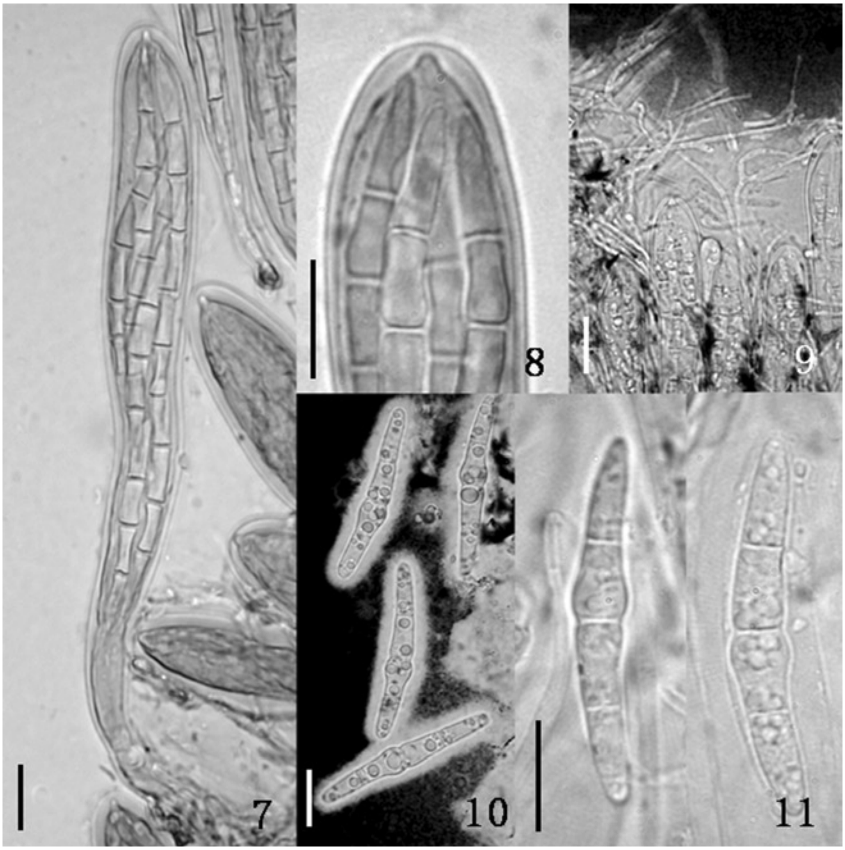
Inflatispora pseudostromatica Yin. Zhang, J. Fourn. & K.D. Hyde, **gen. et sp. nov.** – Figs. 1–11.
MycoBank no.: MB 561845.

Ascomata 250–280 µm diametro, globosa, semi-immersa, dispergere vel gregariculus, nigra. Asci 125–137 × 14–17 µm, 8-spori, clavati, bitunicati. Ascosporae 35-38 × 5-6 µm, supra-medius cella amplifico, hyalinae.



Figs. 1–6. – ***Inflatispora pseudostromatica*** (IFRD 8989, **holotype**). **1, 2.** Ascomata gregarious on the host surface (bar = 0.5 mm). **3-6.** Habit section of ascomata showing the pseudostroma and peridium structure (bars: 3-5 **=** 100 µm, 6 = 20 µm).

Ascomata 250–280 µm diam., single or in groups of 2–3, often gregarious and coalescent, erumpent, semi-immersed, globose to subglobose, hard, forming under a black pseudostroma of 50–80(–100) µm thickness forming a large black crust when ascomata are densely gregarious, opening through a broad rounded pore (Figs. 1–3). Perid-



Figs. 7–11. – ***Inflatispora pseudostromatica*** (IFRD 8989, **holotype**). **7.** Eight-spored clavate ascus with conspicuous ocular chamber. **8.** Apical part of an ascus showing the small ocular chamber. **9.** Narrowly cellular pseudoparaphyses. **10, 11.** Ascospores surrounded with mucilaginous sheath. Bars 10 µm.

ium 18–20 µm thick at base and on sides, colourless to black, of thin-walled polygonal cells at the inside, becoming thicker towards the outside, eventually being an undifferentiated amorphous black mass combined with the similar structured stroma, apex of small, thick-walled cells, nearly opaque outwardly, base mixed with host cells and often indistinguishable (Figs. 4–6). Hamathecium of dense, long narrowly cellular pseudoparaphyses 1.5–1.8 µm broad, embedded in mucilage (Indian Ink Fig. 9), diverticulate and sparsely anastomosing between and above the asci. Asci 125–137 × 14–17 µm, 8-spored, bitunicate, cylindro-clavate, with a short pedicel, with a conspicuous ocular chamber (Figs. 7, 8), fissitunicate dehiscence not observed. Ascospores 35–38 × 5–6 µm, biseriate to triseriate near the apex, narrowly fusiform to almost cylindrical with broadly to narrowly rounded ends, straight to slightly curved, hyaline, 3-septate, first septum supramedian, strongly constricted, cell above supramedian septum enlarged, smooth, containing some oil guttules (Figs. 10, 11), surrounded by a 3-4 µm thick mucilaginous sheath, visible in India ink (Fig. 10), progressively vanishing with age.

Etymology. – From the Latin "*pseudostromatica*", in reference to the *pseudostroma* possessed by the fungus.

Holotypus. – FRANCE: Aude, Belcaire, Clot de la Plaine, 950 m, on dead decorticated wood of *Rhamnus alpina* L. in forest litter, 950 m, 30 Apr 2007, leg. & det. J. Fournier (IFRD 8989), ex-type culture CBS 123110.

