Simocybe ramosa, a New Species from the Boston Harbor Islands National Recreation Area

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Abstract - A new saprotrophic species was discovered during our fungal inventory at the Boston Harbor Islands National Recreation Area (Massachusetts), which consists of 34 islands and peninsulas. *Simocybe ramosa* sp. nov. (Agaricales, Crepidotaceae) is described based on morphology and molecular phylogenetic data. The holotype collection was found in a *Quercus* (oak)–*Carya* (hickory) forest under bark of a dead oak tree on World's End peninsula, the largest land mass of the archipelago. Phylogenetic reconstruction of a dataset of the internal transcribed spacer region (ITS) resolved *S. ramosa* and *S. rhabarbarina* as sister species. *Simocybe rhabarbarina* is here redescribed based on the holotype and newly reported material from the Netherlands, and its presence on the island of Jersey, off the coast of northern France, is confirmed based on an ITS sequence. Finally, we compare morphological features of *S. ramosa* with *S. rhabarbarina* and the 20 species in the genus that have thus far been recorded in Canada, the US, and Mexico.

Introduction

The kingdom Fungi is one of the most diverse and species-rich branches of the tree of life, estimated at 2.5 million species (Niskanen et al. 2023). Within the kingdom, phylum Basidiomycota is second in number of species (after Ascomycota), with the order Agaricales encompassing the majority of that described diversity (17,291 out of 41,270 species; He et al. 2019). Crepidotaceae is one of 46 recognized families within Agaricales (Kalichman et al. 2020). The family is characterized in part by basidiomata producing pale yellow to dark-brown, smooth to ornamented basidiospores, with a saprotrophic mode of nutrition, and includes the closely related genera *Crepidotus*, *Neopaxillus*, and *Simocybe* (Aime et al. 2005, Watling and Aime 2013) in addition to *Episphaeria*, *Nanstelocephala*, *Phaeosolenia*, and *Pleuroflammula* (Kalichman et al. 2020).

Whereas species of *Crepidotus* are mostly pleurotoid and those of *Neopaxillus* are centrally stipitate, *Simocybe* comprises both centrally stipitate and pleurotoid forms. Microscopically, *Simocybe* species can be recognized by (1) the wide abundance of cystidia in all parts of the basidiomata that causes their pruinose appearance and (2) the basidiospores that are smooth and applanate to depressed at the adaxial side. In comparison, the basidiospores of *Crepidotus* are either smooth

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or ornamented (i.e., punctate, echinulate, rugulose, or verrucose), globose or cylindrical to ellipsoid in shape, and without depression, whereas those of *Neopaxillus* are subglobose and strongly echinulate (Hesler and Smith 1965, Watling and Aime 2013). In addition, basidiomata of *Simocybe* species often have a typically olivaceous tint in the pileus.

All 70 species included in the genus *Simocybe* (Index Fungorum 2023) are saprotrophic and grow on rotting plant debris such as wood, bark, leaves, and stems, or on decaying moss (Horak and Ronikier 2011). Worldwide, a total of 3549 georeferenced records of *Simocybe* have been made between 1786 and 2023 (GBIF Secretariat 2023a). Most occurrences come from North America and Europe, followed by Oceania. Africa is the continent least represented for the genus, with only a handful of records from 3 countries: Gabon, Madagascar, and South Africa (GBIF Secretariat 2023a, iNaturalist 2023). The observed distributional unevenness—with the majority of records originating from North America and Europe—is also seen in other groups of fungi (Haelewaters et al. 2024, Quandt and Haelewaters 2021) and necessitates caution in conclusions about ecological preferences and distributional patterns.

Several new species of *Simocybe* have been described in the past 30 years—from Canada (Horak and Miller 1997), Spain (Bandala et al. 2008), Switzerland (Horak and Ronikier 2011), Italy (Poli et al. 2015, Simmel and Gröger 2015), and New Zealand (Horak 2018). The aim of this paper is to formally describe a previously undiscovered *Simocybe* species resulting from a fungal inventory project at the Boston Harbor Islands National Recreation Area (BHI) in Massachusetts (Haelewaters et al. 2018b).

Materials and Methods

Description of the research area

We made a collection of *Simocybe* (BHI-F724) on World's End peninsula, a 1-km² park and conservation land, part of the BHI. The peninsula is bordered by the Weir River to the North and East and Hingham Harbor to the West. The outer side of World's End is rocky and exposed to wind and sea waves. The inner habitat is varied but at all places subjected to human disturbances like agriculture (Haelewaters et al. 2018b). World's End is the site on BHI hosting the largest number of plant species (301) and one of the only parts of the recreation area with a proportion of native species exceeding 60% of the total flora. This significant floral diversity is the result of its large size, varied topography, habitat diversity, connection to the mainland, and relative lack of historical disturbance (Elliman 2005). Hopping (2000) identified some upland habitats on World's End of particular regional significance.

We collected the sample in a *Quercus* (oak)–*Carya* (hickory) forest on the southeast of the peninsula, to the east of the Damde Meadows marshland and close to the Weir River Road. This forested community is the only closed-canopy habitat in BHI and is dominated by native trees. The most common tree species belong to the genus *Quercus*, specifically *Q. velutina* Lam. (Black Oak), *Q. rubra* L. (Northern Red Oak), and *Q. alba* L. (White Oak). The following species were also identified: *Acer rubrum* L. (Red Maple), *Carya cordiformis* (Wangenh.) K. Koch (Bitternut

Hickory), *Ostrya virginiana* (Mill.) K. Koch (Hop Hornbeam), *Pinus strobus* L. (White Pine), and *Tsuga canadensis* (L.) Carrière (Eastern Hemlock).

