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Macrolepiota macilenta and *M. pallida*, two new species from North America

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Abstract: Two new species of *Macrolepiota, viz*. *Macrolepiota macilenta* and *M. pallida*, are formally described from eastern and midwestern North America based on molecular data, morphological characters and geographic distribution pattern. They are found in summer and fall in hardwood forests including *Fagaceae* (*Quercus*, *Fagus*) and in grassy clearings and nutrient-rich soils. They can be distinguished morphologically from one another by their general colours, spore size, pileus covering structure and shape of the cheilocystidia. Photographs of the species and of their micromorphological characters are provided.

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INTRODUCTION

The genus *Macrolepiota* was erected by Singer (1948) for medium to large species [*e.g*. pileus 100–300 mm broad, stipe 100–400 × 10–20 mm in *M. procera*, the type species (Vellinga 2001)] in *Agaricaceae* characterized by their large to giant spores (*e.g*. 12.5–16.5 × 7.5–11.0 µm in *M. procera*; Vellinga 2001), metachromatic in Cresyl blue, white to cream in mass, and their clamped hyphae. Singer (1986) later refined his concept of the genus as comprising species with pure white to pale pink, smooth and very voluminous, strongly metachromatic spores, with a broad germ pore; an absence of pleurocystidia; a usually scaly pileus smooth on disk ("calotte") made of a palisade of long, usually straight elements sometimes becoming appressed; and a movable annulus. In the early 2000s, Vellinga *et al.* (2003) emended the genus to encompass only the species with a trichodermial pileus, a stipe covering made of hymeni-trichodermal patches and spores with a rounded apex and a covered germ pore. They transferred to *Chlorophyllum* some species then belonging to *Macrolepiota* (*e.g. M. rhacodes*, *M. olivieri*), redefining the former as including species with a hymenodermal pileus covering, a smooth stipe and spores with or without a germ pore, without a hyaline covering.

The genus *Macrolepiota* has been the subject of several systematic phylogenetic studies in the last two decades (Johnson & Vilgalys 1998, Johnson 1999, Vellinga 2003, Vellinga *et al*. 2003, Ge *et al*. 2010, Vizzini *et al.* 2011), and many of these studies included sequences generated from North American collections, but the actual taxonomy of the North American species remained unresolved. Rarely featured in popular western North American field guides ["*Lepiota procera*" reported from southern California by Arora (1986)], these species are occasionally found in eastern and midwestern North America, where they have been applied European names such as *Lepiota procera* (*e.g.* Murrill 1914, Kauffman 1918, Pomerleau 1980), *Macrolepiota procera* (Lincoff 1981, Barron 1999, Roody 2003, Bessette *et al*. 2007, Baroni 2017, Bessette *et al.* 2019) and *M. prominens* (McNeil 2019). Our molecular work based on sequences of the nuclear ribosomal internal transcribed spacer (nrITS) and work performed by previous workers (Vellinga *et al*. 2003, Ge *et al*. 2010, Vellinga *et al*. 2011, Cho *et al*. 2019) has shown that the genus was represented by several undescribed species in North America, including two from eastern and midwestern North America that we aim to describe here.

MATERIALS & METHODS

Sample collection and morphological examination

Basidiomata of *Macrolepiota* were collected in Eastern Canada (Québec) and in eastern and midwestern states of the USA (Maine, Indiana), photographed *in situ*, then dried at 40 °C. The habitat, altitude, and nearby trees were noted. Macromorphological characters were described from fresh basidiomata and from photographs, with the colour codes of Kornerup & Wanscher (1978). Micromorphological studies were conducted on exsiccata with a Nikon Labophot microscope (Nelville, NY) and a Moticam 2500 digital camera. Microscopical elements were rehydrated in isopropanol 70 %, hand-sectioned, and observed in 3 % potassium hydroxide (KOH) and sodium dodecyl sulphate (SDS) Congo Red. Spores were measured in

3 % KOH, and their dextrinoidity, metachromasy, cyanophily and congophily were observed in Melzer's reagent, Cresyl blue, Cotton Blue in lactic acid, and SDS Congo Red, respectively. Microstructures were measured with the aid of an optical micrometre, and descriptive terminology follows Vellinga (1988). A minimum of 30 basidiospores per basidioma, obtained from spore print or from lamellae, were randomly selected and measured in profile view using the following notation: [a/b/c] (d–)e–f(–g), where 'a/b/c' represent respectively the total number of spores, basidiomata, and collections, 'e' and 'f' the 5th and 95th centile of the measured values, and 'd' and 'g' the extreme values. Q (minimum and maximum length/width ratio) and Q_{av} (average length/width ratio) were calculated. Collections are kept in public fungaria *sensu* Thiers (2024) when indicated, or in the private fungaria of the collectors.

DNA extraction, amplification and sequencing

Specimens were prepared for DNA sequencing using one of two sequencing workflows outlined below: 1) extraction using a modified version of the Promega Wizard Protocol for Sanger sequencing found in Russell (2023a) or 2) a workflow from Russell (2023b) for nanopore sequencing.

Sanger sequencing protocol
DNA extraction was performed following the protocol in Russell (2023a). PCR amplifications of the nuclear internal transcribed spacer (ITS) ribosomal DNA (nrDNA) region were carried out using the ITS1F forward primer and the ITS4 reverse primer (White *et al.* 1990, Gardes & Bruns 1993). Each amplification reaction contained 12.5 µL of MeanGreen 2× Master Mix (Empirical Bioscience, Grand Rapids, Michigan, USA), 9 µL water, 1·25 µL forward primer, 1·25 µL reverse primer, and 1 µL DNA template for a total PCR volume of 25 µL· The following PCR protocol was used: (i) initial denaturation at 94 °C for 60 s; (ii) 30 cycles of denaturation at 94 °C for 60 s, annealing at 51 °C for 60 s, and extension at 72 °C for 60 s; (iii) hold at 72 °C for 8 min. Electrophoresis with a 1 % agarose gel was used to verify successful amplification.

PCR amplicons were sent to Genewiz (Genewiz, Inc., Boston, Massachusetts, USA) for sequencing of both the forward and reverse DNA strands. The two reads were assembled using Sequencher v. 5.0.1 (Gene Codes Corp., Ann Arbor, Michigan) and the consensus sequences were deposited in GenBank. Raw DNA sequence data [trace files (AB1)] are available within the *Macrolepiota* of North America MycoMap project (MycoMap 2023).