Discussion

Representative species of core families of *Pleosporales* as well as genera morphologically comparable to *Inflatispora* such as *Massarina*, *Lophiostoma* and *Lentithecium* were included in the phylogenetic analysis. According to the dendrogram in Plate 1, *Massarineae* got moderate support with *Inflatispora pseudostromatica* basal to other taxa of this suborder. Herein we assign *I. pseudostromatica* to *Massarineae*. The familial status is, however, undetermined because of its morphological and molecular phylogenetic separation from other families of *Massarineae* (Zhang *et al*. 2012).

Morphologically, *I. pseudostromatica* shares the character which is typical of *Nodulosphaeria* in having ascospores with an enlarged supramedian cell, which is considered the most significant character in this genus (Shoemaker 1984). The ascospores of *Nodulosphaeria,* however, have an appendage at each end (Holm 1957, Shoemaker 1984, Shoemaker & Babcock 1987), while *I. pseudostromatica* has an entire sheath. In particular, *Nodulosphaeria* occurs mostly on herbaceous dicotyledons, mainly *Asteraceae*, and usually is highly specific, at least to host genus, with one species known from *Pinaceae* and one from *Liliaceae* (Shoemaker 1984). *Inflatispora pseudostromatica* differs, however, as it occurs on the dead decorticated wood of *Rhamnus alpina* on the forest floor and has a thick pseudostroma above half-immersed ascomata as well as a widely porate ostiole. *Nodulosphaeria* is considered a genus under *Phaeosphaeriaceae* (*Pleosporineae*) (Shoemaker 1984, Zhang *et al*. 2012) although this has not been proven by DNA sequence data. *Inflatispora* seems more typical of *Massarineae* (Figs. 1–11).

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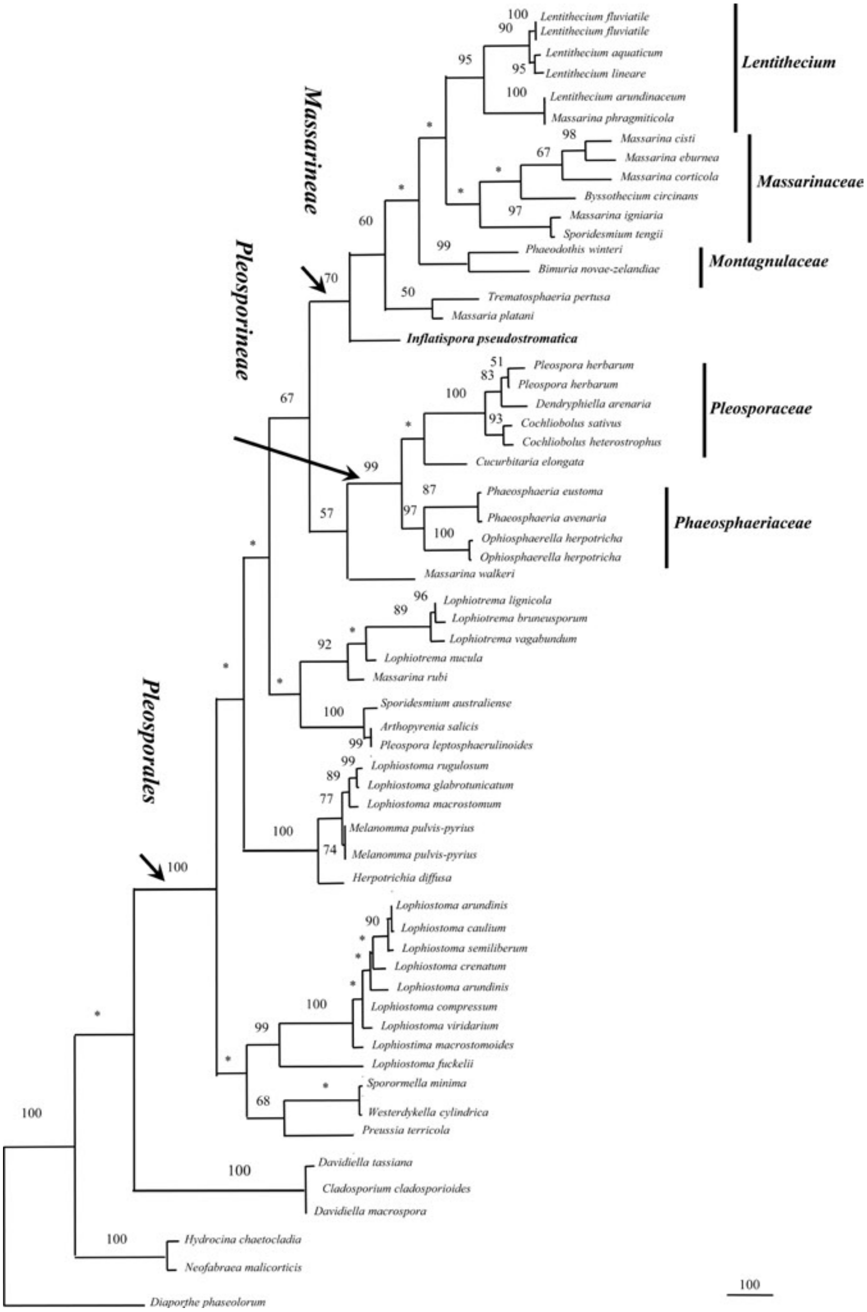

Plate 1. – The maximum parsimony tree generated based on analysis of combined 28S, 18S *nur*DNA and RPB2 sequences dataset. Designated out group is *Diaporthe phaseolorum*. Maximum parsimony bootstrap support values above 50 % shown at nodes are based on 1000 replicates.

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