Morphology

We studied macroscopic characteristics from dried material using a Novex RZB-PL 65.500 dissecting microscope (Arnhem, The Netherlands). We photographed the collection BHI-F724 using a Euromex HD-Ultra camera (Euromex Microscopen, Arnhem, The Netherlands) attached to a Nikon SMZ8000 binocular microscope (Tokyo, Japan). We observed microscopic characteristics in a 1% solution of Congo red in modified L4 (Clémençon 1972) by replacing Invadin IFC with SDS (Sodium dodecyl sulfate) after 10-s pre-treatment in 10% KOH. When necessary, we performed an extended pre-treatment of 15 min up to 1 h in 10% KOH to obtain better swelling of microscopic structures. We observed basidiospores at magnification of 1000× under a Zeiss Axioskop 2 phase-contrast microscope (Dublin, CA), took measurements with a crosshair eyepiece, and created line drawings using a camera lucida at magnification of 6000×. We measured a total of 20 basidiospores in side view (length and width). Basidiospore measurements are herein presented as (MIN–)[Avg - SD]–Avg–[Avg + SD](–MAX), with Avg = mean value for the measured basidiospores, SD = standard deviation, MIN = lowest extreme value, and MAX = greatest extreme value. The Q-value (quotient length/width) is given as (MIN Q–)[Qavg - Qsd]–Qavg–[Qavg + Qsd](–MAX Q), with Qavg = mean ratio for the measured basidiospores, Qsd = standard deviation, MIN Q = lowest extreme ratio, and MAX Q = greatest extreme ratio. For other elements, we made measurements using an eyepiece micrometer (at magnification of 1000×) and prepared line drawings (at original magnifications of 1500×) with the aid of a camera lucida (Olympus U-DA) mounted on an Olympus CX21 compound light microscope (Tokyo, Japan). Measurements are presented as Lmin–Lmax × Wmin–Wmax, with Lmin = lowest value for the length of an element, Lmax = greatest value for the length of an element, Wmin = lowest value for the width of an element, and Wmax = greatest value for the width of an element. We measured lengths of basidia without sterigmata. The number of structures measured is shown in square brackets. We edited drawings in Adobe Photoshop CS (San Jose, CA). We took microscopic images with a Euromex HD-Ultra camera attached to a Nikon eclipse E600 compound microscope. We compared morphological features to those described in the literature (Breitenbach and Kränzlin 2000, Knudsen and Vesterholt 2018, Ludwig 2000, Seifert et al. 2011).

DNA extraction, PCR amplification, and sequencing

We removed a rice-sized piece of tissue from each fresh or dried specimen used in this study and stored them at -20 °C until molecular work could be performed. We extracted DNA using the QIAamp DNA Micro Kit or DNeasy Plant Pro Kit (Qiagen, Valencia, CA), following the manufacturer's instructions. We macerated fungal tissue in 1.5-mL tubes with 1.5-ml pellet pestles (Kimble, Rockwood, TN, #749521-1500), prior to adding buffer ATL (QIAamp) or AP1 (DNeasy). We stored DNA extractions at -20 °C until performing PCR amplification.

PCR amplification targeted the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) and part of the nuclear large subunit rDNA (nrLSU). We used the following primers: ITS1f/ITS4 for ITS and LR0R/LR5 for nrLSU (Gardes and Bruns 1993, Hopple 1994, Vilgalys and Hester 1990, White et al. 1990). PCR reactions (25 µL total) consisted of 13.3 µL of RedExtract Taq polymerase (Sigma-Aldrich, St. Louis, MO), 2.5 µL of each 10-µM primer, 5.7 µL of ddH_2O, and 1.0 µL of DNA extract. PCR conditions were as follows: for ITS, initial denaturation at 94 °C for 3 min; 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 45 s, and extension at 72 °C for 90 s; and final extension at 72 °C for 10 min (Haelewaters et al. 2018a); for LSU, initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 45 s, and extension at 72 °C for 1 min; and final extension at 72 °C for 7 min (Liu et al. 2020). We purified PCR products using 1.5 µL of Exo-FAP (0.5 µL exonuclease I and 1 µL FAST alkaline phosphatase; Thermo Fisher Scientific, Waltham, MA) per 10 µL of PCR product at 37 °C for 15 min, followed by deactivation at 85 °C for 15 min. We sequenced purified PCR products using an automated ABI 3730 XL capillary sequencer (Life Technology at Macrogen, Amsterdam, The Netherlands). We assembled forward and reverse sequence reads and edited the contig sequence in Sequencher v5.4.6 (Gene Codes Corporation, Ann Arbor, MI). We submitted newly generated sequences to NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/; Table 1).

Sequence alignment and phylogenetic analysis

We BLAST searched newly generated ITS sequences against NCBI GenBank's standard *nr*/*nt* nucleotide database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). We downloaded related sequences for phylogenetic analysis and selected *Pleuroflammula flammea* (Murrill) Singer and *P. praestans* E. Horak as outgroup taxa based on the phylogeny by Matheny et al. (2020). We aligned ITS sequences using the command-line version of MUSCLE version 5.1.0 (Edgar 2022) and trimmed them at the conserved motifs 5'–CATTA–3' and 5'–GACCT–3' (Dentinger et al. 2011). We selected models of nucleotide substitution for each partition (ITS1, 5.8S, ITS2) using ModelFinder (Kalyaanamoorthy et al. 2017) according to the Akaike information criterion corrected for small sample size (AICc). Maximum likelihood (ML) was inferred under partitioned models using IQ-TREE version 1.6.7 for Windows 64-bit (Chernomor et al. 2016, Nguyen et al. 2015), with 1000 UFBOOT2 replicates (Hoang et al. 2017). We visualized the phylogenetic reconstruction with ultrafast bootstrap values in FigTree version 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) and edited in Adobe Illustrator 26.5. The aligned and trimmed alignment (in NEXUS format) and unedited tree (in TXT format) are available in the following GitHub directory: https://github.com/dannyhaelewaters/haelewaters-group/tree/main/simocybe_ramosa_paper.

Results

Nucleotide alignment and phylogenetic inference

The ITS dataset consists of 720 characters (311 in the ITS1, 158 in the 5.8S, and 251 in the ITS2), of which 252 are parsimony informative (134 in the ITS1, 1 in the

Table 1. Details of all isolates used in this study. Accession numbers of newly generated sequences indicated with "*".

