FUSE
Fungal Systematics and Evolution
VOLUME 15
JUNE 2025
PAGES 225–234

doi.org/10.3114/fuse.2025.15.10

Requienella populi sp. nov. (*Requienellaceae*, *Xylariales*) from the bark of living aspen trees in Western Norway

M. Andreasen[1]*, J.B. Jordal[2], B. Nordén[1]

[1]Norwegian Institute for Nature Research, 0855 Oslo, Norway
[2]Miljøfaglig Utredning, Gunnars veg 10, NO 6630 Tingvoll, Norway

*Corresponding author: mathias.andreasen@nina.no

Key words:
Molecular phylogeny
new taxon
Requienella
taxonomy
Xylariales

Abstract: The new species *Requienella populi* in the *Requienellaceae* is described from Western Norway. Multigene analysis of the four molecular markers ITS, LSU, *RPB2* and *TUB* revealed it as a strongly supported sister clade within the genus. The new species appears to be restricted to old aspen *Populus tremula* trees and can be morphologically distinguished by submuriform and somewhat smaller ascospores compared to the other species of the genus. A table comparing species of *Requienella* is provided. The *Requienellaceae* received a moderate statistical support as a sister group to the *Cainiaceae* in our analysis and the circumscriptions of the two families need to be studied further using additional genetic markers.

Citation: Andreasen M, Jordal JB, Nordén B (2025). *Requienella populi sp. nov.* (*Requienellaceae*, *Xylariales*) from the bark of living aspen trees in Western Norway. *Fungal Systematics and Evolution* **15**: 225–234. doi: 10.3114/fuse.2025.15.10
Received: 23 October 2024; **Accepted:** 5 February 2025; **Effectively published online:** 28 February 2025
Corresponding editor: P.W. Crous

INTRODUCTION

As part of a biodiversity mapping project in Norway, we focused our interest on bark-living species on old aspen trees, *Populus tremula*. During fieldwork in the oceanic parts of Western Norway we encountered a species macroscopically reminiscent of *Requienella fraxini*, which we knew well from previous studies. *Requienella* is a small genus of prominent bark-living *Ascomycota* with only three species hitherto known, *R. fraxini*, *R. seminuda* and the newly published *R. shangrilana*, the two former being specific to trees in the *Oleaceae* (on *Fraxinus* and *Olea*, respectively) while the latter was encountered from wood of an unknown host species. Macroscopically *R. fraxini* and *R. seminuda* are easily identified in the field by their prominently protruding cone-shaped black papillae surrounded by white rings of amorphous matter, an aspect shared with our new species but seemingly not with *R. shangrilana*. Under the microscope, however, the species on aspen differed clearly from the three previous species of the genus by having submuriform ascospores.

Requienella has been considered a member of the *Dothideomycetes* due to its bitunicate asci, or of the *Pyrenulales* due to the distoseptate ascospores with lenticular lumina. However, Jaklitsch *et al*. (2016) showed that it belongs to the *Xylariales* based on molecular evidence. For an exhaustive historical account of the systematic treatment and a detailed morphological account of *Requienella*, please refer to Jaklitsch *et al*. (2016).

We here describe the new species as *R. populi* based on morphological data and multigene analysis of four molecular markers. To aid identification, we provide a table comprising all species of *Requienella.*

MATERIALS AND METHODS

Sampling and morphological investigation

Ascomata were rehydrated with autoclaved water and studied using a Nikon SMZ 745T stereomicroscope and a Nikon Eclipse Ci-L or a Zeiss Axio Imager A2 compound microscope. Images of ascomata were captured with a Nikon DS-Fi2 or Tucsen DigiRetina 16 camera, using stacking software Lite Helicon Focus 8 v. 8.2.2. Microslides were created with contents of the ascomata mounted in sterile water or 5 % KOH. Photomicrographs were produced using a Zeiss Axiocam 503 camera and measurements were made with Zeiss AxioVision v. 4.9.1 software (Carl Zeiss AG), and images were processed in GIMP v. 2.10.34 (Kimball & Mattis 1996).

DNA extraction and sequencing

Genomic DNA was extracted from hymenial material, placed in inhibiting buffer solution, and sent to Eurofins, Germany for DNA isolation, amplification, and Sanger sequencing of the nuclear ribosomal DNA (nrDNA) regions of internal transcribed spacer (ITS) containing ITS1, 5.8S NRDNA and ITS2 and the 28S large subunit nrDNA (LSU), RNA polymerase II second largest subunit (*RPB2*) and the beta-tubulin gene (*TUB*) using the primer pairs ITS4/ITS5 (White *et al.* 1990), LR5/V9G (Vilgalys & Hester 1990/De Hoog & Gerrits van den Ende 1998), fRPB2-5/rRPB2-7C (Novakova *et al.* 2012), and Bt2a/Bt2b (Hsieh *et al.* 2005), respectively.

