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The *Podospora anserina* (Rabenh.) Niessl species complex in metropolitan and overseas France with description of a new species, *Podospora reunionensis* Silar, sp. nov.

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ABSTRACT
Podospora anserina (Rabenh.) Niessl is a model fungus that was shown to belong to a complex of seven species with morphologically indistinguishable sexual fruiting bodies. Through the morphological analyses and ITS (Intergenic Transcribed Spacer) sequences of 86 newly-isolated strains, as well as the genome sequences of five strains, we show that members of the *P. anserina* species complex present different distribution ranges in metropolitan and overseas France. *Podospora anserina* is present all-over metropolitan France, including Corsica, while *P. comata* Milovtz. is restricted to the Northern part and *P. pauciseta* (Ces.) Traverso to the Southern part of continental France. The Guadeloupe hosts *P. pseudoanserina* C.Boucher, T.S.Nguyen & P.Silar and La Réunion island a species new to science, described here as *Podospora reunionensis* Silar, sp. nov. This species is closely related to *P. comata*, but exhibit clear morphological and biological differences.

KEY WORDS
Podospora anserina species complex, Podosporaceae, Sordariales, geographical structuration, genome sequence, new species.

RÉSUMÉ

Le complexe d'espèces Podospora anserina *(Rabenh.) Niessl en France métropolitaine et outre-mer, avec la description d'une nouvelle espèce,* Podospora reunionensis *Silar, sp. nov.*

Podospora anserina (Rabenh.) Niessl est un champignon modèle qui appartient à un complexe de sept espèces indistinguables sur le critère de la morphologie de leurs fructifications sexuelles. À travers les analyses de la morphologie et des séquences ITS (Intergenic Transcribed Spacer) de 86 souches nouvellement isolées, ainsi que la détermination des séquences des génomes de cinq souches, nous montrons ici que les membres du complexe ont différentes aires de distribution en France métropolitaine et d'outremer. *Podospora anserina* est présent partout en France métropolitaine, y compris en Corse ; *P. comata* Milovtz. est restreinte dans le nord et *P. pauciseta* (Ces.) Traverso dans le sud de la partie continentale du pays. La Guadeloupe héberge *P. pseudoanserina* C.Boucher, T.S.Nguyen & P.Silar et l'île de La Réunion une nouvelle espèce décrite ici comme *Podospora reunionensis* Silar, sp. nov. Cette nouvelle espèce est très proche génétiquement de *P. comata*, mais montre de claires différences morphologiques et biologiques avec celle-ci.

MOTS CLÉS

Podospora anserina
complexe d'espèces,
Podosporaceae,
Sordariales,
structuration
géographique,
séquence génomique,
espèce nouvelle.

INTRODUCTION

Podospora anserina (Rabenh.) Niessl is a model fungus used in several laboratories to study various biological processes such as sexual reproduction, prions, ageing, signal transduction, heterokaryon incompatibility, epigenetics, plant biomass degradation and genome maintenance and evolution (Silar 2020; Bhunjun *et al.* 2024). Molecular analyses of four small DNA regions of the genome (Rchr3, Rchr4, Rchr6 and the Intergenic Transcribed Spacer-ITS) have shown that the species formerly known as "*P. anserina*" is actually a species complex with presently seven members that are indistinguishable based on morphological criteria (i.e., their perithecia, asci and ascospores appear identical), but may differ in the distribution ranges (Boucher *et al.* 2017). The different species can, however, be differentiated by their behavior when cultivated in different media, i.e., the efficiency and timing with which they produce fruiting bodies. In particular, perithecia are distributed onto the thallus differently in different species (Boucher *et al.* 2017). High quality genome sequences supported the subdivision in different species and the differences in distribution range (Vogan *et al.* 2019; 2021b; Hartmann *et al.* 2021; Ament-Velásquez *et al.* 2024). However, the phylogenomic analyses could not resolve the relationships between the different species so far, likely due to their rapid diversification (Boucher *et al.* 2017; Ament-Velásquez *et al.* 2024).

Some controversies remain regarding the naming of *P. anserina* and other species of the complex. Indeed, Wang *et al.* renamed the fungus as *Triangularia anserina* (Rabenh.) X. Wei Wang & Houbraken and placed it in the Podosporaceae family within the Sordariales order (Wang *et al.* 2019). However, this suggestion met strong resistance, especially from the scientific community working with *P. anserina* as a genetic model (Silar 2020; Ament-Velásquez *et al.* 2020; Bhunjun *et al.* 2024). The three genera defined by Wang *et al.* (2019) to accommodate the species of the Podosporaceae (i.e., *Podospora*, *Cladorrhinum* Sacc. & Marchal and *Triangularia* Boedijn) were synonymized (Ament-Velásquez *et al.* 2020) and a proposal to change the type species of the *Podospora* genus from *P. fimiseda* (Ces. & De Not.) Niessl to *P. anserina sensu stricto* (*s.s.*), as defined in Boucher *et al.* (2017), was issued (Vogan *et al.* 2021a). While the decision regarding the type species chosen for the genus *Podospora* is pending, it is best to continue to use *P. anserina* as the name for the fungus (Bhunjun *et al.* 2024).

Species from the *P. anserina* complex are coprophilous and thus frequently found on various herbivore dung, especially those of large animals, such as cows and horses (Silar 2020). However, they can also be found in soil (Silar 2020), and a strain was even isolated as an endophyte (Matasyoh *et al.* 2011). At the present time, the distribution of the various species is not well-known. In order to better understand the range distribution of the species complex, we isolated numerous new strains from herbivore dung and also from soil collected in metropolitan and overseas France. To this end, we describe here a method that enables the preferential recovery of Sordariales species from soil samples. Among the 86 strains that we isolated, two strains displayed differences in their Internal Transcribed Spacer (ITS) with those of the seven described species. We sequenced the genomes of these two strains, as well as three other strains. We calculated their average nucleotide identity (ANI) with the known type species of the complex using FungANI (Lalanne & Silar 2025) to assign them to species. Based on the ITS sequences of the 86 strains of the complex that we isolated, we found that: 1) *P. anserina s.s.* is present all-over metropolitan France, including the Corsica island, while *P. comata* Milovtz. appears more specific to the Northern part of France and *P. pauciseta* to the Southern part; 2) *P. pseudoanserina* C.Boucher, T.S.Nguyen & P.Silar is present in the Guadeloupe island from the French Antilles; and 3) a species new to science is present in the La Réunion Island from the Indian ocean (Fig. 1). This latter species is described here as *Podospora reunionensis* Silar, sp. nov. Unlike the other species from the complex that cannot be differentiated by morphology, this species produced plumpier ascospores carrying a much longer primary appendage and a bigger and much more prominent secondary appendage(s) at the base of the pedicel than the other members of the complex.