Species	ID (isolate, voucher), type status	Country: State	Accession #
Crepidotus cesatii	LUGO ECC19120501	Spain	MW376643
Crepidotus cesatii	HBAU15207		MW855601
Crepidotus eucalyptinus	XAL 3165	Spain	KT715780
Crepidotus eucalyptinus	XAL 3275	Spain	KT715782
Crepidotus sphaerosporus	KMCC04845	South Korea	MN823140
Crepidotus sphaerosporus	HMAS 255466		MK966515
Crepitodus sp.		South Korea	MN294875
Naucoria decolorata	AH 13310, paratype	Spain	KT715784
Pleuroflammula cf. *ragazziana*	K(M):195255	Jersey	MZ159526
Pleuroflammula flammea	FLAS-F-60007	USA: Florida	KY654720
Pleuroflammula flammea	AFTOL-ID 1381 (MCA 339)	USA: Virginia	DQ494685
Pleuroflammula flammea	iNaturalist 103385419	USA: New York	OM403095
Pleuroflammula praestans	PERTH08242151	Australia	HQ832450
Simocybe centunculus	GC 92113	Italy	KT715787
Simocybe centunculus	HGASMF01-14743	China	MZ666425
Simocybe centunculus	iNaturalist 8526166	USA: Indiana	MN892572
Simocybe centunculus	Ghobad-Nejhad 125	Iran	MT535746
Simocybe centunculus	DUKE 0352598	USA: South Carolina	OL342391
Simocybe centunculus	ILLS:00112905	USA: Illinois	PP179169*
Simocybe centunculus	ILLS:00112903	USA: Missouri	PP179168*
Simocybe haustellaris	AH 34371	Spain	KT715794
Simocybe haustellaris	Gessi 07473	Italy	KT715790
Simocybe haustellaris	AH 31147	Spain	KT715792
Simocybe ramosa sp. nov.	PUL F29417, holotype	USA: Massachusetts	OM534632*
Simocybe reducta	GC 05229	Italy	KT715796
Simocybe rhabarbarina	GENT:D. Haelew. 3972	The Netherlands	PP177462*
Simocybe rhabarbarina	4583-15, isotype	Italy	KT934412
Simocybe serrulata	FLAS-F-60369	USA: Florida	MF153085
Simocybe serrulata	MushroomObserver 303044	USA: Indiana	MK607565
Simocybe serrulata	LUGO ECC17091302	Spain	MW376709
Simocybe serrulata	27-B10-ITS4-C19	USA: Arkansas	MN872846
Simocybe sp.	PDD 95840	New Zealand	HQ533028
Simocybe sp.	FLAS-F-61723	USA: Florida	MH212069
Simocybe sp.	FLAS-F-61129	USA: Florida	MH211762
Simocybe sp.	FLAS-F-61130	USA: Florida	MH211763
Simocybe sp.	iNaturalist 67063815	USA: California	MZ663994
Simocybe sp.	FLAS-F-64957	Chile	MH930383
Simocybe sp.	TENN:075712	USA: Vermont	ON503065
Simocybe sp.	MES-2782	Chile	MH930300
Simocybe sp.	PUL F26065	Canada	PP179164*
Simocybe sp.	BW1 (TENN)	USA: Tennessee	MF773645
Simocybe sp.	PBM3031 (TENN)	USA: Tennessee	GQ893023
Simocybe sp.	D. Haelew. 4207a	Guinea	PP179166*
Simocybe sp.	D. Haelew. 4207b	Guinea	PP179167*
Simocybe sp.	FLAS-F-63236	Argentina	PP179165*
Simocybe sp.	ILLS:00112902	USA: Illinois	PP179170*
Simocybe sp.	ILLS:00112897	USA: Illinois	PP179172*
Simocybe sp.	ILLS:00112899	USA: Illinois	PP179171*
Simocybe sumptuosa	RA718-1	USA: Arkansas	MK217451

5.8S, and 117 in the ITS2). The evolutionary models as selected by ModelFinder are as follows: TN+F+I+G4 (ITS1, -lnL = 2277.0421), JC+R2 (5.8S, -lnL = 266.1745), and TVM+F+I+G4 (ITS2, -lnL = 1834.4240). The resulting ITS tree (Fig. 1) shows

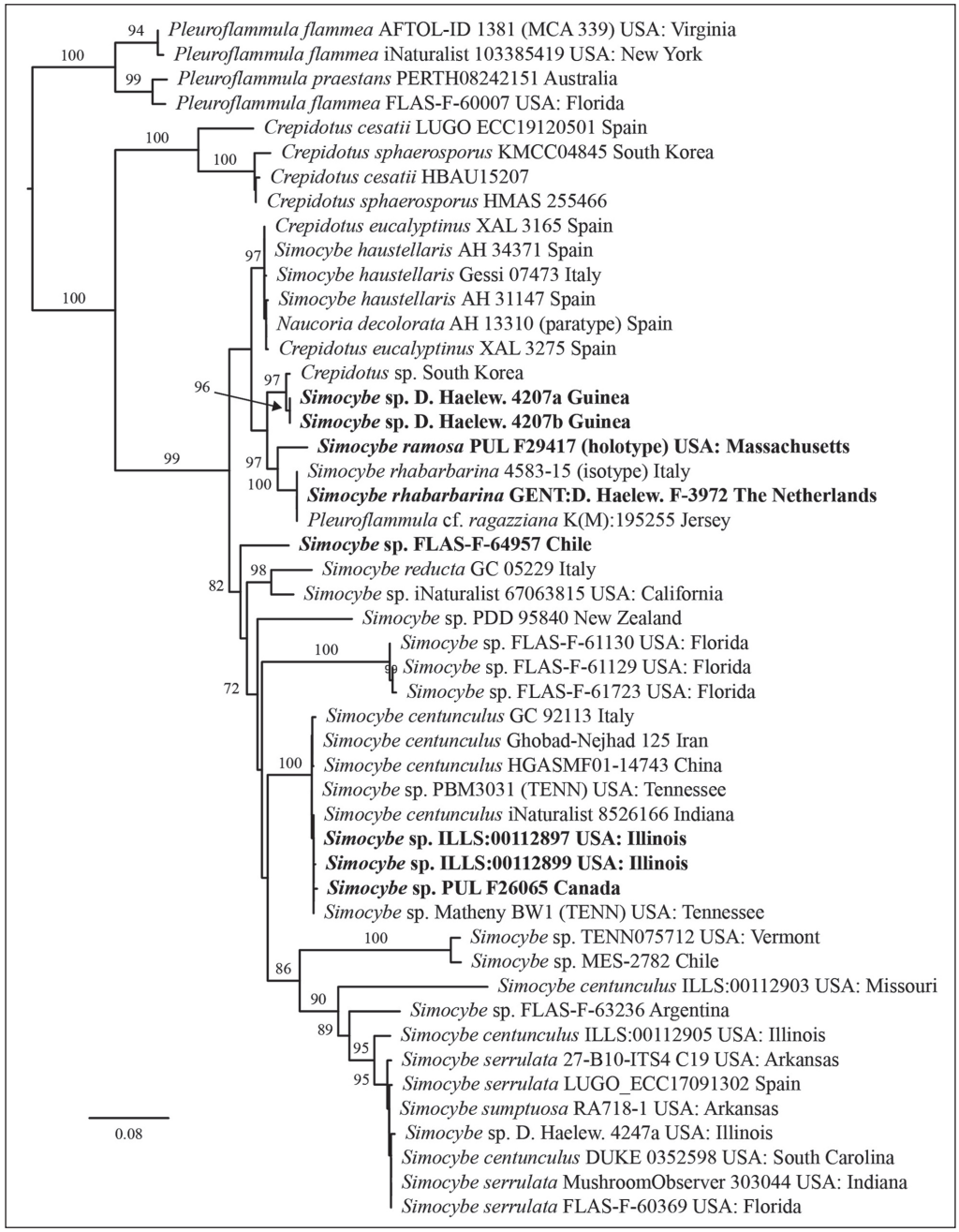

Figure 1. Phylogeny of *Simocybe* species. The tree with the highest log likelihood (-lnL= 4386,964686) is shown, resulting from ML inference performed with IQ-TREE. For each node, the ML UFBoot2 support value is presented if >70. Newly generated sequences highlighted in bold; the bar length indicates the expected number of substitutions per site.