Nanopore sequencing protocol
A nanopore sequencing workflow was used per the protocol from Russell (2023b). In brief, tissue was extracted into 0.2 mL 8-strip PCR tubes. DNA extraction began by adding 20 µL of X-Amp (IBI Scientific, Peosta, Iowa, United States) into each well of the 8-strip tubes and heating at 80 °C for 1 h. Fifty microliters of water were then added to each well to create the PCR template. PCR was performed for the full ITS region using ITS1F & ITS4 primers. Dual-index primer-master mix plates were created by hand or on an Opentrons OT-2 robotic pipetting platform following the protocol in Russell (2023c). Amplification reaction components and thermocycling conditions were the same as reported above for Sanger sequencing, aside from a 12.5uL total reaction volume for nanopore sequencing.. The resulting amplicons were pooled, a library was created with the Oxford Nanopore Technologies (ONT) LSK 112 or LSK114 chemistry (Oxford Nanopore Technologies, Oxford, UK), and sequencing was performed on an ONT Minion & Flongle device with a Flongle 9.4.1 or 10.4.1 flowcell. Raw data were basecalled, demultiplexed, consensus sequences were formed, and the final data were uploaded to MycoMap using the pipeline from Russell (2023b). All curated sequence data were compared against data contained in public (GenBank, BOLD & UNITE) and in-house databases. Raw DNA sequence data (FASTQ) are available within the *Macrolepiota* of North America MycoMap project (MycoMap 2023). Final consensus sequence data were deposited in GenBank.

Phylogenetic analysis

We assembled all available sequences for North American collections of *Macrolepiota*. This includes newly generated nrITS sequences for this study (or as part of general mycological diversity surveys), sequences generated in previous studies, and/or available in public databases and biodiversity repositories (GenBank, iNaturalist, Mushroom Observer). A total of 53 nrITS sequences generated from North American collections were included in the dataset. We then included representative sequences of all other species of *Macrolepiota* sequenced to date. The final dataset consists of 104 *Macrolepiota* sequences, with *Bovista plumbea* and *Lycoperdon perlatum* as outgroup taxa, following previous phylogenies of the family *Agaricaceae* (Vellinga *et al*. 2011) (Table 1).

Sequences were aligned using MAFFT v. 7 (Katoh *et al.* 2019) and the strategy FFT-NS-i. The alignment was inspected and manually corrected in AliView (Larsson 2014). Two separate phylogenetic analyses were run, maximum likelihood (ML) and Bayesian inference (BI). The ML analyses were run in RAxML v. 8.2.12 under a GTRGAMMAI model as recommended (Stamatakis 2014) with 1 000 rapid bootstrap (BS) replicates. The BI analyses were run using MrBayes v. 3.2.7 (Ronquist *et al*. 2012) for 10 M generations under a GTRGAMMAI model with four chains, and trees sampled every 1 000 generations. The initial burn-in phase was set to 2.5 M generations, and this value was confirmed to be adequate by checking the graphic representation of the likelihood scores of the sampled trees. Additionally, we also confirmed that the standard deviation of split frequencies was < 0.05, and that potential scale reduction factor (PRSF) values were close to 1, as detailed in Ronquist *et al.* (2011). All analyses were run using resources at the CIPRES Science Gateway (Miller *et al.* 2010).

When comparing sequences of closely related species, the number of evolutionary changes is given as the minimum number of events, including indels (multiple base indels treated as one change), transitions, and transversions. Only differences shared by all sequences of the same species were counted (missing data for sequences of different lengths not considered). For brevity, the term "evolutionary events" is used to refer to all the possible evolutionary changes between two sets of sequences.

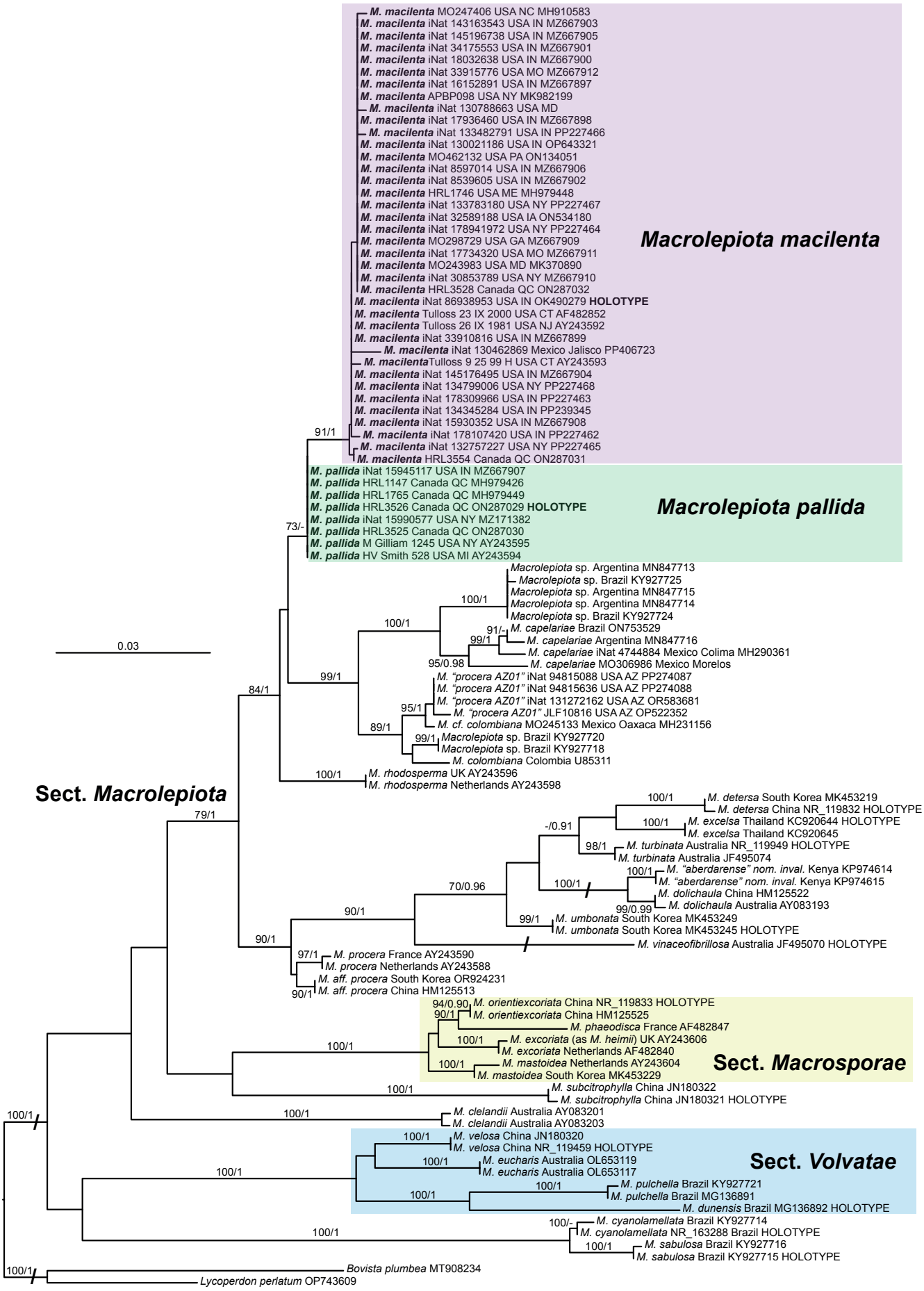

Fig. 1. Best tree from the ML analysis of the nrITS dataset. Support values from the ML and BI analyses (Bootstrap ≥ 70 % / Posterior Probabilities ≥ 0.90) are shown on or below the branches. Branches crossed with a "/" have had their length reduced to facilitate graphical representation.