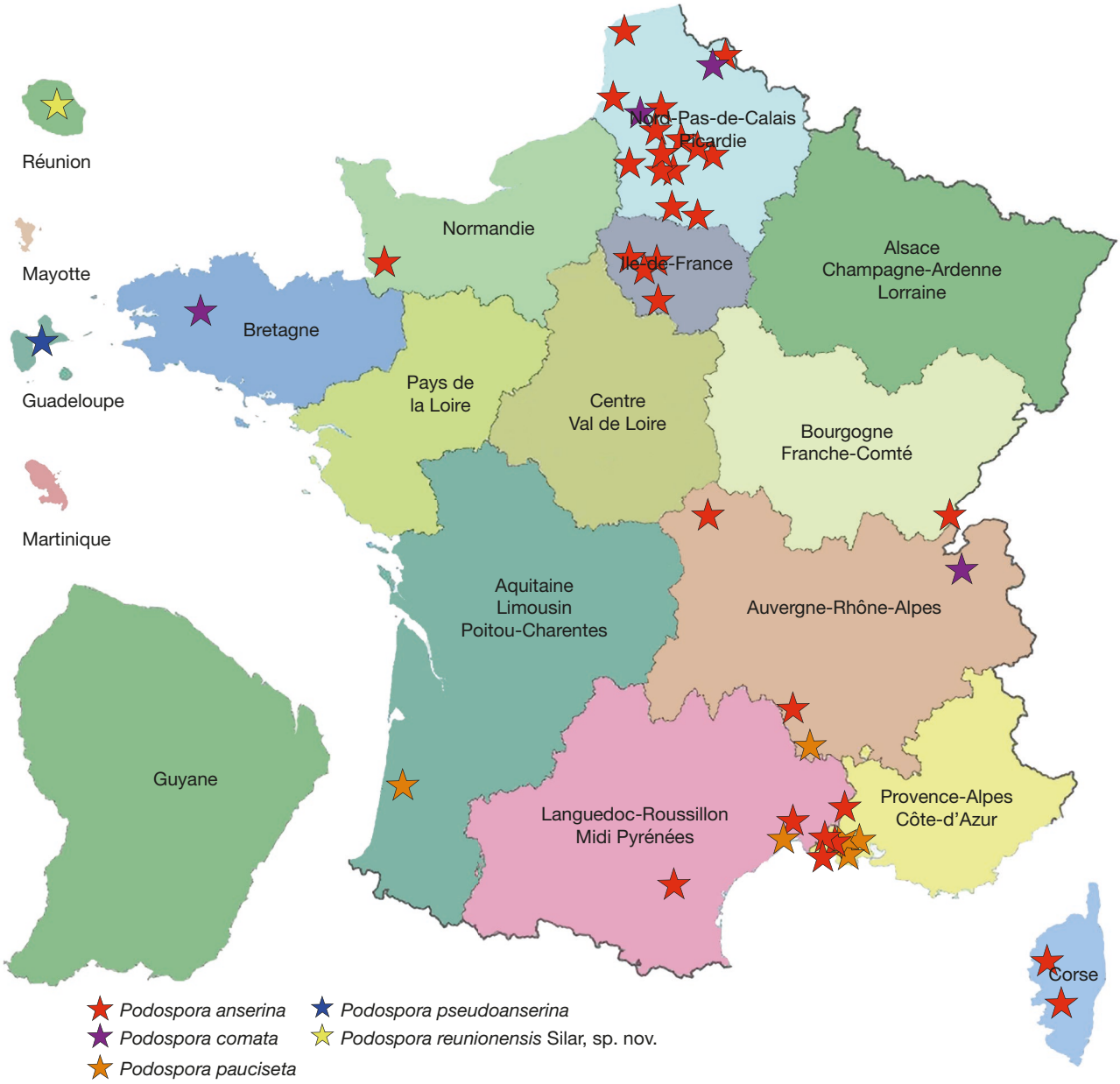
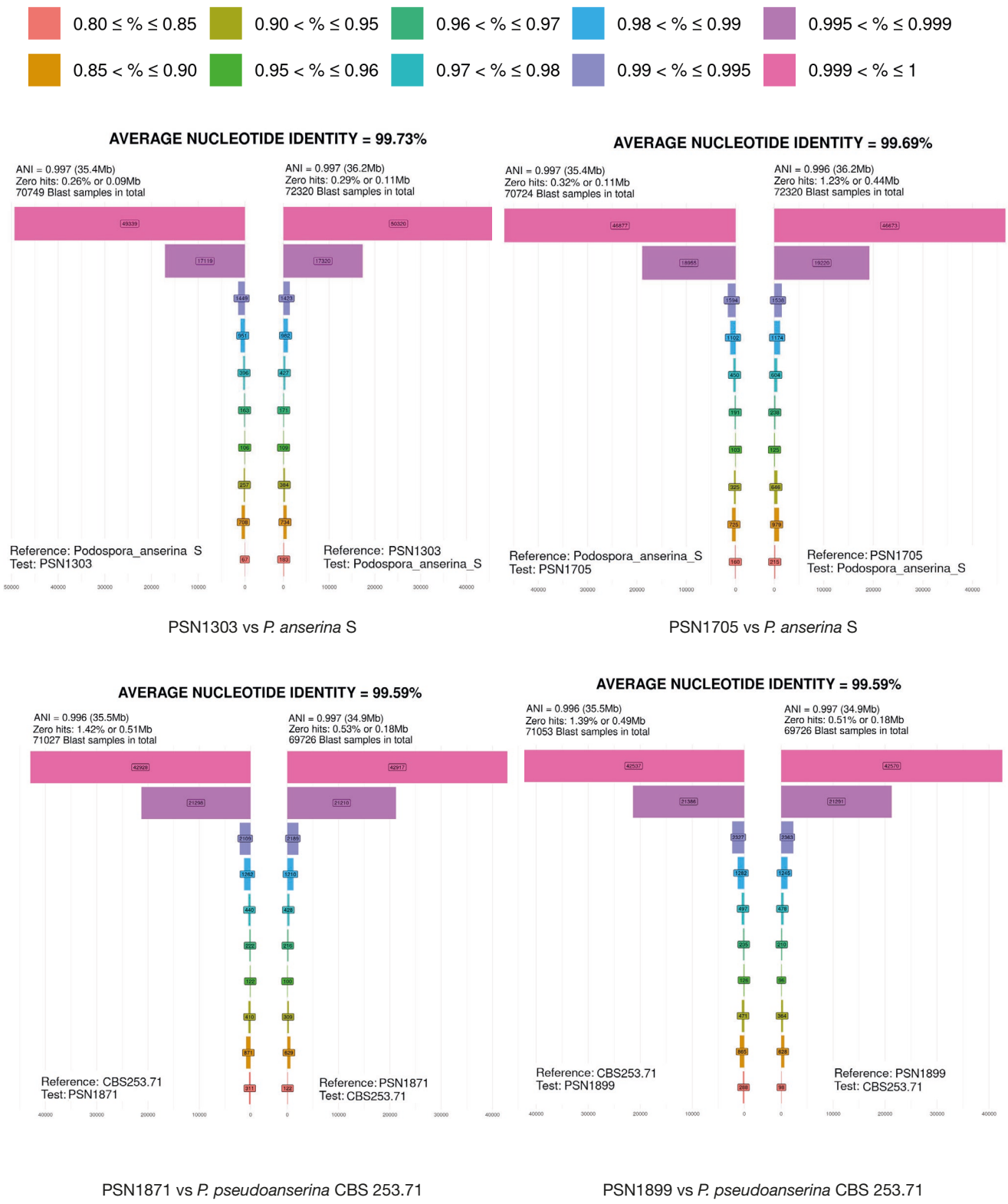


Fig. 1. — Map of France with **stars** pointing to locations where the strains of the present study originated from.

MATERIAL AND METHODS

Recovery of new strains from the *P. anserina* species complex

Strains were recovered from dung as described (Silar 2020). To isolate Sordariales strains from soils, M0 plates (0.25 g/L KH_2PO_4, 0.3 g/L K_2HPO_4, 0.25 g/L $MgSO_4$-$7H_2O$, 0.5 g/L urea, 0.05 mg/L thiamine, 0.25 µg/L biotin, 2.5 mg/L citric acid, 2.5 mg/L $ZnSO_4$, 0.5 mg/L $CuSO_4$, 125 µg/L $MnSO_4$, 25 µg/L boric acid, 25 µg/L sodium molybdate, 25 µg/L iron alum, 10 g/L agar) were supplemented with 0.5 g of shredded miscanthus by adding the complex biomass onto the plates after the agar rigidified. These were then inoculated with *c.* 0.5 mL of soil. For each soil sample, three plates were prepared. One was left at room temperature, a second one was incubated at 65°C for 30 minutes and then transferred to room temperature; the third one was incubated overnight at 37°C and then transferred to room temperature. The three plates were then left at room temperature in the presence of light for up to two months. Note that the plates were not treated with antibiotics or chemicals to prevent the growth of bacteria or the presence of small animals (such as mites and collembolans), because the stress brought by their presence and/or grazing may promote fruiting body development of Sordariales (Silar, unpublished observations). The plates were regularly checked (once or twice weekly) for the presence of perithecia typical of Sordariales fungi. If such fruiting bodies developed,



PSN1303 vs *P. anserina* S

PSN1705 vs *P. anserina* S

PSN1871 vs *P. pseudoanserina* CBS 253.71

PSN1899 vs *P. pseudoanserina* CBS 253.71

Fig. 2. — FungANI analyses of the *Podospora* Rabenh. strains PSN1303, PSN1705, PSN1871 and PSN1899. The Average Nucleotide Identities (ANIs) are at the top, and at the bottom are given the ANIs obtained when comparing the genomes in the two directions (reference and tested genome IDs are given below the graphic), as well as the percentages of zero hits, the estimated size of the region specific for each genome and the numbers of BLAST made during the analysis. The graphic represents the number of BLAST detecting sequences with the color-coded percentages and in the boxes are the actual number of BLAST with such hit percentages. In all cases, ANIs were higher than 99.5% and the strains had less than 1.5% of specific sequences. These were scattered all over the genomes. For more information see (Lalanne & Silar 2025).