Simocybe ramosa sp. nov. as a sister species to the recently described *S. rhabarbarina* L. Poli, Musumeci & P. Alvarado with high support (UFBOOT2 97%). The isotype of *S. rhabarbarina* is retrieved in a clade with a collection from the island of Jersey, located off the coast of northern France, originally identified as *Pleuroflammula* cf. *ragazziana* (Bres.) E. Horak and a new collection from the Netherlands that we studied here. This set (*S. ramosa*, *S. rhabarbarina*) is placed as sister to a clade holding a *Simocybe* sp. (as *Crepidotus*) from South Korea and an undescribed species of *Simocybe* from Guinea (UFBOOT2 67%).

Taxonomy

Simocybe ramosa Mortier, Haelew. & Verbeken, **sp. nov. (**Figs. 2–4)

Index Fungorum. IF900583.

Diagnosis. Different from other species of *Simocybe* by the combination of small and round basidiospores measuring 6.2–8.1 × 4.8–6.1, sometimes with a slight germ pore-like thinning; large, 2–3-spored basidia; and pileocystidia that are apically slightly thick-walled and often branched.

Typification. USA. MASSACHUSETTS: Plymouth County, Boston Harbor Islands National Recreation Area, World's End peninsula, oak–hickory forest east of Damde Meadows marshland, close to Weir River Road, under bark of dead standing *Quercus* sp. tree, 26 March 2017, J.K. Mitchell, BHI-F724 (PUL F29417, **holotype**), GenBank (isolate BHI-F724a): ITS = OM534632.

Description. Habitus pleurotoid. Pileus 1–3 mm, rounded flabelliform, center depressed, margin irregularly eroded, surface very grainy to hairy, beige to light brown, center a little darker than edge, hairs white. Lamellae adnexed, edge undulating, irregularly eroded, beige to light brown, darker brown with age. Stipe eccentric to lateral, very small, hairy, tapering downwards, producing rhizomorphs, concolorous with pileus, beige to light brown. Subiculum visible under and connected to stipe, white. Smell and taste not recorded.

Basidiospores 6.2–*7.1*–8.1 × (4.5–)4.8–*5.4*–6.1 µm, Q = 1.1–*1.3*–1.5 [20], round, slightly thick-walled, smooth, with a clear apiculus, content visible, sometimes with a slight germ pore-like thinning. Basidia 17–22 × 6.7–8.7 µm [5], mostly 2-spored, sometimes 3-spored, often present as immature basidioles; sterigmata large, distinct, bowing inward. Pleurocystidia absent. Cheilocystidia 27–41 × 4.0–6.7 µm [6], cylindrical, straight, apically rounded. Pileipellis with parallel and horizontal hyphae perpendicular to oblique pileocystidia. Pileocystidia 16.7–40.0 × 3.3–6.7 µm [6], tortuous, often branched, apically slightly thick-walled. Caulocystidia 35.3–52.7 × 4.0–6.0 µm [6], not abundant, cylindric, tortuous, rarely branched. Clamp connections abundant at hyphal septa and base of cheilocystidia. Subiculum composed of a dense mat of hyphae.

Etymology. Referring to the branched pileocystidia of this species, a rarely reported characteristic in the genus.

Habitat and distribution. Under bark of dead standing oak tree in a coastal oak–hickory forest. Thus far, only found on World's End peninsula, MA, USA.

Notes. In some parts of the subiculum, helicospores are present. The helicospores are coiled, hooked, and have less than 1 complete coil, similar to those of

Slimacomyces monosporus (W.B. Kendr.) Minter or *Troposporopsis atroapicis* Whitton, McKenzie & K.D. Hyde (Seifert et al. 2011).

Simocybe rhabarbarina L. Poli, Musumeci & P. Alvarado, Boll. Assoc. Micol. Ecol. Romana 96:23 (2015). (Fig. 5).

Specimens examined. ITALY. LOMBARY: Seveso, 17 May 2015, L. Poli (LUG 19069, **holotype**). THE NETHERLANDS. GELDERLAND: Arnhem, Akkerstraat, on a fallen branch of *Tilia* sp., 18 February 2022, M. Groenendaal (GENT:D. Haelew. F-3972), GenBank: ITS = PP177462, LSU = PP177463.