Table 1. Collection details and GenBank accession numbers of specimens studied and/or included in the phylogenetic analysis.

Species	Collection data[1]	Specimen-voucher (Fungarium)[2]	Collector(s)	Origin	ITS sequence source
Macrolepiota aberdarense	—	MAT08 (**Type**)	C. Mbaluto, D. Otieno	Kenya	KP974615
	—	KIA08	C. Mbaluto, D. Otieno	Kenya	KP974614
Macrolepiota capelariae	iNat 4744884	—	A. Rockefeller	Colima, Mexico	MH290361
	MO 306986	—	A. Tux	Morelos, Mexico	MO observation
	—	DSF02	D.S. Freitas	Brazil	ON753529
	—	GA73	N/A	Argentina	MN847716
Macrolepiota cf. colombiana	MO 245133	NA	A. Rockefeller	Oaxaca, Mexico	MH231156
Macrolepiota cf. procera	—	(HKAS 5722)	N/A	China	HM125513
	—	(KMCC05413)	K.-H. Lee	South Korea	OR924231
Macrolepiota clelandii	—	MEL 229171	K.R. Thiele	Australia	AY083201
	—	Wembley E5627	K. Syme	Australia	AY083203
Macrolepiota colombiana	—	NY-EFM694	N/A	Colombia	PP724363 (Unite)
Macrolepiota cyanolamellata	—	1165 (ICN 187663)	A.C. Magnago	Brazil	KY927714
	—	516 (ICN 187662) (**Type**)	E.P. Fazolino	Brazil	NR_163288
Macrolepiota detersa	—	(HKAS 55306) (**Type**)	N/A	China	NR_119832
	—	SFC20160712-42	N/A	South Korea	MK453219
Macrolepiota dolichaula	—	901 (CANB)	R.P.J. de Kok	Australia	AY083193
	—	(HKAS 43813)	N/A	China	HM125522
Macrolepiota dunensis	—	NMJ161 (SP466944) (**Type**)	N. Menolli Jr.	Brazil	MG136892
Macrolepiota eucharis	—	FBT821 (MEL)	S.J.M. Mcmullan-Fisher	Australia	OL653119
	—	FBT2102 (MEL 2432046)	S.J.M. Mcmullan-Fisher	Australia	OL653117
Macrolepiota excelsa	—	ecv3553 (UC)	E.C. Vellinga	Thailand	KC920645
	—	ecv3572 (UC)	E.C. Vellinga	Thailand	KC920644
Macrolepiota excoriata	—	5-IX-1999 (L)	R. Tofts	UK	AY243606
	—	11-IX-1997 (L)	R. Chrispijn	Netherlands	AF482840
Macrolepiota macilenta	—	23-IX-2000 (UC)	R. Tulloss	Connecticut, USA	AF482852
	—	25-IX-1999 (UC)	R. Tulloss	Connecticut, USA	AY243593
	—	26-IX-1981 (UC)	R. Tulloss	New Jersey, USA	AY243592
	—	APBP098	A.T. Hudon, T.R. Horton	New York, USA	MK982199
	iNat 130021186	341A (MYCO)	R.M. Hallock	Indiana, USA	OP643321
	iNat 130462869	(IBUG)	A. Rockefeller	Jalisco, Mexico	PP406723
	iNat 130788663	—	jsharper	Maryland, USA	iNat observation
	iNat 132757227	DMc249 (MYCO)	D. McCheyne	New York, USA	PP227465
	iNat 133482791	HMS22-02518 (MYCO)	J.W. Mead	Indiana, USA	PP227466

Table 1. (Continued).

Species	Collection data[1]	Specimen-voucher (Fungarium)[2]	Collector(s)	Origin	ITS sequence source
	iNat 133783180	DMc246 (MYCO)	D. McCheyne	New York, USA	PP227467
	iNat 134345284	2341 (MYCO)	B. Hunt	Indiana, USA	PP239345
	iNat 134799006	DMc250 (MYCO)	D. McCheyne	New York, USA	PP227468
	iNat 143163543	20160915SDR016 (PUL00031000)	S. Russell	Indiana, USA	MZ667903
	iNat 145176495	20160915FMK001	F. Kuerbs-Buckley	Indiana, USA	MZ667904
	iNat 145196738	20170918SDR085 (PUL00036473)	S. Russell	Indiana, USA	MZ667905
	iNat 15930352	64543	S. Russell	Indiana, USA	MZ667908
	iNat 16152891	RSK0145IN and 66336	R. Kerner	Indiana, USA	MZ667897
	iNat 17734320	72559	P. Harvey	Missouri, USA	MZ667911
	iNat 178107420	8245 (MYCO)	R.M. Hallock & M. Seefeldt	Indiana, USA	PP227462
	iNat 178309966	3858 (MYCO)	J. Logterman	Indiana, USA	PP227463
	iNat 178941972	11837 (MYCO)	R. Butzer	New York, USA	PP227464
	iNat 17936460	MC-2018-42	S. Klink	Indiana, USA	MZ667898
	iNat 18032638	65813	M. Dahl	Indiana, USA	MZ667900
	iNat 30853789	2409	P. Gladkov	New York, USA	MZ667910
	iNat 32589188	59723 (S. Delong-Duhon pers. fungarium)	D. Layton	Iowa, USA	ON534180
	iNat 33910816	**70501 (DAOM 985166)**	**M. Seefeldt**	**Indiana, USA**	**MZ667899**
	iNat 33915776	BFF77 (MYCO)	A. Peters	Missouri, USA	MZ667912
	iNat 34175553	**1670 (DAOM 985165)**	**S. Russell**	**Indiana, USA**	**MZ667901**
	iNat 8539605	8081	S. Russell	Indiana, USA	MZ667902
	iNat 8597014	3024	F. Kuerbs-Buckley	Indiana, USA	MZ667906
	iNat 86938953	**AS70 (MICH 346530; DAOM 985164) (Type)**	**M. Russell**	**Indiana, USA**	**OK490279**
	MO 243983	243983	R. P. Pridgeon	Maryland, USA	MK370890
	MO 247406	NAMA 2015-011	G. Taylor	North Carolina, USA	MH910583
	MO 298729	MO-298729	J. Knapp	Georgia, USA	MZ667909
	MO 462132	**DAOM 985167**	**D. Wasilewski**	**Pennsylvania, USA**	**ON134051**
	MP 13373733	**HRL3528 (DAOM 984980)**	**G. Boyer**	**Québec, CA**	**ON287032**
	MP 13374027	**HRL3554 (DAOM 984981)**	**C. Lapointe**	**Québec, CA**	**ON287031**
	MP 2424833	**HRL1746 (R.L. pers. fungarium)**	**C. Michaud**	**Maine, USA**	**MH979448**
Macrolepiota mastoidea	—	E.C. Vellinga 2294 (L)	N.J. Dam	Netherlands	AY243604
	—	SFC20171012-09	N/A	South Korea	MK453229
Macrolepiota orientiexcoriata	—	(HKAS 23040)	N/A	China	HM125525
	—	(HKAS 45863) (**Type**)	N/A	China	NR_119833
Macrolepiota pallida	—	1245 (MICH 53357)	M. Gilliam	New York, USA	AY243595

Table 1. (Continued).