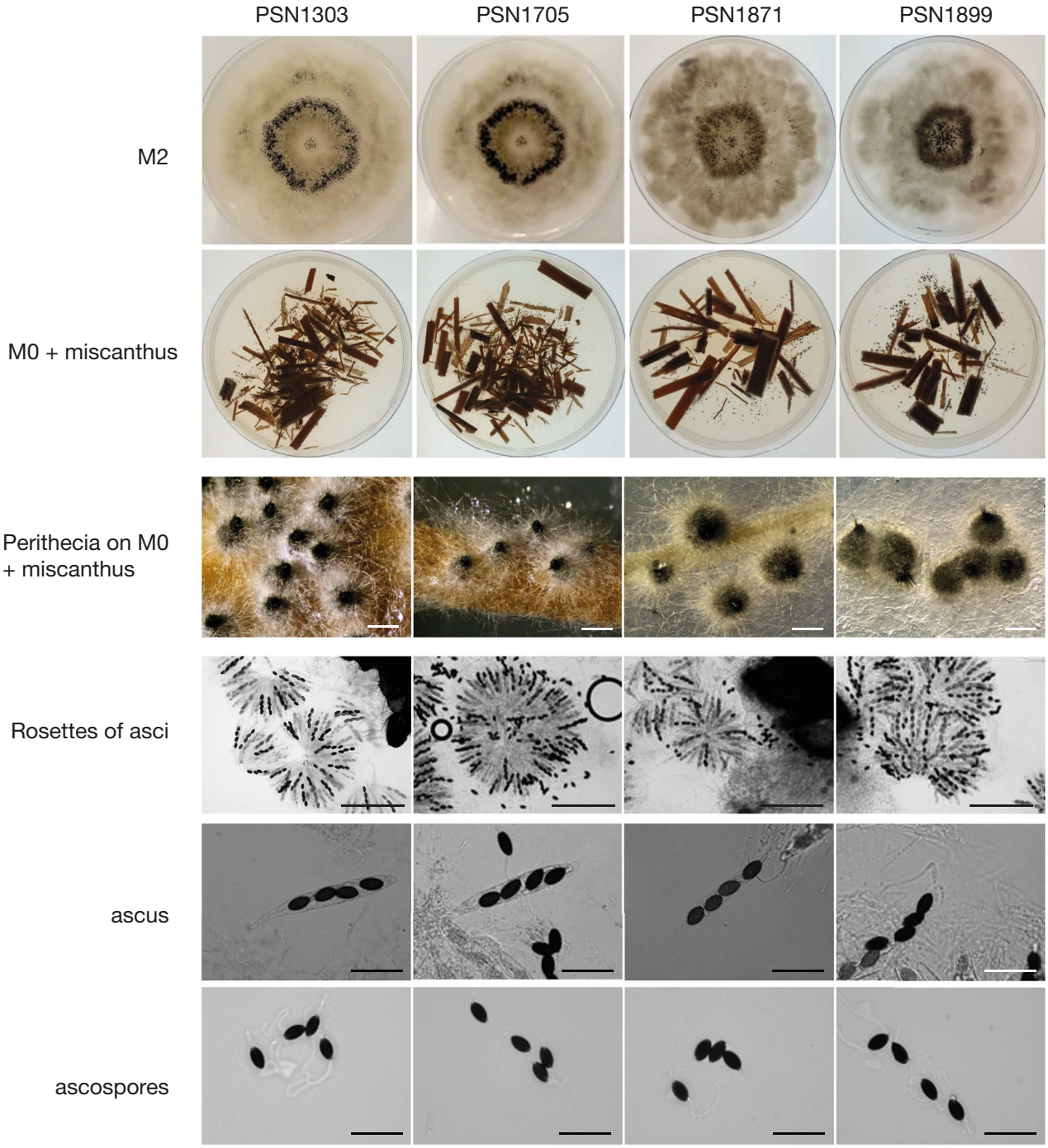


FIG. 3. — Main morphological features of PSN1303, PSN1705, PSN1871 and PSN1899. Scale bars: Perithecia on M0 + miscanthus, rosettes of asci, 250 µm; ascus, ascospores, 50 µm.

ascospores ejected from them were collected onto projection plates as described previously (Silar 2020), except that the plates were supplemented with three antibiotics (chloramphenicol 25 µg/mL, tetracycline 50 µg/mL and kanamycin 50 µg/mL) to prevent bacterial contaminations. Ascospores were then transferred onto G medium (ammonium acetate 4.4 g/L, bactopeptone 15 g/L and agar 13 g/L) supplemented with 5 g/L of yeast extract and three antibiotics (chloramphenicol 25 µg/mL, tetracycline 50 µg/mL and kanamycin 50 µg/mL). A 30-minutes heat-shock at 65°C was immediately applied to promote ascospore germination. If this failed, an overnight heat-shock at 37°C was then applied to a second batch of freshly collected ascospores. Note that in the case of strains of the *P. anserina* species complex, the presence of yeast extract in G medium and heat-shock are dispensable, while it is often mandatory for most other species of Sordariales. For species the *P. anserina* complex, the four ascospores of several asci were collected