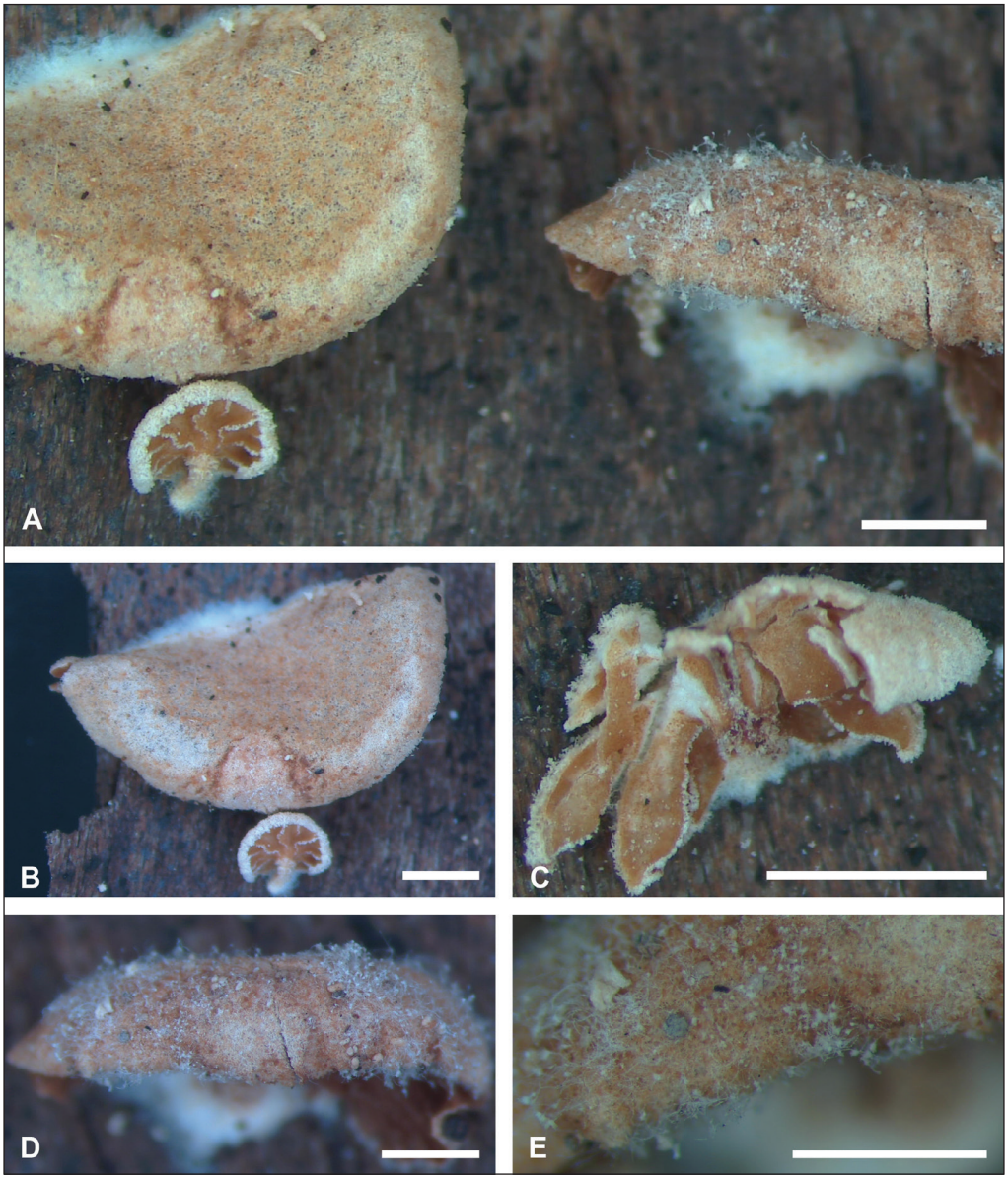

Figure 2. *Simocybe ramosa* (PUL F29417, holotype). Basidiomata in lab. Bars = 0.1 cm.

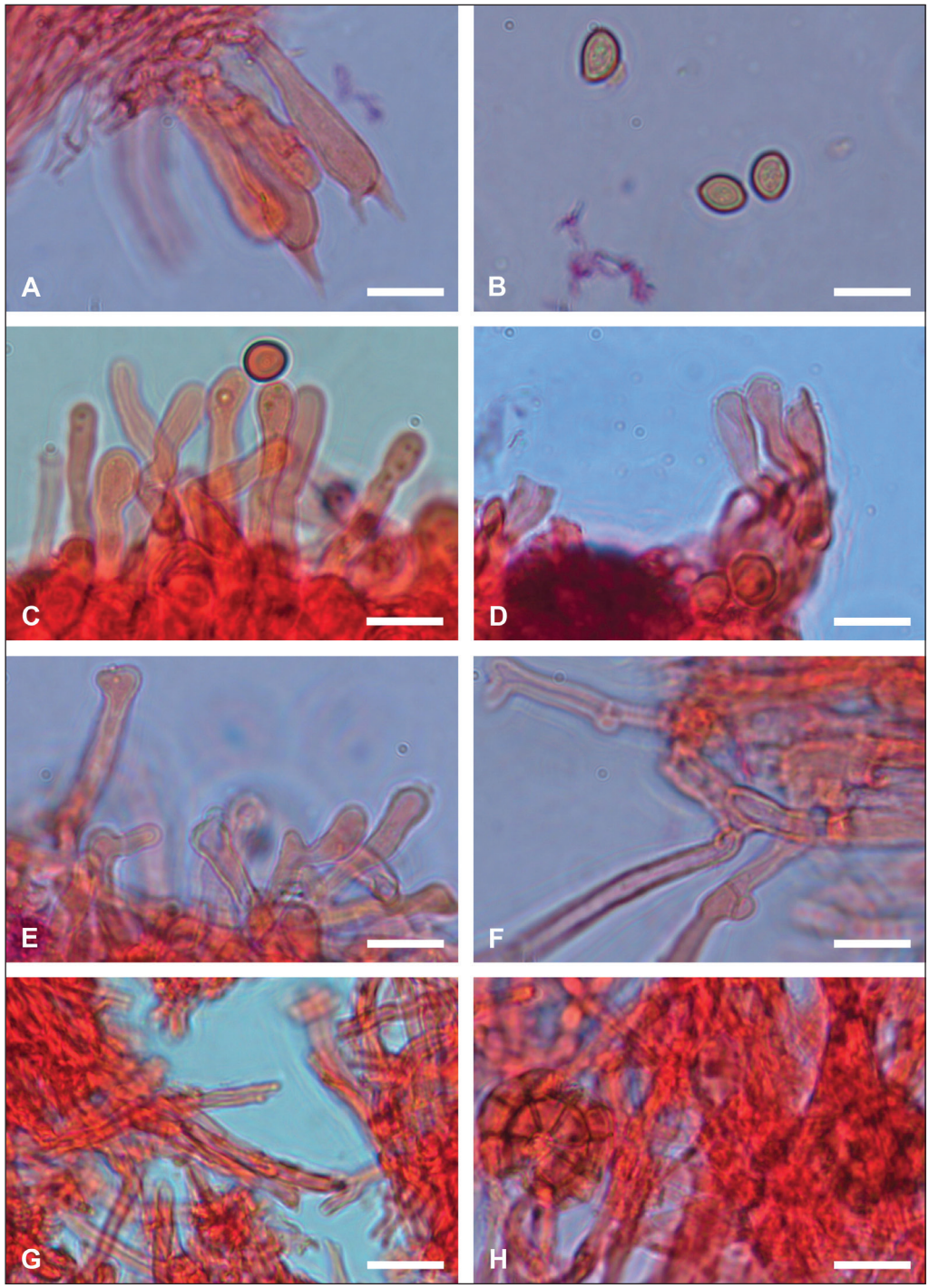

Figure 3. *Simocybe ramosa* (PUL F29417, holotype). (A) Basidia. (B) Basidiospores. (C) Cheilocystidia. (D) Caulocystidia. (E) Pileocystidia. (F) Clamp connections. (G) Subiculum. (H) Helicospores in the subiculum. Bars = 10 µm.