Species	Collection data[1]	Specimen-voucher (Fungarium)[2]	Collector(s)	Origin	ITS sequence source
	—	528 (MICH 42440)	H.V. Smith	Michigan, USA	AY243594
	iNat 15945117	68489	S. Russell	Indiana, USA	MZ667907
	iNat 15990577	**DAOM 985168**	**G. Taylor**	**New York, USA**	**MZ171382**
	MP 892513	**HRL1147 (DAOM 984977)**	**J. Nuzzolese**	**Québec, CA**	**MH979426**
	MP 2498026	**HRL1765 (R.L. pers. fungarium)**	**R. Demers**	**Québec, CA**	**MH979449**
	MP 13373730	**HRL3525 (DAOM 984978)**	**I. Pouliot**	**Québec, CA**	**ON287030**
	MP 13373731	**HRL3526 (DAOM 984979) (Type)**	**R. Lebeuf, A. Paul**	**Québec, CA**	**ON287029**
Macrolepiota phaeodisca	—	994 (L)	P. Roux	France	AF482847
Macrolepiota procera	—	1049 (L)	T. Boekhout	Netherlands	AY243588
	—	9289 (L)	C. Bas	France	AY243590
Macrolepiota '*procera* AZ01'	iNat 131272162	—	H. Singer	Arizona, USA	OR583681
	iNat 136469748	JLF10816	J. Frank	Arizona, USA	OP522352
	iNat 94815088	—	M.C. Torres-Grant	Arizona, USA	PP274087
	iNat 94815636	—	M.C. Torres-Grant	Arizona, USA	PP274088
Macrolepiota pulchella	—	JJS0386 (SP455944)	N/A	Brazil	MG136891
	—	683 (UFRN-Fungos 2690)	E.P. Fazolino	Brazil	KY927721
Macrolepiota rhodosperma	—	4-VIII-2999	P.H. Kelderman	Netherlands	AY243598
	—	3666 (Y)	P.D. Orton	UK	AY243596
Macrolepiota sabulosa	—	689 (UFRN-Fungos 2693) (**Type**)	E.P. Fazolino	Brazil	KY927715
	—	70 (UFRN-Fungos 2694)	M.D. Xavier	Brazil	KY927716
Macrolepiota sp.	—	362	E.P. Fazolino	Brazil	KY927718
	—	643	E.P. Fazolino	Brazil	KY927720
	—	SB194	N/A	Argentina	MN847714
	—	126 (ICN139341)	M.S. Rother	Brazil	KY927725
	—	SB192	N/A	Argentina	MN847713
	—	SB80	N/A	Argentina	MN847715
	—	124 (ICN139339)	M.S. Rother	Brazil	KY927724
Macrolepiota subcitrophylla	—	(HKAS 58248)	Z.H. Chen	China	JN180322
	—	(HKAS 61624) (**Type**)	Z.W. Ge	China	JN180321
Macrolepiota turbinata	—	H0219 (PERTH) (**Type**)	N. Malajczuk	Australia	NR_119949
	—	14576	R. Watling	Australia	JF495074
Macrolepiota umbonata	—	SFC20180924-08	N/A	South Korea	MK453249
	—	SFC20160909-16 (**Type**)	N/A	South Korea	MK453245
Macrolepiota velosa	—	(HKAS 59720)	Y.C. Li	China	JN180320

Table 1. (Continued).

Species	Collection data[1]	Specimen-voucher (Fungarium)[2]	Collector(s)	Origin	ITS sequence source
	—	Z.L. Yang 2172 (HKAS 29487) (**Type**)	Z.L. Yang	China	NR_119459
Macrolepiota vinaceofibrillosa	—	H0047 (**Type**)	N/A	Australia	JF495070
Bovista plumbea (outgroup)	—	AG1825	S.L. Miller, A. Gongloff	N/A	MT908234
Lycoperdon perlatum (outgroup)	iNat 137771783	—	S. Russell	Missouri, USA	OP743609

[1]iNat: iNaturalist; MO: Mushroom Observer; MP: MyCoPortal; NA: not available.

[2]Records in **bold**: Collections examined.

RESULTS

Phylogeny

The topologies of the ML and BI trees are essentially identical, so only the ML analysis is shown in Fig. 1, with support values from both phylogenetic reconstructions. The three sections previously recognized within *Macrolepiota* (*viz*. *Macrolepiota, Macrosporae* and *Volvatae;* see Ge *et al*. 2010) are recovered with good support in our analysis. However, several species previously recovered as part of sect. *Macrolepiota* (*M. clelandii*, *M. cyanolamellata*, *M. sabulosa*, *M. subcitrophylla*) appear in unresolved positions within the genus in our analyses.

All North American sequences are recovered as part of sect. *Macrolepiota*, and more specifically as part of a well-supported clade together with *M. capelariae*, *M. colombiana*, *M. rhodosperma* and an undescribed species from Brazil and Argentina. All taxa in this lineage occur exclusively in the Americas, except for *Macrolepiota rhodosperma* that occurs in Europe. All sequences of the species described here as *M. macilenta* form a well-supported clade, while the sequences of *M. pallida* are recovered as separate from *M. macilenta*, but not forming a single clade themselves (see under Discussion).

An additional set of sequences from Arizona (USA) is recovered as closely related to the South American *M. colombiana*. These sequences are currently known under the provisional code "*Macrolepiota procera* AZ01", but they are not discussed in detail in the present paper. An additional sequence from Mexico (GenBank MH231156) might represent the same or a closely related species.

Taxonomy

Macrolepiota macilenta Lebeuf, S.D. Russell & Justo, ***sp. nov.*** MycoBank MB 852291. Figs 2, 3.

Etymology: From the Latin *macilentus*, meaning "lean" or "thin", referring to the stipe of this species.

Diagnosis: Medium to large species with general pale brown colours found in eastern and midwestern North America. Pileus 50–160 mm broad, covered with greyish brown, brown, rusty brown to dark brown uplifted patches over a pale cream, greyish orange to brownish orange fibrillose background; stipe 120–260 mm long, 4–9 mm at apex, 16–45 mm at base, clavate to bulbous, densely covered with small scales concolourous with pileus fibrils in a zigzag pattern on a pale beige background; basidiospores 13.0–18.0 × 9.5–12.0 µm, on average 13.9–16.8 × 10.1–11.5 µm; cheilocystidia predominantly fusiform to clavate; pileus covering a trichoderm made of erect hyphae 125–200 µm long; stipe scale terminal elements attenuated towards apex. *M. procera* differs in its geographic distribution (Europe), nrITS sequences (26 evolutionary events) and several morphological characters: larger pileus 100–300 mm in diameter; longer and broader stipe measuring 100–400 × 10–20 mm; narrower basidiospores on average 13.8–15.7 × 9.2–9.9 µm, variously-shaped cheilocystidia not predominantly fusiform or clavate; and very thick-walled pileal hyphae.