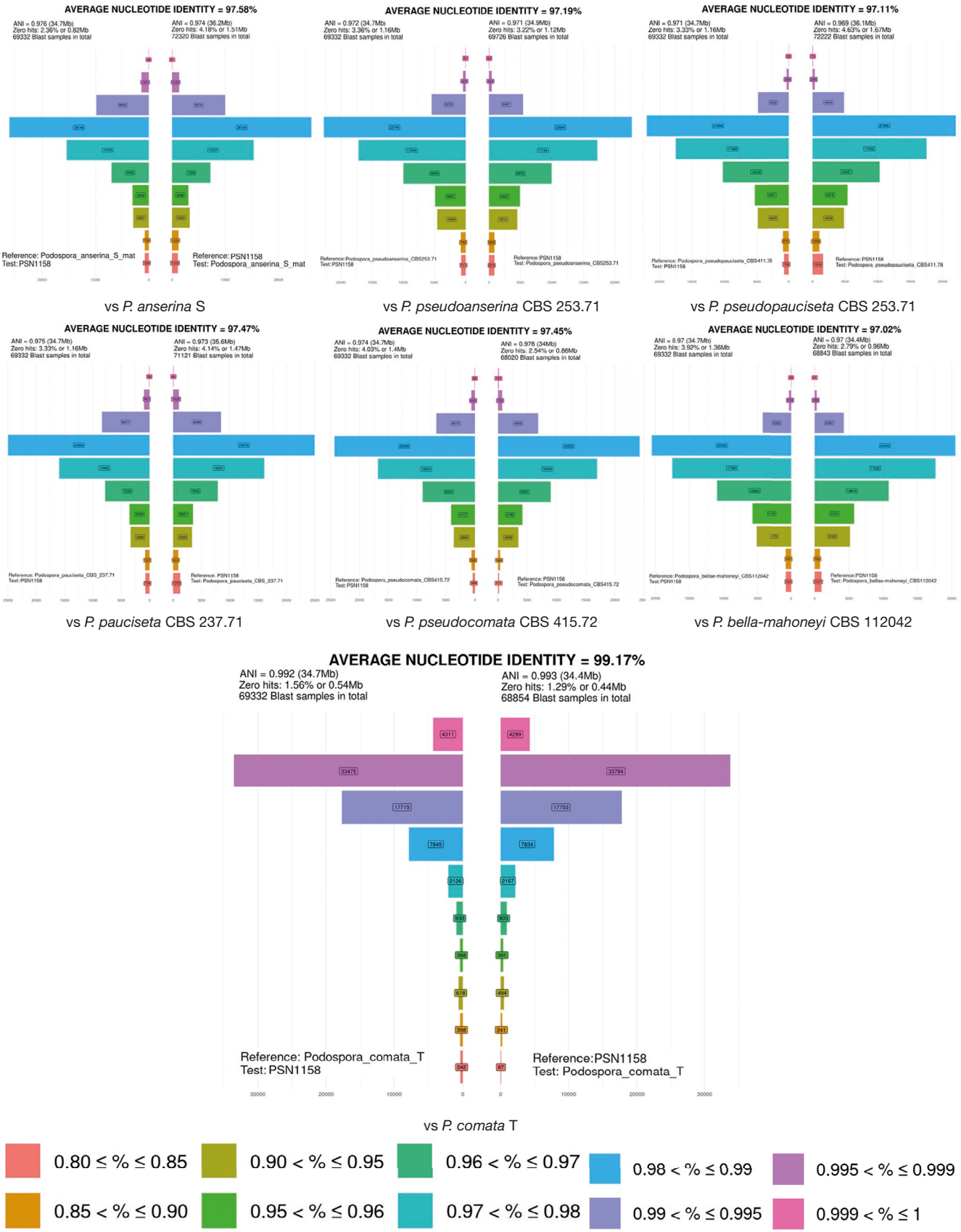


FIG. 4. — FungANI analysis of the genome of *Podospora reunionensis* Silar, sp. nov. strain PSN1158 with those of all type strains of the *P. anserina* (Rabenh.) Niessl species complex. The layouts are the same as on Figure 2.

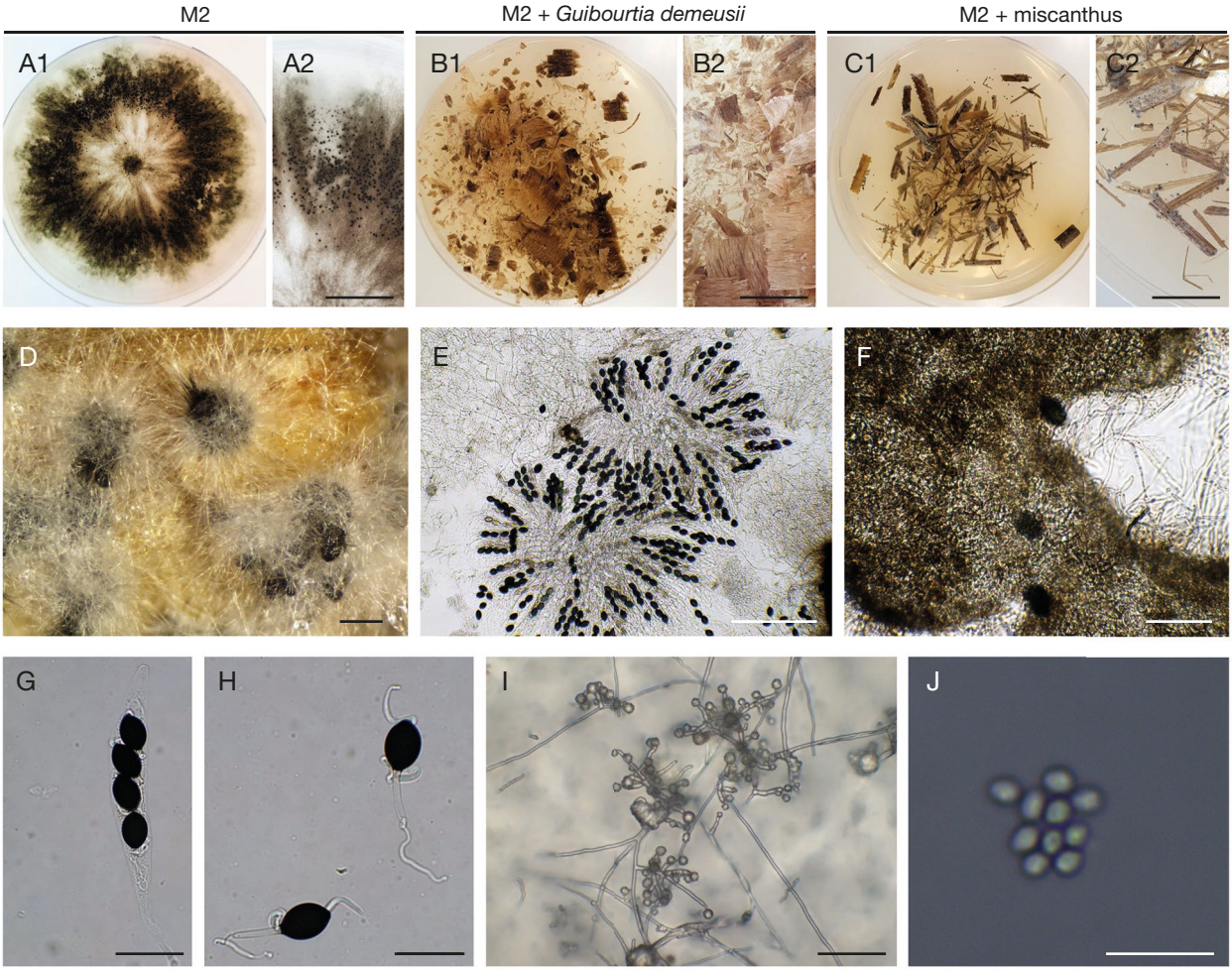


FIG. 5. — Morphology of *Podospora reunionensis* Silar, sp. nov: **A**, mycelium morphology after eight days of incubation on M2; **B**, M0 + *Guibourtia demeusii* L. wood shavings; **C**, M0 + miscanthus; on the right are enlargements to see the presence/absence and positions of the perithecia; **D**, Perithecia on M0 + miscanthus; **E**, rosettes of asci; **F**, peridium showing a textura intricata; **G**, ascus; **H**, ascospores; **I**, cladorrhinum-like anamorph; **J**, spermatia. Scale bars: A-C, 1 cm; D, E, 250 µm; F-H, 50 µm; I, J, 10 µm.

separately; when possible, one ascus with four germinated ascospores was then selected, and the four thalli obtained from the four ascospores of this F0 generation were stored separately at –80°C (Silar 2020).

DNA was extracted using a quick method adapted from plant (Bellstedt *et al.* 2010) and yeast (Liu *et al.* 2011) DNA extraction protocol. A 5 mm × 5 mm × 5 mm plug of agar taken from a plate onto which the fungus grew was put in a 1.5 mL Eppendorf tube along with 200 µL of a DNA extraction buffer that was made as follows. Firstly, 8 mL of 0.2 M stock solution of anhydrous sodium in water was freshly mixed with 17 mL of 0.2 M stock solution of sodium bicarbonate in water into 100 mL final volume of sterile water to make a 0.05 M carbonate buffer with pH = 9.6. The extraction buffer was then produced by mixing 96 mL of the carbonate/bicarbonate solution with 4 mL of 0.5 M of polyvinylpyrrolidone, 200 mg of bovine serum albumin fraction V (Sigma-Aldrich cat#A4503) and 50 µL of tween 20. The mycelium plug was then broken at speed 4.0 for 20 s in a TeSeE Precess 24 (Bio-Rad, Hercules, CA, United States), and the tubes were incubated at 95°C for 15 minutes. The tube was gently shaken with a finger and transferred on melting ice for one minute. The tube was then vortexed for 10 seconds and centrifuged at *c.* 20 000 g for 10-20 seconds in an Eppendorf centrifuge machine. The ITS (Intergenic Transcribed Spacer from the rDNA cluster) was amplified with the ITS5 and ITS4 or ITS1 and ITS5 primer pairs directly on 5 µL of supernatant. The PCR products were sent to Genewiz from AZENTA (Takeley, UK) for sequencing with ITS1 (or ITS5) and ITS4. Note that for Sordariales, ITS5 and ITS4 amplifications and sequencing with ITS5 gave more reliable results than ITS1 and ITS4 amplifications and ITS1 sequencing, respectively.