Description. Pileus 1–3 mm; convex, not umbonate, not hygrophanous; under lens subtomentose (no darker fibrils seen); on drying becoming somewhat granulose, pale yellowish buff; on drying and aging somewhat darker, slightly brownish ochraceous. Stipe 1–2 × ~0.2–0.3 mm, eccentric, not truly lateral, concolorous with

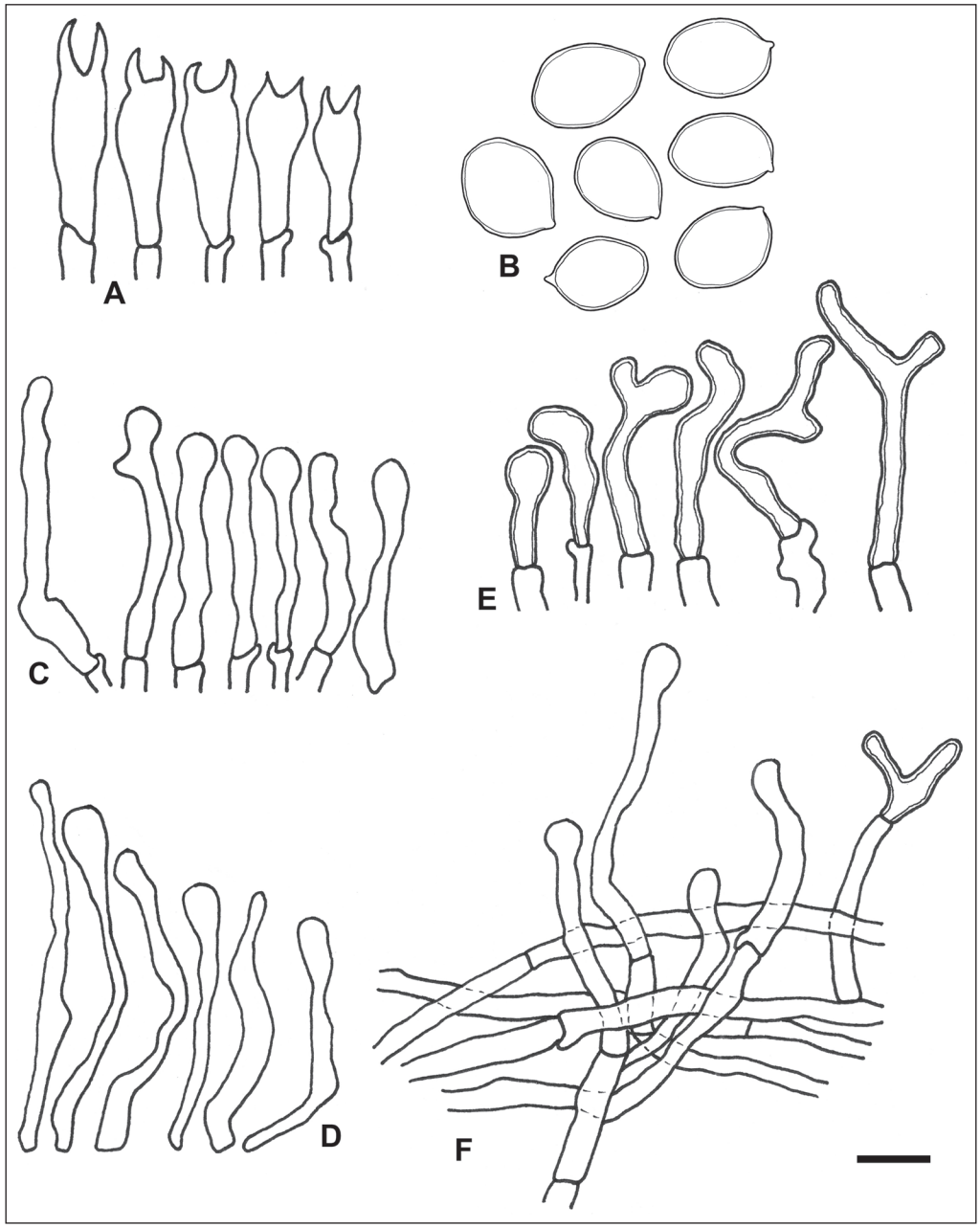

Figure 4. *Simocybe ramosa* (PUL F29417, holotype). (A) Basidia. (B) Basidiospores. (C) Cheilocystidia. (D) Caulocystidia. (E) Pileocystidia. (F) Pileipellis. Bars: A, C–F = 10 µm; B = 5 µm.

pileus, somewhat pubescent under lens. Lamellae brown with white-fimbriate edge. Smell indistinct. Taste not recorded.

Basidiospores (7.2–)7.4–*9.0*–10.5 × 5.6–*6.5*–7.5 µm, Q = 1.17–*1.38*–1.59 [20] (LUG 19069), (7.4–)7.6–*8.3*–9.0(–9.6) × (5.9–)6.3–*6.6*–6.9, Q = (1.16–)1.18–*1.26*–1.33(–1.41) [10] (GENT:D. Haelew. F-3972), smooth, slightly thick-walled, with a clear apiculus, with obtuse apex, with thin germ pore, sometimes difficult to see (not observed in GENT:D. Haelew. F-3972). Basidia 18–24 × 6.7–8.0 µm [4] (LUG 19069), 2-spored. Pleurocystidia absent. Cheilocystidia 25–49 µm × 4.0–5.3 µm [6] (LUG 19069), 30–45 × 5.0–6.0 µm [10] (GENT:D. Haelew. F-3972), abundant (edge sterile), long, erect, cylindrical, sometimes slightly swollen near apex, tending to subcapitate (to 6.0–8.0 µm), flexuose, thin-walled, hyaline. Hymenophoral trama regular, hyphae narrow, 3.0–4.0(–5.0) µm wide (GENT:D. Haelew. F-3972), hyaline, no incrustations observed. Stipitipellis not studied. Pileipellis a cutis with ascending hyphae and often erect pileocystidia (but not a trichoderm). Pileocystidia similar to cheilocystidia, but more irregular, sometimes with a basal branch, more distinctly flexuose, slightly thick-walled. Clamp connections observed in pileipellis and hymenophoral trama.