Fig. 2. *Macrolepiota macilenta.* **A, B.** Basidiomata *in situ*, collection MICH 346530 (holotype). **C.** Basidiomata (*ex situ*), collection HRL1746. **D.** Details of stipe, collection iNat 30853789. **E.** Basidiomata *in situ*, collection PUL00036473. **F.** Details of pileus surface, collection DAOM 985165. **G.** Details of pileus surface, collection HRL3554. **H.** Details of annulus, collections HRL3554, iNat 8597014, iNat 86938953 (from top to bottom). Photos: A, B, E, F: S. Russell; C: R. Lebeuf; D: P. Gladkov; G: C. Lapointe.

Typus: **USA**, Indiana, Marion, Monroe Township, 40.527882, -85.553596, 269 m a.s.l., grassy clearing in hardwoods, in soil, 13 Jul. 2021, *M. Russell*, AS70 (**holotype** MICH 346530; **isotype** DAOM 985164). GenBank OK490279.

Description: *Pileus* 50–160 mm diam, at first oblong, ovoid to subglobose with central umbo, expanding via paraboloid with low umbo and deflexed margin to convex with low umbo, at times with depressed centre, when very young completely closed, greyish brown, brown, rusty brown to dark brown (6E(3–8), 6EF6, 7EF8) from the covering which later breaks into a central calotte and uplifted squamulose patches easily detachable from the pileus over the pale cream, orange-grey, greyish orange to brownish orange (4A2, 5B2, 5B3, 5C4) fibrils below; margin fibrillose-squarrose with white fibrils from the annulus, exceeding lamellae. *Lamellae* free, remote from stipe, close to crowded, 88–96 complete with 0–2 tiers of lamellulae, convex, white, with white, fimbriate edge. *Stipe* 120–260 mm long, 4–9 mm at apex, slightly widening downwards to a clavate to bulbous base 16–45 mm across, stuffed then hollow, densely covered up to the apex with small scales that are concolourous with the pileus fibrils and form a zigzag pattern with elongation of the stipe on a pale beige background. *Annulus* with beige, ascending or descending, cuff-like part around stipe, double crowned, with upperside white to pale brown and fringed, and underside with a brown rim concolourous with calotte. *Context* thick in pileus, white, brown in larval perforations, with an indistinct odour and a mild taste. *Spore print* pinkish white (8A2). *Basidiospores* (Fig. 3A) [244/8/7] (11–)13.0–18.0(–26.0) × (8.5–)9.5–12.0(–16.0) µm, av. 13.9–16.8 × 10.1–11.5 µm, Q = 1.17–1.71, $Q_{av.}$ = 1.36–1.52, ellipsoid, ellipsoid-amygdaloid to ovoid in profile view, ellipsoid to ovoid in face view, dextrinoid, metachromatic, cyanophilous, congophilous, smooth, thick-walled (1–2 µm), with a mostly central or at times eccentric germ pore 1–1.5 µm wide covered with a hyaline cap. *Basidia* 33–60 × 13–17 µm, 4-spored, clavate. *Cheilocystidia* (Fig. 3B, C) 10–57 × 8–20 µm, numerous, forming a sterile band or intermixed with basidioles and basidia, variously shaped but predominantly fusiform to clavate with a broad base, also oblong, ellipsoid, (sub)cylindrical, catenulate or not, thin-walled. *Pileus covering* (in the calotte) (Fig. 3D–F) a trichoderm made of erect hyphae 125–200 µm long, septate, with thin to slightly thickened walls (~0.5 µm); terminal elements predominantly tapering towards apex, less frequently cylindrical with round apex, with a diffuse

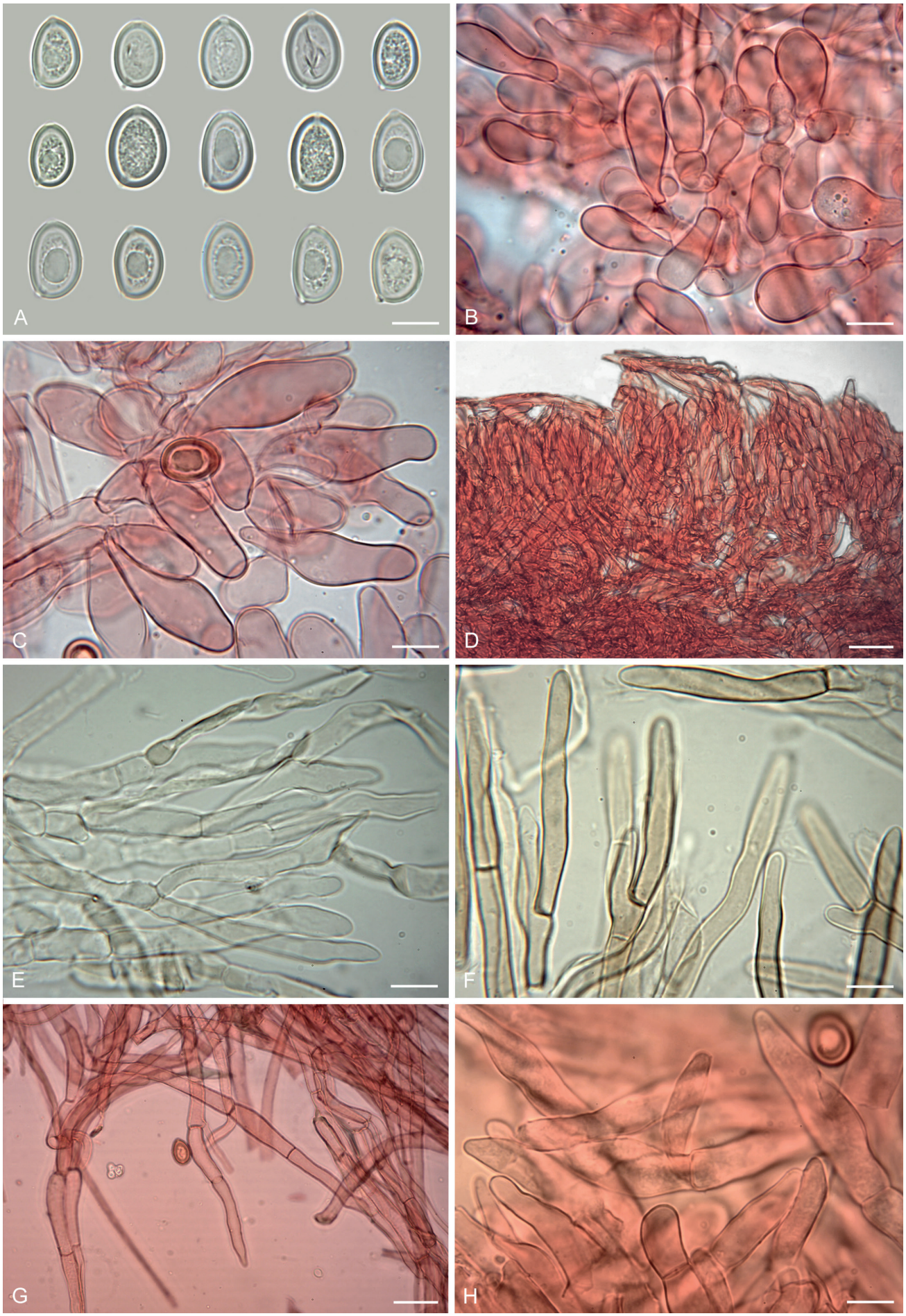

Fig. 3. *Macrolepiota macilenta.* **A.** Basidiospores. **B, C.** Cheilocystidia, collections Mushroom Observer 462132 (B) and HRL3528 (C). **D.** Pileus covering section in SDS Congo Red, collection HRL1746. **E, F.** Pileus covering in KOH, collection HRL1746 (E) and MICH 346530 (holotype) (F). **G, H.** Stipe scale terminal elements, collections MICH 346530 (holotype) (G) and HRL3528 (H). Scale bars: A–C, E, F, H = 10 µm; D, G = 25 µm.