GENOME SEQUENCING OF STRAINS FROM THE *P. ANSERINA* SPECIES COMPLEX.

We sequenced the genome of mat1-1 (aka mat+) and mat1-2 (aka mat-) homokaryotic isolates. To obtain them, a self-fertile dikaryotic F0 thallus was used to produce homokaryotic self-sterile F1 progenies. These were confronted to each other to

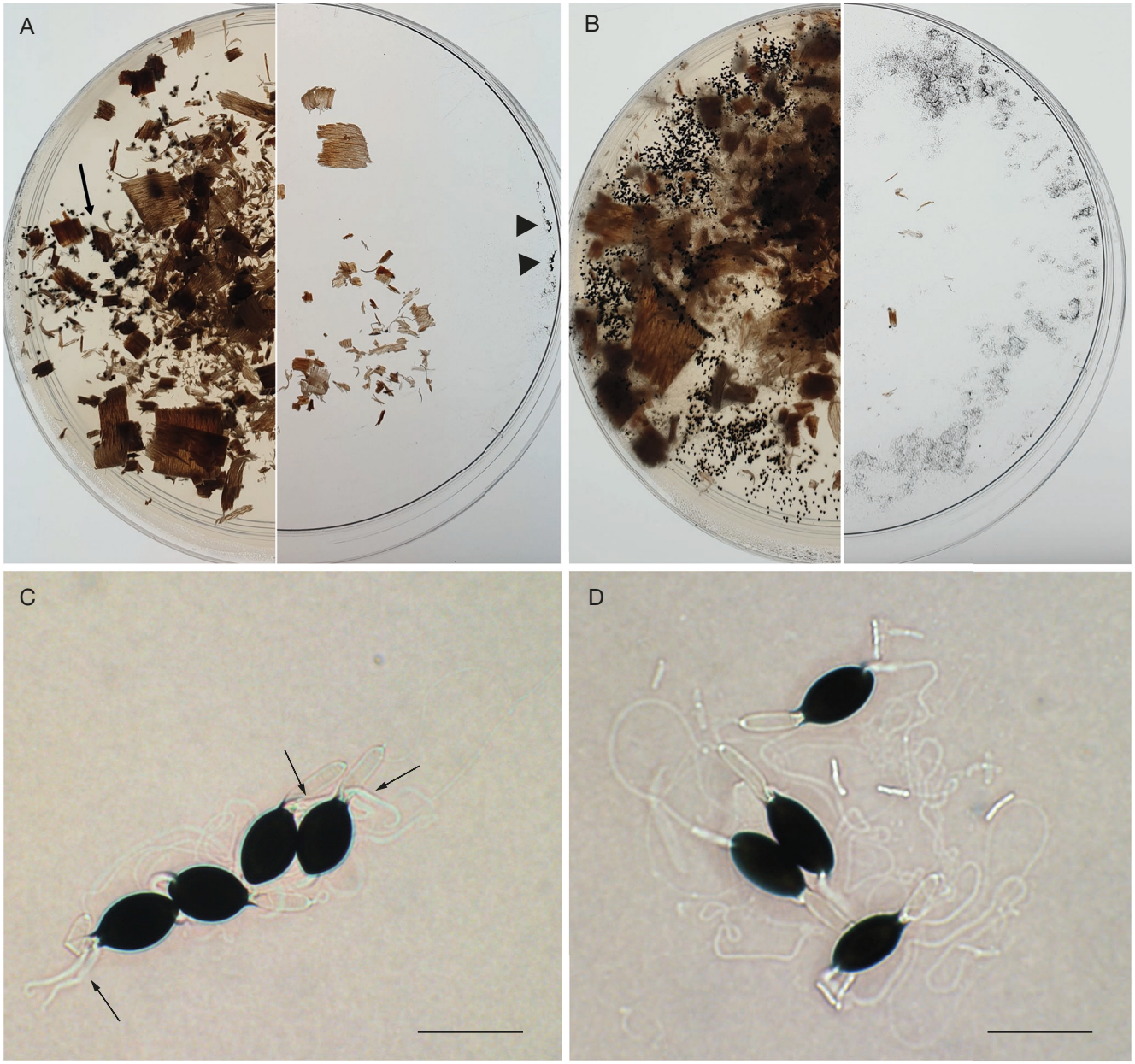


Fig. 6. — Comparison of *Podospora reunionensis* Silar, sp. nov. and *Podospora comata* Milovtz.: **A**, production of perithecia; **B**, production of ascospores (right: ejected on the lid) on M0 and *Guibourtia demeusii* L. wood shavings; **A**, **arrows** point to where PSN1158 differentiated perithecia that ejected ascospores on the lid (**arrowheads**). **C**, **D**, ascospores; **C**,The **arrows** point towards the conspicuous secondary appendages in PSN1158. These are also present in the *P. comata* Milovtz. strain T, but are shorter and rarer (i.e., they appear to be missing on most ascospores). Scale bars: 50 µm.

determine their mating compatibility, and the DNA from two sexually compatible progenies were extracted using the NucleoSpin® Soil from Machery Nagel (Düren, Germany) and submitted to 2×150 bp Novaseq illumina sequencing by Novogene (Cambridge, United Kingdom). The resulting FastQ files were then assembled using Unicycler (Wick *et al.* 2017), as previously described for other strains of the *P. anserina* species complex (Lalanne & Silar 2025). Mining the genomes with BLAST and manual annotation of the mating type loci allowed to determine whether the isolates were mat1-1 or mat1-2.

ANI calculation

ANIs were calculated with FungANI (Lalanne & Silar 2025) using the default parameters on the mat1-2 genome assemblies.

Determination of the region lacking recombination

To compute synonymous divergence (dS) values between the mat1-1 and mat1-2 genomes of PSN1158, we first performed SNP calling against the *P. anserina* S mat+ genome (Grognet *et al.* 2014) as described previously (Hartmann *et al.* 2021). For SNP calling, we used the version of the *P. anserina* S mat+ assembly available from the Joint Genome Institute