Habitat and distribution. Found on fallen branches of *Tilia* sp. (basswood). Thus far only known from collections in Italy (holotype) and the Netherlands. An ITS sequence with accession number MZ159526, identified as *Pleuroflammula* cf. *ragazziana*, from Jersey near the coast of La Manche (France) shares 99.7%

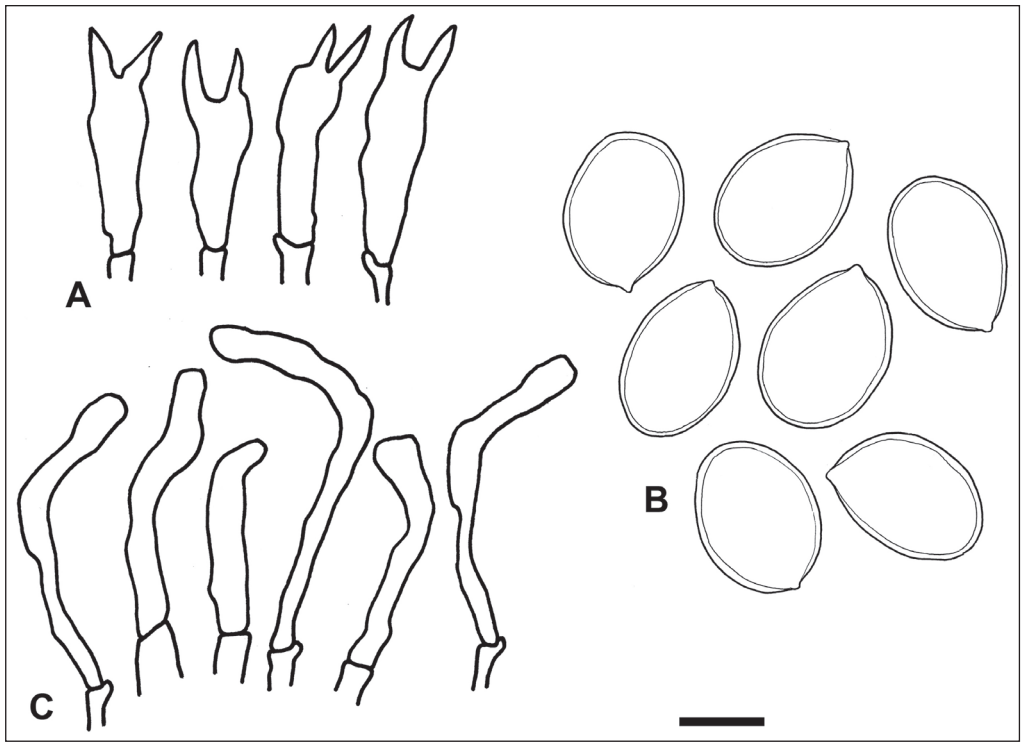

Figure 5. *Simocybe rhabarbarina* (LUG 19069, holotype). (A) Basidia. (B) Basidiospores. (C) Cheilocystidia. Bars: A, C = 10 µm; B = 5 µm.

identity with the ex-type sequence of *S. rhabarbarina* (identities = 666/668 bp, gaps = 2/668) as well as with the sequence of our new collection from the Netherlands (identities = 696/698 bp, gaps = 1/698). This sequence was generated from a basidioma on the bark of a small fallen, decayed branch of *Salix cinerea* L. (Large Gray Willow) and indicates a broader distribution in western Europe.

Discussion

Simocybe ramosa is based on a single collection. The question could be asked whether it would be better to wait until more collections are obtained before making a formal description. We have pondered this question ourselves and decided to move forward with the description based on the following rationales: (1) Currently and in the foreseeable future, no active fungal collections are being made at or planned for the Boston Harbor Islands National Recreation Area. Waiting would unnecessarily increase the so-called "shelf time" between time of collection and time of publication (Fontaine et al. 2012). And (2) The description of singleton-based species represents responsible science given the large gap between described and estimated species of fungi (Cazabonne et al. 2024). We are fully aware the best practice is to include multiple collections when describing a new species (Aime et al. 2021). However, the same authors also mentioned that when multiple collections are unavailable (e.g., for rare taxa or taxa from specialized or remote locations), multiple lines of evidence are recommended. We have provided such evidence by presenting comparative morphological descriptions of the new species and its phylogenetically closest relative and a phylogenetic analysis including sequences from previously unsequenced collections of *Simocybe* (several from North America). We hope that with our description of *S. ramosa*, professional and amateur mycologists may be encouraged to collect more specimens of *Simocybe* in North America.

Simocybe ramosa can be distinguished from other species of *Simocybe* by a combination of micro-morphological characteristics: smooth, small, and round basidiospores sometimes with a slight germ pore-like thinning, large, 2–3-spored basidia, and (often) branched pileocystidia that are apically slightly thick-walled. The ecology of the new species is unclear. In general, species of Crepidotaceae occur more abundantly on hardwoods than on conifers, mostly without preference (Hesler and Smith 1965, Ludwig 2000). While the holotype of *S. ramosa* was collected on the bark of a dead oak tree, no conclusion can be made about host preference without more collections.

The phylogenetically most closely related species to *S. ramosa* is *S. rhabarbarina* (Fig. 1). Their ex-type ITS sequences share 95.8% identity. Using an identity threshold of 98.5% (Lücking et al. 2020), this finding suggests that they are separate species. Morphologically, *S. ramosa* is different in having a beige to light brown pileus (vs. more yellowish in *S. rhabarbarina*) and smaller basidiospores (6.2–8.1 × 4.8–6.1 µm vs. 7.4–10.5 × 5.6–7.5 µm in *S. rhabarbarina*). In addition, the presence of both 2-spored and 3-spored basidia is a characteristic of *S. ramosa* not observed in *S. rhabarbarina*. In both species, the pileocystidia are irregular;

they are slightly thick-walled apically and sometimes (in *S. rhabarbarina*) to often (in *S. ramosa*) branched.

Twenty species of *Simocybe* have been reported from North America (Canada, the USA, Mexico): *S. aestivalis* Singer, *S. alachuana* (Murrill) Singer, *S. amara* (Murrill) Singer, *S. americana* E. Horak & O.K. Mill., *S. atomacea* (Murrill) Singer, *S. centunculus* (Fr.) P. Karst., *S. citrinipes* (Murrill) Singer, *S. coniferarum* Singer (var. *quercuum* Singer), *S. fulvifibrillosa* (Murrill) Singer, *S. haustellaris* (Fr.) Watling, *S. melleiceps* (Murrill) Singer, *S. puberula* (Peck) Singer, *S. quebecensis* Redhead & Cauchon, *S. reducta* (Fr.) P. Karst., *S. reductoaffinis* Singer, *S. semiglobata* (Murrill) Singer, *S. serrulata* (Murrill) Singer, *S. subolivacea* (Murrill) Singer, *S. tepeitensis* (Murrill) Singer, and *S. tiliophila* (Peck) Singer (Breitenbach and Kränzlin 2000; Horak and Miller 1997; Peck 1898; Redhead and Cauchon 1989; Singer 1973, 1987). Of these, *S. aestivalis*, *S. alachuana*, *S. atomacea*, *S. centunculus*, *S. melleiceps*, *S. reducta*, *S. reductoaffinis*, *S. semiglobata*, *S. serrulata*, and *S. subolivacea*, and *S. tepeitensis* are species that are centrally stipitate and thus easily separated from *S. ramosa*.