Fig. 4. *Macrolepiota pallida.* **A–E**. Basidiomata *in situ*. **A, D, E.** Collection DAOM 984979 (holotype). **B.** Collection HRL1765. **C.** Collection HRL3525. **E. (insert)** Details of annulus from above, collection DAOM 984979. Photos: A, B, D, E: R. Lebeuf; C: I. Pouliot.

brownish yellow to yellowish brown pigment in KOH, 25–150 × 5–12 µm; elements below shorter, often inflated, 15–60 × 7–12 µm, thin-walled or slightly thick-walled, with a diffuse or spirally-incrusting pale yellow to brownish yellow pigment in KOH; basal elements incrusted with a pale yellow pigment. *Scales on stipe* (Fig. 3G, H) made of bundles of multiseptate hyphae, with a yellowish brown diffuse pigment in KOH, sometimes with fine spiral-like incrustations; terminal elements tapering towards apex to aciculate or more rarely with rounded apices, mostly thin-walled, sometimes with slightly thickened walls (~0.5 µm), smooth or spirally incrusted, 20–130 µm long, 5–12 µm at their widest; elements below 25–50(–80) × 5–16 µm, inflated, with thin or slightly thickened walls (~0.5 µm), sometimes slightly incrusted. *Clamp connections* rare to frequent at the base of basidia, absent to abundant in the stipitipellis hyphae. Absent in pileus covering hyphae, cheilocystidia and stipe scales.

Habitat and distribution: In small groups or gregarious, sometimes many isolated basidiomata present in a vast area, in hardwoods with *Quercus* or in clearings, from August to October, on nutrient-rich soils. Widely distributed in eastern and midwestern North America, from the southernmost part of Québec to Georgia in the east, and from Iowa to Missouri in midwestern USA, and collected from the state of Jalisco in Mexico.

Edibility: Consumed and considered a good edible.

Additional materials examined: **Canada**, Québec, Venise-en-Québec, mixed forest of *Q. rubra*, *F. grandifolia*, *Acer* sp. and *Pinus strobus* in litter, 17 Sep. 2021, *C. Lapointe*, HRL3554 (DAOM 984981; R.L. personal fungarium); Frelighsburg, sunny clearing enriched with compost and straw, in rather sandy soil, 18 Sep. 2021, *G. Boyer*, HRL3528 (DAOM

984980; R.L. pers. fung.). **USA**, Maine, Brunswick, under *Q. rubra* in litter, 7 Aug. 2014, *C. Michaud*, HRL1746 (R.L. pers. fung.); Indiana, Monterey, Tippecanoe River State Park, deciduous forest with *Quercus* sp., 5 Oct. 2019, *M. Seefeldt*, 70501, iNaturalist 33910816 (DAOM 985166); Angola, Jamestown Township, Pokagon State Park, deciduous forest (*Quercus*, *Acer*), in litter-covered soil, 10 Oct. 2019, *S. Russell*, 1670, iNaturalist 34175553 (DAOM 985165); Pennsylvania, Luzerne County, Harvey's Lake, on ground in *Quercus* woods, 23 Jul. 2021, *D. Wasilewski*, Mushroom Observer obs. 462132 (DAOM 985167).

Macrolepiota pallida Lebeuf, S.D. Russell & Justo, ***sp. nov.*** MycoBank MB 852292. Figs 4, 5.

Etymology: Named for the general pale colours of the basidiomata.

Diagnosis: Large species with general pale colours found in eastern and midwestern North America. Pileus 70–260 mm broad, covered with brownish orange to greyish orange concentric uplifted patches over a white fibrillose background; stipe 250–375 mm long, 5–14 mm at apex, 25–40 mm at base, clavate to bulbous, with orange-white covering over a paler background, finely scaly in the upper part, in a zigzag pattern below; basidiospores 11–16.5 × 8–11 µm, on average 12.6–14.3 × 9.0–10.0 µm; cheilocystidia predominantly cylindrical to broadly cylindrical; pileus covering a trichoderm made of a dense palisade up to 100 µm thick of slightly thick-walled hyphae intermixed with less numerous and longer thick-walled hyphae up to 350 µm long; stipe scale terminal cells cylindrical to clavate. Differs from *M. macilenta* in the paler colours of the pileus and stipe, slightly smaller basidiospores, predominantly cylindrical cheilocystidia, pileus covering structure, shape of the stipe scale terminal elements, and nrITS sequences (9 evolutionary events).

Typus: **Canada**, Québec, Québec City, Boisé Marly, 46.751611, -71.329083, 95 m a.s.l., in a deciduous forest of *Q. rubra*, *Fraxinus* sp. and *Acer* sp., in soil covered with leaf litter, 23 Sep. 2021, *R. Lebeuf & A. Paul*, HRL3526 (**holotype** DAOM 984979; **isotype** R.L. pers. fung.). GenBank ON287029.