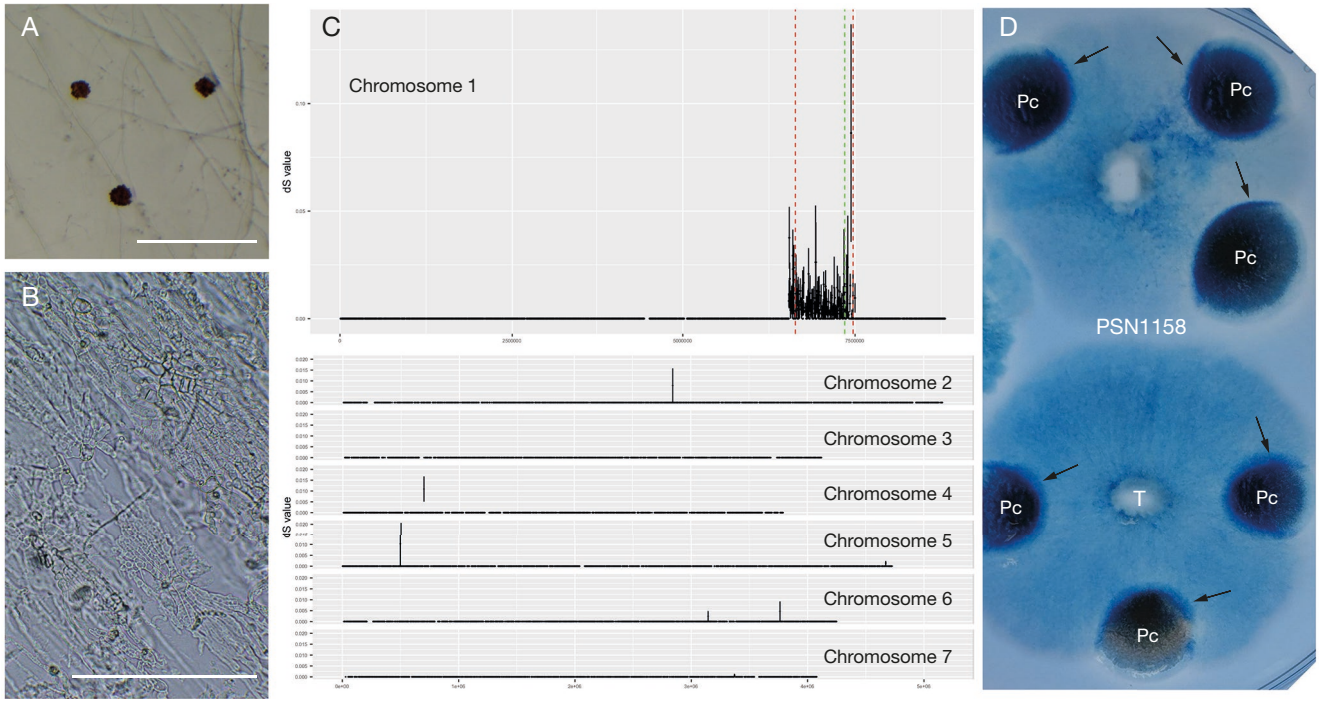


FIG. 7. — Additional features of the ex-type strain PSN1158 of *Podospora reunionensis* Silar, sp. nov: **A**, acellular microsclerotic-like structures differentiated on M0 + miscanthus; **B**, palm-like structures differentiated during appressorium-like-directed cellophane penetration; **C**, Per-gene synonymous divergence (dS) between the mat1-1 and mat1-2 homokaryotic genomes of the strain PSN1158 plotted against the location on *P. anserina* (Rabenh.) Niessl chromosome 1 carrying the mating-type locus, and the six other chromosomes; location of the mating-type locus is indicated with a green dotted vertical line and boundaries of the non-recombining region around the mating-type locus of the *P. anserina* strain S genome with red dotted vertical lines. High dS values indicate recombination suppression or outcrossing; **D**, hyphal interference visualized by accumulation of Evans Blue in dead cells (**arrows**) at the confrontation of Penicillium chrysogenum (Pc) and PSN1158 or *P. comata* Milovtz. strain T. Scale bars: A, B, 50 µm.

MycoCosm website (https://mycocosm.jgi.doe.gov/mycocosm/home, last accessed November 15, 2018) as "Podan2" (Espagne *et al.* 2008; Grognet *et al.* 2014) and annotations improved by (Vogan *et al.* 2019), available from the GitHub repository https://github.com/johannessonlab/SpokBlock-Paper (last accessed June 1, 2020). Briefly, we mapped Illumina trimmed reads against the *P. anserina* S mat+ genome assembly with the bowtie2 v2.3.4.1 program (Langmead *et al.* 2009), with the following software options: – very-sensitive-local – phred33-X 1000. To remove PCR duplicates, we used the MarkDuplicates tool of Picard tools version 1.88 (http://broadinstitute.github.io/picard, last accessed October 30, 2019). We used the RealignerTargetCreator and IndelRealigner tools of the Genome Analysis Toolkit (GATK) to locally realign the mapped reads and improve alignment accuracy in indel regions. To perform SNP calling, we run the HaplotypeCaller tool of GATK version 3.7 (McKenna *et al.* 2010) in the haploid mode on each genome individually. Then, we performed joint variant calls with GenotypeGVCFs on a merged gvcf variant file. SNP calls were filtered for quality with VariantFiltration, in accordance with GATK Good Practice for the hard filtering of variants (QUAL<250; QD<2; MQ<30.0; –10.5>MQRankSum>10.5; –5>ReadPosRankSum>5; FS>60; SOR>3). We performed additional filtering steps with vcftools to retain only biallelic and polymorphic SNPs. For each genome, we used a customized script (available on request) to build pseudogenome sequences, replacing the nucleotide bases of the *P. anserina* S mat+ genome with the base pair ID present in the focal genome as inferred from SNP calling and we retrieved gene coding sequences based on *P. anserina* S mat+ gene models with the gffread program (available from https://ccb.jhu.edu/software/stringtie/gff.shtml#gffread, last accessed April 15, 2020). We performed pairwise sequence comparisons using the codon-based approach implemented in translatorX, with default parameters (Abascal *et al.* 2010) and used the nucleotide alignment as input for the yn00 program in the PAML package, to calculate dS values (Yang & Nielsen 2000; Yang 2007).

MORPHOLOGICAL AND PHYSIOLOGICAL ANALYSES

All strains, including those of the new species PSN1158, grew and produced sexual fruiting bodies as previously described for species of the *P. anserina* complex (Silar 2020). Morphological and physiological analysis of PSN1158, including the ability to produce microsclerotic-like and appressorium-like structures as well as to display Hyphal Interference towards *Penicillium chrysogenum*, was performed as previously described (Boucher *et al.* 2017). For PSN1158, ascospore spore head and spermatia measurements were made on 50 ascospores and 50 spermatia, respectively. Perithecia were measured on 10 fruiting bodies and ascospore primary and secondary appendages on 15 ascospores. The perithecia analyzed for their peridium and centrum were those obtained on the M2 medium. Comparisons of fruiting body production and

morphology of PSN1158 and *P. comata* T were made in triplicate with the same batches of media and in parallel to avoid the influence of medium and growth condition differences on fruiting body production and repartition, as well as on ascospore morphology.

Strain availability

The F0 heterokaryotic isolates for all strains listed in Table 1 and their F1 homokaryotic progenies whose genomes were sequenced (Table 2) can be obtained upon request to the corresponding author. The type specimen of *P. reunionensis* Silar, sp. nov. PSN1158 was deposited in the Herbarium of the Museum national d'Histoire naturelle (MNHN, Paris, France; PC), and an ex-type living heterokaryotic F1 culture of PSN1158 was deposited in the "Centre International de Ressources Microbiennes-Champignons Filamenteux" (CIRM-CF, Inrae, France).

RESULTS AND DISCUSSION

Identification of strains of the *P. anserina* species complex and species distribution ranges

To understand the geographical distribution in France of the different species of the *P. anserina* complex, we isolated new strains not only dung but also from soil. For the latter substrate, a new method that proved efficient in isolating Sordariales fungi was designed (see Material and Methods). Among the isolates gathered from the soil, some corresponded to *P. anserina sensu lato* (*s.l.*) (i.e., belonged to the *P. anserina* species complex). Dung and soil samples were collected from different regions in metropolitan and overseas France for several years, and 86 isolates with morphologies corresponding to *P. anserina s.l.* were recovered (Table 1). To identify the species to which they belonged, we first sequenced their ITS barcodes and compared them with those of the seven species of the complex (Boucher *et al.* 2017). We could identify five *P. comata*, ten *P. pauciseta*, two *P. pseudoanserina* and 67 *P. anserina s.s.* strains. Another strain, PSN1303, presented a difference with the reference *P. anserina s.s.* ITS from strain S at position 460 where an additional C was present in PSN1303. The genome sequence of PSN1303 (see below) showed that this strain also belonged to *P. anserina s.s.*, showing that two different ITS barcode sequences exist for this species. In total, 68 *P. anserina s.s.* strains were thus isolated. Another strain, PSN1158, had one difference with the *P. comata* ITS sequence, having a G instead of an A at position 47 and two differences with the reference ITS sequence of the *P. anserina* strain S, with an A instead of G at position 22 and the presence of two additional Cs after position 467. Genome sequence and morphological analyses of PSN1158 (see below) showed that it belonged to a species new to science.