The basidiospores of *S. amara* are different in shape (ellipsoid or ovoid) and slightly smaller (5.0–7.0 × 4.0–5.0 µm) compared to those of *S. ramosa* (Murrill 1943). *Simocybe citrinipes* has 4-spored basidia, wider cheilocystidia (6.5 – 7.5 µm vs. 4.0–6.7 µm in *S. ramosa*), and pileocystidia that are variable and unlike those observed in *S. ramosa* (Singer 1973). *Simocybe coniferarum* differs from *S. ramosa* in the following characteristics: a much wider pileus (up to 17 mm vs. 1–3 mm in *S. ramosa*); elliptical, ovate, to phaseoliform basidiospores measuring 6.5–7.5 × 4.2–4.5 µm, without germ pore; longer basidia (26 µm vs. 17–22 µm) that are 4-spored (vs. 2–3-spored); and larger cheilocystidia (36–48 × 5.5–7.7 µm vs. 27–41 × 4.0–6.7 µm) (Singer 1987). The North American form from rotten oak wood, *S. coniferarum* var. *quercuum*, is described to have a smaller pileus and shorter cheilocystidia—but no measurements were presented (Singer 1987). *Simocybe fulvifibrillosa* has an entire pileus margin (vs. eroded in *S. ramosa*), basidiospores equal in size but without germ pores, and considerably wider cheilocystidia (9.5–10.8 µm). In addition, these 2 species grow in completely different habitats: *S. fulvifibrillosa* has only been collected in primary tropical montane cloud forests and coffee plantations in Mexico (Singer 1973). *Simocybe haustellaris* differs from *S. ramosa* in several characteristics: longer basidiospores (7.0–9.5 µm vs. 6.2–8.1 in *S. ramosa*), longer basidia (23–34 µm vs. 17–22 µm), presence of pleurocystidia, and larger cheilocystidia (36–61 × 4–12 µm vs. 27–41 × 4.0–6.7 µm) (Hesler and Smith 1965, Knudsen and Vesterholt 2018). *Simocybe puberula*, described from California, has a much wider pileus (6–10 mm vs. 1–3 mm in *S. ramosa*) and larger, somewhat elliptical basidiospores measuring 9–10 × 5–6 µm (Peck 1898). Finally, *S. tiliophila* lacks clamps connections, has slightly smaller basidiospores (5.5–7.0 × 4.0–4.5 µm), narrower basidia (5.0–6.0 µm vs. 6.7–8.7 µm in *S. ramosa*), and shorter cheilocystidia (18–29 µm vs. 27–41 µm in *S. ramosa*).

Two additional North American species are stipitate with a stipe that can be centrally to eccentrically attached. *Simocybe americana* is different from *S. ramosa* in

its larger basidiomata (4–12 mm), 4-spored basidia, and the presence of pleurocystidia scattered near the lamellar edge (Horak and Miller 1997). The second species, *S. quebecensis*, has coralloid cheilocystidia, pileocystidia, and caulocystidia, "with convoluted, branched structures mainly towards the apices" (Redhead and Cauchon 1989:292). Other differences from *S. ramosa* include the paler color of the pileus, smaller basidiospores (5.5–6.1 × 4.4–5.0 µm), and 2- and 4-spored basidia. While *S. americana* grows on logs of *Populus tremuloides* Michx. (Quaking Aspen) and is thus far only reported from Canada, *S. quebecensis* was discovered on peeling inner bark of dying *Quercus*, also in Canada. To date, *S. quebecensis* has also been reported in Belgium (2 collections: one on *Acer* sp. [maple] and the other on Northern Red Oak), France, and the Netherlands (Walleyn et al. 2012).

Boston Harbor Islands fungal survey

Thirty-four islands and peninsulas between Boston's inner harbor and its vulnerable outskirts comprise a drumlin archipelago that is the BHI. The islands and peninsulas vary in size from less than 0.1 ha (Nixes Mate island) to 105 ha (World's End peninsula). Many different plant communities are found on the BHI: native and non-native forests and woodlands, maritime shrub communities, fields, beach-strand communities, maritime cliff communities, and dune systems. Between December 2012 and August 2019, more than 900 collections were made from 8 islands and peninsulas as part of a fungal inventory (Haelewaters et al. 2018b).

From those, several new species have been described: *Orbilia japonica* Baral, M.L. Wu & Y.C. Su (Orbiliales) from Thompson Island and World's End; *O. nemaspora* Baral, Bin Liu, A.I. Romero, Healy & Pfister from Peddocks Island and Thompson Island (Baral et al. 2020); *O. renispora* Y.Y. Shao, Quijada, Baral, Haelew. & Bin Liu from World's End (Shao et al. 2018); *Trochila bostonensis* Quijada & Haelew. (Helotiales) from Great Brewster Island (Gómez-Zapata et al. 2021); and *Xylaria finismundoensis* Vandegrift (Xylariales) from World's End (Vandegrift 2021). The BHI collections also revealed new geographic records: *Orbilia aprilis* Velen. from Slate Island represented the first report for this species in North America, and *Durella melanochlora* (Sommerf.) Rehm (Helotiales) and *Resupinatus urceoloides* J.V. McDonald & Thorn (Agaricales), both from Grape Island, were the first records in the US. Note that *R. urceoloides* was presented as an undescribed species (*Resupinatus* sp. 1) in the fungal checklist by Haelewaters et al. (2018b) but was formally described based on Canadian material the year after (McDonald and Thorn 2019). While *Eutypa maura* (Fr.) Fuckel (Xylariales) from World's End was not the first record from the US, it was the first with a voucher (Vandegrift 2021).

Other collections were not new species records but represented significant geographic range extensions. Examples include *Nemania beaumontii* (Berk. & M.A. Curtis) Y.M. Ju & J.D. Rogers (Xylariales) from Great Brewster Island and World's End, previously only known from Alabama and Brazil (Ju and Rogers 2002, Vandegrift 2021) and *Proliferodiscus earoleucus* (Berk. & Broome) J.H. Haines & Dumont (Helotiales) from Slate Island, which was previously only known from South Carolina, Bermuda, the Caribbean, Brazil, Colombia, Venezuela, and Sri Lanka (Haelewaters et al. 2018b, Haines and Dumont 1983).

Simocybe ramosa is the sixth new species to be described based on material from the BHI, showing the potential to discover fungal species new to science during multi-year inventory projects. The same could be said for other multi-year studies: in tropical lowlands of Chiriquí Province in Panama (Piepenbring et al. 2012), Cusuco National Park in northwestern Honduras (Haelewaters et al. 2021, Martin et al. 2021), and the Pakaraima Mountains in southwestern Guyana (e.g., Aime et al. 2003, 2010; Henkel et al. 2012; O.K. Miller et al. 2001; S.L. Miller et al. 2012).

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