Description: *Pileus* 70–260 mm diam, at first ovoid to oblong with central umbo, expanding via convex with low umbo and inflexed margin to applanate with low umbo, when very young completely closed, brownish orange to greyish orange [5A3, 5B3, 6(B–C)(4–5)] from the covering, concolourous or paler towards the margin, the covering soon breaking up into a central calotte and concentrically arranged and uplifted squamulose patches easily detachable from the pileus over the white radial coarse fibrils below; margin fibrillose-squarrose, exceeding lamellae. *Lamellae* free, remote from stipe, close to crowded, 84–92 complete with 1–2 tiers of lamellulae, subventricose, 10–15 mm broad, white with an orange hue, with white, fimbriate edge. *Stipe* 250–375 mm long, 5–14 mm at apex, widening downwards to a white tomentose clavate to bulbous base 25–40 mm across, hollow, overlain by fine orange-white (5A2) scales from the apex to about 20–40 mm under the annulus, and below by orange-white (5A2) appressed fibrils in a zigzag pattern on a pale beige background. *Annulus* thick with a broad edge (double crown), with upperside white and fringed and underside concolourous with calotte, sometimes with a narrow white cuff-like part around stipe. *Context* thick in pileus, white, slowly browning when bruised, with an indistinct odour and a mild taste. *Spore print* pinkish white on a white tissue. *Basidiospores* (Fig. 5A) [271/9/6] (11–)11.5–15(–18) × (8–)8.5–10.5(–11) µm, av. 12.6–14.3 × 8.9–10.0 µm, Q = 1.25–1.83, $Q_{av.}$ = 1.36–1.49, ellipsoid to ellipsoid-amygdaloid in profile view, ellipsoid to ovoid in face view, dextrinoid, metachromatic, cyanophilous, congophilous, smooth, thick-walled (1–1.5 µm), with a central germ pore 1–2 µm wide covered with a hyaline cap. *Basidia* 36–52 × 12–16 µm, 4-spored, clavate. *Cheilocystidia* (Fig. 5B, C) 10–62(–70) × 6.5–14 µm, forming a sterile band, cylindrical, broadly cylindrical, subventricose, more rarely narrowly clavate or fusiform, rarely bearing an apical digital excrescence up to 30 × 2–3 µm, very often catenulate or 1-septate, colourless, thin-walled. *Pileus covering* (in the calotte) (Fig. 5D–F) a trichoderm made of a dense palisade up to 100 µm thick of slightly thick-walled (~0.5 µm) septate hyphae, cylindrical or inflated, colourless or with a pale yellow to sand-brown intracellular pigment in KOH; terminal elements 20–80 × 5–12 µm, cylindrical with rounded apices, less frequently clavate or subcapitate; elements below slightly shorter, 18–60 × 4–14 µm. Intermixed with the palisade hyphae are less numerous and longer 1–4(–5)-septate hyphae, 100–350 × 7–11 µm, colourless or pale yellow in KOH, thick-walled (1–2 µm), with 0.5–1.5 µm thick septa, erect or lying horizontally on top of the palisade; terminal elements smooth, 40–115(–180) × 4–8 µm, tapering towards apex or less frequently cylindrical with rounded apices, sometimes constricted at septa; basal elements incrusted. *Scales on stipe* (Fig. 5G, H) made of bundles of multiseptate hyphae, colourless or pale yellow to yellow-brown in KOH, with terminal elements 13–75(–110) × 5–14 µm, thin-walled, predominantly (broadly) cylindrical with rounded apices, less frequently (narrowly) clavate or tapering towards apex, rarely subcapitate or bearing an apical digital excrescence up to 30 µm × 3 µm; elements below cylindrical or inflated, 13–60(–90) × 5–14 µm. *Clamp connections* present at the base of basidia, occasional at the base of cheilocystidia, and rare in the stipitipellis hyphae; absent in the pileus covering hyphae and stipe scales.

Habitat and distribution: Solitary or gregarious, sometimes many isolated basidiomata present in a vast area, in deciduous or mixed forests with *Fagaceae* (*Quercus*, *Fagus*), from August to October, on nutrient-rich soils covered with leaf litter. Presently confirmed from Québec, in Canada, and from the states of New York, Indiana and Michigan, in the United States.

Edibility: Consumed and considered a good edible.

Additional materials examined: **Canada**, Québec, Mascouche, deciduous forest of *Q. rubra* and *Fagus grandifolia* in litter, 17 Aug. 2012, *J. Nuzzolese*, HRL1147 (DAOM 984977; R.L. pers. fung.); Saint-Alexis-des-Monts, mixed forest, 16 Aug. 2014, *R. Demers*, HRL1765 (R.L. pers. fung.); La Prairie, mixed forest of *Q. rubra*, *F. grandifolia*, *Acer rubrum*, *Carpinus caroliniana* and *Pinus strobus* in litter, 16 Sep. 2021, *I. Pouliot*, HRL3525 (DAOM 984978; R.L. pers. fung.); Saint-Alexis-des-Monts, chemin Yvon-Plante, mixed forest of *F. grandifolia*, *Acer* sp. and *A. balsamea*, 16 Aug. 2023, *R. Lebeuf & A. Paul*, HRL4269 (R.L. pers. fung.). **USA**, New York, Salamanca, Allegany State Park, mixed woods and deciduous woods in litter, 28 Aug. 2018, *G. Taylor*, iNaturalist 15990577 (DAOM 985168).