As seen in Figure 1, the different species had distinct distribution ranges in metropolitan and overseas France. *Podospora anserina s.s.* is found all over metropolitan France, including Corsica, while *P. comata* was found restricted so far to the Northern part of continental France and *P. pauciseta* to the southern part. *P. comata* has been isolated from the Netherlands (Ament-Velásquez *et al.* 2024), suggesting that this species may prefer colder climates. This is confirmed by our own isolation of a strain from Britany and one from the Alps mountains, both of which are regions colder than the rest of France. On the contrary, *P. pauciseta* may prefer hotter areas, as it seems fairly common near the Mediterranean Sea; note that we also found this species in Greece (Silar, unpublished), confirming its preference for the southern part of Europe.

In overseas France, we found members of the species complex only in Guadeloupe, that is located in the Caribbean's near Martinique, and in La Réunion island, located in the Indian ocean near Madagascar, although we also analyzed samples from Martinique (but not from Mayotte or Guyane). Guadeloupe hosts *P. pseudoanserina*, and La Réunion a new species, here described as *P. reunionensis* Silar, sp. nov.

Genome sequencing for accurate species identification

To clarify which species some strains actually belonged to, we sequenced the genomes of mat1-1 and mat1-2 homokaryotic isolates for five strains. These were: 1) PSN1303 and PSN1158 because of their differences with the previously known ITS barcodes; 2) PSN1705 because it originated from Corsica, an island distant from continental France; and 3) PSN1871 and PSN1899, because the two strains presented a different mycelium morphology and genome sequences were available for only two strains of *P. pseudoanserina*. The main features of genome assemblies are given in Table 2. All genomes were around 35 MB, a size similar to the genomes of strains previously sequenced (Espagne *et al.* 2008; Grognet *et al.* 2014; Silar *et al.* 2019; Ament-Velásquez *et al.* 2024) and moderately fragmented (505 to 999 contigs).

The FungANI analysis confirmed that PSN1303 and PSN1705 belonged to *P. anserina s.s.*, because the genomes of these two strains displayed high similarity to the one of the type *P. anserina* strain *s.s.* S *mat+* (ANI> 99.5%; Fig. 2). In addition, their morphology, especially the repartition of the perithecia on the M2 medium as a ring onto the mycelium was typical of *P. anserina s.s.* ((Boucher *et al.* 2017); Fig. 3). The FungANI analysis also confirmed that PSN1871 and PSN1899 belonged to *P. pseudoanserina* (ANI> 99.5% with the *P. pseudoanserina* type strain CBS253.71; Fig. 2); their morphology further corresponded to that of *P. pseudoanserina*, perithecia being formed on M2 as a disk in the center of the mycelium (Boucher *et al.* 2017; Fig. 3). Note that PSN1871 and PSN1899 displayed different mycelium morphologies, PSN1899 producing more aerial hyphae.

FungANI analyses of PSN1158 showed that it did not correspond to any of the seven previously known species (Fig. 4). Indeed, the PSN1158 genome was only about 97% identical to those of the other species, except *P. comata* T, with which it had 99.17% identity (Fig. 4). PSN1158 and *P. comata* strain T genomes share 48% of sequences with similarity between 99.5% and 99.9% (magenta bar on the FungANI graphic comparing PSN1158 with T of Fig. 4), 25% with 99.0% and 99.5% similarity (dark blue bar on the same graphic) and 11%

TABLE 1. — Strains isolated for this study.

Strain	Location (French department number)	Isolation year	Substrate
Podospora anserina (Rabenh.) Niessl			
PSCJ14	Bures/Yvette (91)	2003	dung
10b-P1-A1	Bures/Yvette (91)	2014	dung
5b-P1-A1	Bures/Yvette (91)	2014	dung
DMC1	Versailles (78)	2014	dung
PSN14	Chantilly (60)	2007	dung
PSN42	Froimont (60)	2008	dung
PSN214	Versailles (78)	2015	dung
PSN295	Granville (50)	2017	dung
PSN338	Nanterre (93)	2019	dung
PSN341	Amiens (80)	2019	dung
PSN429	St Quentin en Tourmon (80)	2019	soil
PSN430	St Quentin en Tourmon (80)	2019	soil
PSN513	Chantegrue (25)	2019	soil
PSN536	Bailleul (59)	2020	dung
PSN542	Amiens (80)	2020	dung
PSN543	Belvédère de Frise (80)	2020	soil
PSN562	Amiens (80)	2020	dung
PSN645	Tour du Valat (13)	2021	dung
PSN679	Amiens (80)	2021	dung
PSN691	St Aubin Montenoye (80)	2021	dung
PSN692	St Aubin Montenoye (80)	2021	dung
PSN693	St Aubin Montenoye (80)	2021	dung
PSN694	St Aubin Montenoye (80)	2021	dung
PSN695	St Aubin Montenoye (80)	2021	dung
PSN728	Tour du Valat (13)	2021	dung
PSN729	Hangest (80)	2021	dung
PSN730	Tour du Valat (13)	2021	dung
PSN731	Tour du Valat (13)	2021	dung
PSN732	Longpré les corps saints (80)	2021	dung
PSN734	Hangest (80)	2021	dung
PSN736	Cap Blanc Nez (62)	2021	dung
PSN737	Tour du Valat (13)	2021	dung
PSN738	Hangest (80)	2021	dung
PSN739	Reugny (03)	2021	dung
PSN740	Tour du Valat (13)	2021	dung
PSN741	Reugny (03)	2021	dung
PSN753	Reugny (03)	2021	dung
PSN754	Belvédère de Vaux (80)	2021	soil
PSN755	Hangest (80)	2021	soil
PSN756	Reugny (03)	2021	soil
PSN823	Reugny (03)	2021	dung
PSN825	Hangest (80)	2021	dung
PSN826	Belvédère de Vaux (80)	2021	dung
PSN827	Corticciato (2A) - Corsica	2021	dung
PSN828	Algans (81)	2021	dung
PSN992	Coussouls de Crau (13)	2021	dung
PSN1016	Vigueirat (13)	2021	dung
PSN1023	St Rémy de Provence (13)	2021	dung
PSN1026	Mas St. germain (13)	2021	dung
PSN1027	Mas St. germain (13)	2021	dung
PSN1028	Vigueirat (13)	2021	dung
PSN1029	Domaine Ricard (13)	2021	dung
PSN1030	Domaine Ricard (13)	2021	dung
PSN1031	Mas St. germain (13)	2021	dung
PSN1049	Mas St. germain (13)	2021	dung
PSN1079	Vigueirat (13)	2021	dung
PSN1237	Montpellier (34)	2022	dung
PSN1302	Villefort (48)	2022	soil
PSN1303	Massac (81)	2022	soil
PSN1406	Vaccares (13)	2022	dung
PSN1705	Cargese (2A) - Corsica	2023	dung
PSN1711	Cargese (2A) - Corsica	2023	dung
PSN2086	Boves (80)	2024	dung
PSN2087	Boves (80)	2024	dung
PSN2088	Boves (80)	2024	dung
PSN2089	Vers/Selle (80)	2024	dung
PSN2090	Vers/Selle (80)	2024	dung
PSN2091	Vers/Selle (80)	2024	dung

Table 1. — Continuation.

Strain	Location (French department number)	Isolation year	Substrate
Podospora comata Milovtz.			
PSN537	Bailleul (59)	2020	dung
PSN544	Monts d'Arrée (29)	2020	soil
PSN733	Hangest (80)	2021	dung
PSN735	Hangest (80)	2021	dung
PSN994	La Clusaz (74)	2021	dung
Podospora pauciseta (Ces.) Traverso			
PSN646	Tour du Valat (13)	2021	dung
PSN999	Arles (13)	2021	dung
PSN1020	Vaccares (13)	2021	dung
PSN1021	Ste Marie de la mer (13)	2021	dung
PSN1022	Vaccares (13)	2021	dung
PSN1025	Vaccares (13)	2021	soil
PSN1048	Vaccares (13)	2021	dung
PSN1182	Villenave (40)	2022	dung
PSN1215	Montpellier (34)	2022	dung
PSN1300	St. Paul le Jeune (07)	2022	dung
Podospora pseudoanserina C.Boucher, T.S.Nguyen & P.Silar			
PSN1871	Guadeloupe (971)	2024	soil
PSN1899	Guadeloupe (971)	2024	soil
Podospora reunionensis Silar, sp. nov.			
PSN1158	La Réunion (974)	2022	dung

with 98.0% and 99.0% (light blue bar on the graphic). They, however, had only about 6% of highly similar sequences (i.e., genomic region with a percentage identity > = 99.9% as calculated by FungANI; Fig. 4), suggesting little recent gene flow between these lineages. Importantly, although ANI showed that PSN1158 was closely related to the *P. comata* strain T, they displayed contrasting cultural features (see below) and ascospore morphology, indicating that PSN1158 belong to a new species (Figs 5; 6).