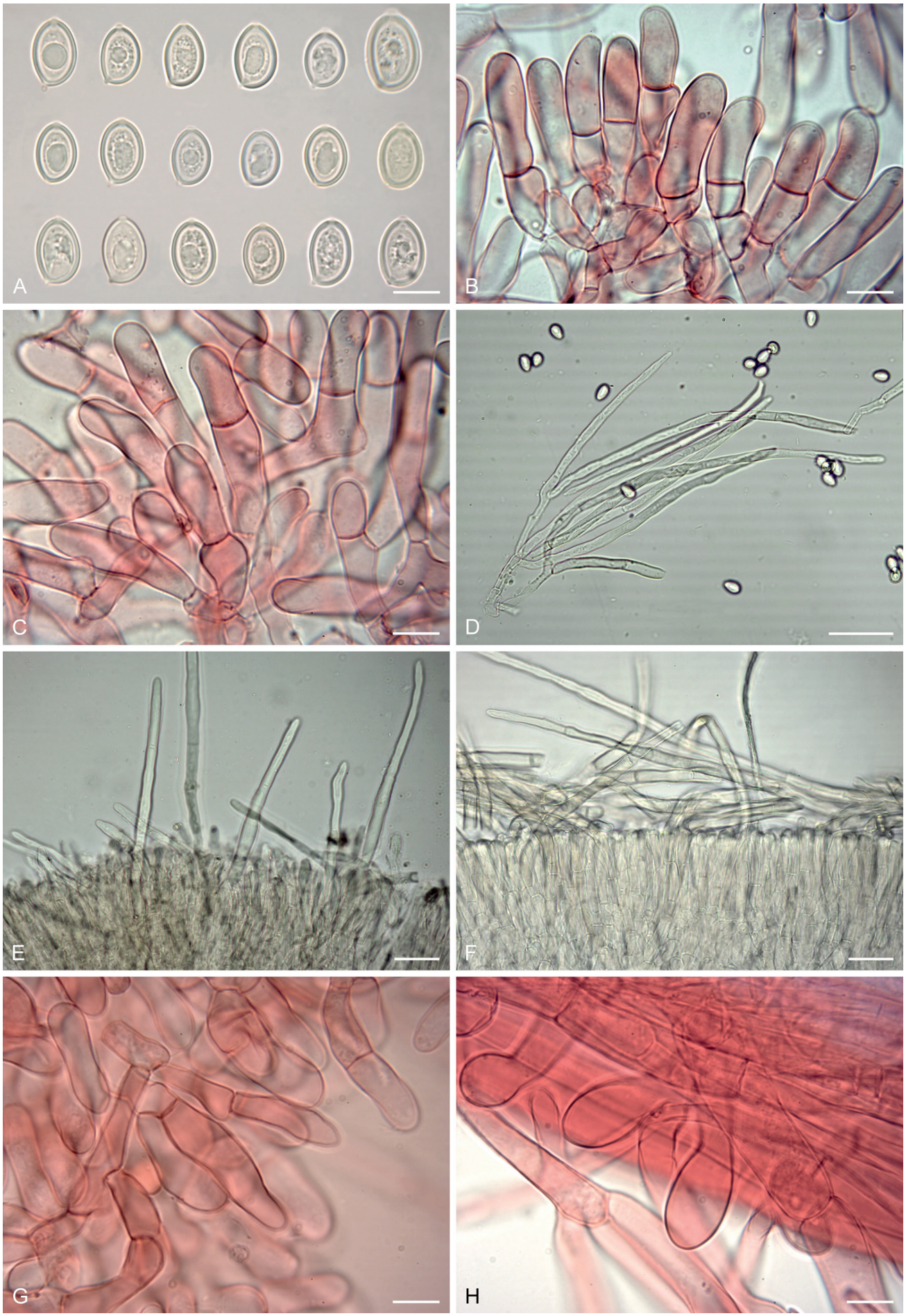

Fig. 5. *Macrolepiota pallida*. **A.** Basidiospores. **B, C.** Cheilocystidia, collections HRL4269 (B) and HRL3525 (C). **D–F.** Pileus covering in 3 % KOH, collections HRL1147 (D), HRL1765 (E) and HRL3525 (F). **G, H.** Stipe scale terminal elements, collections HRL1765 (G) and HRL1147 (H). Scale bars: A–C, G, H = 10 µm; D–F = 25 µm.

DISCUSSION

The two new species described here belong in section *Macrolepiota*, characterized by Singer (1986) by the presence of clamp connections in the basidioma trama, or at least in the lower part of the rind of the stipe and at the base of many basidia. The microscopic data confirm the phylogenetic placement.

Macrolepiota macilenta is very similar to the European *M. procera* and *M. rhodosperma*, none of which are present in North America. It mainly differs in its geographic distribution and nrITS sequences, 26 evolutionary events separating it from *M. procera* and 23 from *M. rhodosperma*. Morphologically, *M. procera* is most similar in its overall colours to the dark basidiomata of *M. macilenta*, but is distinguished by its larger pileus 100–300 mm diam, longer and broader stipe measuring 100–400 × 10–20 mm, narrower basidiospores on average 13.8–15.7 × 9.2–9.9 µm, variously-shaped cheilocystidia not predominantly fusiform or clavate, and very thick-walled pileal hyphae (Vellinga 2001). *Macrolepiota rhodosperma* has a pileus 70–220 mm broad, a stipe often slightly discolouring vinaceous pink or red when scratched, and slightly larger basidiospores on average 13.6–15.2 × 8.6–10.5 µm (Vellinga 2001, as *M. fuliginosa*). See Vellinga (2001) and Vizzini *et al*. (2011) for full descriptions of the two European species, as well as a detailed discussion of their wide morphological variation and nomenclatural history.

Macrolepiota orientiexcoriata, in section *Macrosporae*, is another species that could be confused with *M. macilenta* owing to its similarly-shaped cheilocystidia. It differs in its geographic distribution (China), nrITS sequences (43 evolutionary events), and, morphologically, by its pileus covered with brownish yellow squamules on a white background, shorter stipe 90–110 mm long either glabrous or with shiny hairs, smaller basidiospores measuring (12.0–)13.0–15.0(–16.0) × (7.5–)8.5–10.0(–10.5) µm, and presence of clamp connections at the base of cheilocystidia (Ge *et al*. 2010).

Macrolepiota pallida is morphologically characterized by its white-fibrillose pileus adorned with brownish orange to greyish orange concentrically-arranged scales, pale stipe of orange-white scales or fibrils on a pale beige background, basidiospores on average 12.6–14.3 × 8.9–10.0 µm, pileus covering made of a dense palisade of cylindrical hyphae up to 100 µm long intermixed with less numerous thick-walled hyphae 100–350 µm long, predominantly cylindrical cheilocystidia sometimes with clamp connections, and stipe scale terminal elements predominantly cylindrical to clavate. *Macrolepiota macilenta* differs in its pale cream, brownish orange to greyish orange pileal fibrils, darker stipe ornamentation, larger basidiospores on average 13.9–16.8 × 10.1–11.5 µm, pileus covering hyphae 125–200 µm long, predominantly fusiform to clavate cheilocystidia without clamp connections, and stipe scale terminal elements attenuated towards apex to aciculate. Even though their dimensions can overlap, the basidiomata of *M. pallida* can reach larger sizes than those of *M. macilenta*, with a pileus up to 260 mm diam and a stipe up to 375 mm long. In *M. macilenta*, the pileus can reach 50–160 mm and the stipe 120–260 mm in length. The European *Macrolepiota procera* differs from *M. pallida* mostly by its darker pileus with patches poorly contrasting from the background and darker zig-zag bands on the stipe.

The nrITS sequences of *M. macilenta* and *M. pallida* differ in nine evolutionary events, and both species differ from each other morphologically, as discussed above. However, phylogenetic analyses performed here fail to recover both taxa as reciprocally monophyletic. This is not an uncommon result between species-pairs with 0.5–1.5 % sequence divergence in their nrITS sequences. Similar examples of this situation in *Agaricales* include *Pluteus salicinus* and *P. americanus* (Justo *et al*. 2014); *Lyophyllum fumosum* complex (Bellanger *et al*. 2015); *Hygrophorus fuscoalboides* & *H. korhonenii*, *Hygrophorus agathosmus* & *H. agathosmoides* (Bellanger *et al*. 2021) and many taxa in *Cortinarius* subgenus *Telamonia* (Liimatainen *et al*. 2020).

Macrolepiota capelariae, recently described from Brazil, Argentina and Mexico, can be easily distinguished from the new species by its rimose to finely cracked orange-brown pileus without large scales, less complex annulus, non-catenulate clavate cheilocystidia, and absence of clamp connections, even though it genetically belongs in section *Macrolepiota* (Souza *et al*. 2022).

Based on the molecular data available, the species of *Macrolepiota* found in the USA and Canada are specific to that region of the world, which mimics the observations made by workers from other regions (*e.g*. Ge *et al*. 2010, Fazolino *et al*. 2018, Cho *et al*. 2019, Souza *et al*. 2022). Moreover, judging from the available ITS sequences and the pictures posted on the web sites iNaturalist (https://www.inaturalist.org) and Mushroom Observer (https://mushroomobserver.org), *M. pallida* seems to be restricted to the Maritimes and the southern region of Québec and Ontario in Canada and to the northernmost states of eastern and midwestern USA, occupying a narrow band of territory up to the northern limit of the *Fagaceae*, whereas *M. macilenta* covers a large territory going from the southernmost part of Québec, all the way down to Georgia in the east and west to Iowa and into Mexico. This distribution pattern would have been difficult to establish without the numerous records made by community scientists as part of large sequencing initiatives such as the North American Continental Mycoblitzes (https://mycota.com/continental-mycoblitz) and the Fungal Diversity Survey (https://fundis.org).

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