On M2, PSN1158 produced a mycelium pigmented in dark green with a center nearly colorless, while *P. comata* formed a mycelium with a more homogeneous pigmentation ((Boucher *et al.* 2017); Fig. 5). PSN1158 perithecia were produced along a ring (wider than the one produced by *P. anserina*), while *P. comata* produced perithecia in a disk in the center of the colony ((Boucher *et al.* 2017); Fig. 5). Additionally, *P. comata* was particularly fertile on M0 supplemented with *Guibourtia demeusii* L. wood shavings (Boucher *et al.* 2017), while PSN1158 sexual reproduction was delayed by three days on this medium and produced much fewer fruiting bodies (Fig. 6). It however rapidly produced perithecia on M0 + miscanthus like all the species of the *P. anserina* complex (Fig. 5). The ascospores produced by PSN1158 had clear morphological differences with those produced by *P. comata* strain T, which is the ex-type for this species (Fig. 6). The ascospore spore head was plumpier (35.0 +/– 1.8 × 22.7 +/– 0.8 µm for PSN1158 vs 35.7 +/– 4.1 × 17.8 +/– 1.5 µm for *P. comata* T), presented a longer primary appendage (32.3 +/– 2.5 µm for PSN1158 vs 26.3 +/– 2.8 µm for *P. comata* T) and a much more prominent secondary appendage(s) at the junction between the spore heard and the primary appendage.

Another argument supporting that PSN1158 belonged to a new species was that, as previously stated, *P. comata* appears to like colder climates, and PSN1158 came from a humid tropical island. Finally, crosses between *P. comata* T and PSN1158 were much less fertile than PSN1158 × PSN1158 and T × T selfing crosses. Indeed, only three fruiting bodies, including only one bearing ascospores, were obtained in conditions where hundreds of spore-bearing perithecia were produced for intra-species crosses.

TAXONOMY

Family Podosporaceae
X.Wei Wang & Houbraken

Genus *Podospora* Rabenh.

Podospora reunionensis Silar, sp. nov.
(Figs 5; 6; 7)

IF number. — 901426.

Etymology. — Refers to the place of its discovery, La Réunion island.

Holotype. — PC0799011; ex-type culture: PSN1158.

Habitat and geographic distribution. — PSN1158, the only strain presently available, was isolated from dung collected in La Réunion, an island of the Indian Ocean near Madagascar, in 2022. It is available under reference BRFM 3810 at the Centre international de Ressources microbiennes-Champignons filamenteux (CIRM-CF, Inrae, France).

TABLE 2. — Main features of genome assemblies generated in this paper.

Strain	Assembly size (Mb)	Contig number	Raw data accession number	Assembly URL
PSN1158 mat1-1	34.5	683	SRR31732282	https://github.com/podo-gec/fungi-public-data/blob/master/podospora-reunionensis/PSN1158_mat1-1.fasta
PSN1158 mat1-2	34.5	640	SRR31732281	https://github.com/podo-gec/fungi-public-data/blob/master/podospora-reunionensis/PSN1158_mat1-2.fasta
PSN1303 mat1-1	35.2	633	SRR31732274	https://github.com/podo-gec/fungi-public-data/blob/master/podospora-reunionensis/PSN1303_mat1-1.fasta
PSN1303 mat1-2	35.3	505	SRR31732273	https://github.com/podo-gec/fungi-public-data/blob/master/podospora-reunionensis/PSN1705_mat1-1.fasta
PSN1705 mat1-1	35.2	533	SRR31732276	https://github.com/podo-gec/fungi-public-data/blob/master/podospora-reunionensis/PSN1705_mat1-1.fasta
PSN1705 mat1-2	35.1	752	SRR31732275	https://github.com/podo-gec/fungi-public-data/blob/master/podospora-reunionensis/PSN1705_mat1-2.fasta
PSN1871 mat1-1	34.9	999	SRR31732278	https://github.com/podo-gec/fungi-public-data/blob/master/podospora-reunionensis/PSN1871_mat1-1.fasta
PSN1871 mat1-2	35.3	880	SRR31732277	https://github.com/podo-gec/fungi-public-data/blob/master/podospora-reunionensis/PSN1871_mat1-2.fasta
PSN1899 mat1-1	35.3	852	SRR31732280	https://github.com/podo-gec/fungi-public-data/blob/master/podospora-reunionensis/PSN1899_mat1-1.fasta
PSN1899 mat1-2	35.3	863	SRR31732279	https://github.com/podo-gec/fungi-public-data/blob/master/podospora-reunionensis/PSN1899_mat1-2.fasta

ADDITIONAL FEATURES. — PSN1158 undergoes senescence on M2 but with a huge variation in longevity between the cultures and with no difference between mat1-1 and mat1-2 strains (PSN1158 mat1-1: 54 +/– 38 cm and PSN1158 mat1-2: 55 +/– 39 cm). It does not undergo Crippled Growth like the *P. comata* strain T (Boucher *et al.* 2017; Nguyen *et al.* 2022). Like the other species of the *P. anserina* complex (Boucher *et al.* 2017), it produces acellular orange-turning-to-black acellular microsclerotia-looking structures when cultivated on media with *Guibourtia demeusii* L. wood shavings (Fig. 7A). It differentiates appressorium-like structures enabling to breach cellophane when cultivated onto a layer of cellophane in the absence of glucose (Fig. 7B). SNPs between the PSN1158 mat1-1 and mat1-2 isolates were found only in a region of about 0.9 Mb around the mating-type locus (Fig. 7C). Such a high level of genome-wide homozygosity indicated that the isolate was highly inbred. The differentiation around the mating-type locus showed that recombination is suppressed in this region, like in all the other species of the complex (Hartmann *et al.* 2021). Finally, PSN1158 was able to exert Hyphal Interference against *Penicillium chrysogenum* Thom like all the other species of the complex, i.e., it was able to kill *P. chrysogenum* hyphae upon contacting them (Boucher *et al.* 2017).

DESCRIPTION

Perithecium diameter is 370 +/– 60 µm, pyriform, membranous, semitransparent, pale brown, covered with numerous hyphoid hairs. Neck of varying length and curvature depending on the lighting, blackish, coriaceous, often with a tuft of dark, rigid, agglutinated hairs, sometimes with a few scattered hairs too. Peridium with *textura intricata*. Asci 4-spored, clavate-lageniform. Spores spoon-shaped in the early stages. Mature spores obliquely uniseriate: spore head 35.0 +/– 1.8 × 22.7 +/– 0.8 µm, ellipsoidal, flattened at the base and slightly pointed at the apex, smooth, thick-walled, with an apical central germ pore. Presence of a very long and thin primary appendage (pedicel) nearly as long as the ascospore 32.3 +/– 2.5 × 4.4 +/– 1.1 µm, cylindrical, slightly tapering towards the apex. This appendage swells upon ejection so that its width reaches 7.7 +/– 0.8 µm. Upper secondary appendage (cauda) lash-shaped, not covering the germ pore; lower cauda solid, filiform, arising from the pedicel apex and with two (sometimes one) additional well-defined and long appendage(s) at the pedicel base 30.7 +/– 9.4 µm, near the septum.

Presence of a *Cladorrhinum*-like anamorph producing roundish to pear-shaped spermatia (2.6 +/– 0.4 × 2.0 +/– 0.3 µm) that did not germinate.

PSN1158 ITS sequence differs at two positions from the *P. anserina* reference ITS: A instead of G at position 22 and the presence of two additional Cs after position 467.

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