Sequence alignment and phylogenetic analyses

Sequence editing, assembly and concatenations were done using Geneious Prime v. 2025.0.2 (Kearse *et al.* 2012) and deposited in GenBank (Table 1), and the alignments were uploaded to Figshare (www.figshare.com; doi: 10.6084/m9.figshare.28266224). Sequence data from Han *et al.* 2024 and Jaklitsch *et al.* 2016 were downloaded from GenBank. Preliminary alignments were made using MAFFT v. 7.490 (Katoh & Standley 2013) with standard settings as incorporated in Geneious Prime. All alignments were inspected and manually adjusted. Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI). Substitution models for each locus were determined based on the AICc model selection criterion (small-sample-size corrected version of Akaike information criterion) as implemented in PartitionFinder v. 2.1.1 (Lanfear *et al.* 2016). The search was set to 'all' and branch lengths set to 'linked'. The ML analyses were performed on aligned sequences using RAxML v. 8.2.11 (Stamatakis 2014) as implemented in Geneious. Rapid Bootstrapping and search for best-scoring ML tree algorithms were used with GTR-GAMMA-I substitution model and Bootstrap analyses obtained by 1 000 bootstrap replications.

To examine topological incongruence among data sets, ML bootstrapping analyses were carried out on each of the single-gene data sets. Topological incongruence was assumed if conflicting tree topologies were supported by ≥ 70 % ML support. Since topological incongruence could not be observed, maximum likelihood (ML) bootstrapping analyses were carried out on the concatenated four-locus dataset using the same settings as for the single-gene analyses. The BI analyses were performed with MrBayes v. 3.2.6 (Huelsenbeck & Ronquist 2001) with substitution models for different regions selected with the AICc parameter. Metropolis-coupled Markov chain Monte Carlo (MCMC) runs were performed for 1 M generations with trees sampled every 200 generations. Convergence of the MCMC procedure was assessed and effective sample (EES) size scores > 200 checked by using the MrBayes build in Tracer v. 1.6 (Rambaut *et al.* 2018). The first 10 % of trees were discarded as burn-in, and the remaining trees were used to calculate 50 % majority rule trees and to determine Bayesian posterior probabilities (BPP) for individual branches. Output trees were edited with Inkscape v. 1.4 (Harrington *et al.* 2003).

RESULTS

Phylogenetic analyses

We obtained consensus sequences from three strains for the ITS and two strains for the LSU markers (Table 1). In addition, we added two strains of the ITS markers from identified species of the *Hypoxylaceae* to the dataset. The concatenated alignment for *Apiosporaceae*, *Barrmaeliaceae*, *Cainiaceae*, *Graphostromataceae*, *Hypoxylaceae*, *Lopadostomaceae*, *Requienellaceae* and *Xylariaceae* comprised 4 372 nucleotide characters, including gaps (ITS1, 5.8S and ITS2: 1–740; LSU: 741–1 595; *RPB2*: 1 596–2 684; *TUB*: 2 658–4 372). In total, the alignment was composed of 48 strains of the ITS, 46 strains of LSU, 19 of *RPB2*, 19 *TUB* and the following outgroup taxa: *Hypocrea gelatinosum* (NBRC 104900), *Nectria cinnabarina* (CBS 125165), and *Stromatonectria caraganae* (CBS 125579).

The maximum likelihood (ML) analysis of the combined datasets yielding the best scoring trees for *Xylariales* had an MLn of –37393.72 (Fig. 1). The Bayesian inference (BI) analysis showed congruence with the topology of the ML analyses, and for simplicity, only the ML trees are shown. Values for both ML bootstrap (MLB) above 50 % and Bayesian posterior probabilities (BPP) higher than 0.90 are given at the nodes. The alignments had 60.93 % undetermined nucleotide gaps.

Representatives from the order *Hypocreales* formed a fully supported clade (ML 100 % and BPP 1) and outgroup to all included taxa from *Xylariales*, but the reconstruction of *Xylariales* is not entirely settled as the analysed dataset inferred less significant support for some families and genera. *Requienellaceae* and *Cainiaceae* received high support (ML 92 % and BPP 1) as sister group to *Graphostromataceae*, *Barrmaeliaceae* and *Xylariaceae*, but their sister group relationships were not significantly supported (ML 43 % and BPP 0.7, data not shown). Our analyses showed support for our new species represented by three strains, namely α23-061, α23-076 and α23-076a respectively, within *Requienellaceae* (see Fig. 1). The topology within *Requienella* is highly supported and monophyletic.

Morphological character matrix

Detailed morphological characters of the species of *Requienellaceae* are shown in Table 2.

Taxonomy

Requienellaceae Boise, *Mycologia* **78**: 37. 1986. MycoBank MB 81336.

Type genus: *Requienella* Fabre, *Ann. Sci. Nat., Bot*. ser. 6, **15**: 55. 1883. MycoBank MB 4676.

Ascomata perithecioid, immersed or erumpent, subglobose; ostiolar neck inconspicuous or massive and strongly erumpent, black; extra ascomatal tissue present. *Hamathecium* comprising two types of apically free paraphyses differing in length and width. *Asci* bitunicate, fissitunicate, cylindrical, subfusiform to narrowly clavate, with thick-walled apex and wide ocular chamber comprising a slightly refractive, inversely funnel-shaped dome turning slightly reddish in Congo Red, containing 8 uni- to biseriately arranged ascospores. *Ascospores* ellipsoid to oblong, olivaceous or brown, sometimes with paler ends, with several transverse distosepta and large lumina, sometimes with additional thin longitudinal septa.

Requienella Fabre, *Ann. Sci. Nat., Bot*., sér. 6, **15**: 55. 1883.
Synonym: *Trematomyces* Schrantz, *Bull. Soc. Mycol. France* **76**: 324. 1961.

Type species: *Requienella seminuda* (Pers.) Boise.

Requienella populi Andreasen & Nordén, ***sp. nov.*** MycoBank MB 857429. Fig. 2.

Etymology: With reference to the host species *Populus tremula*.

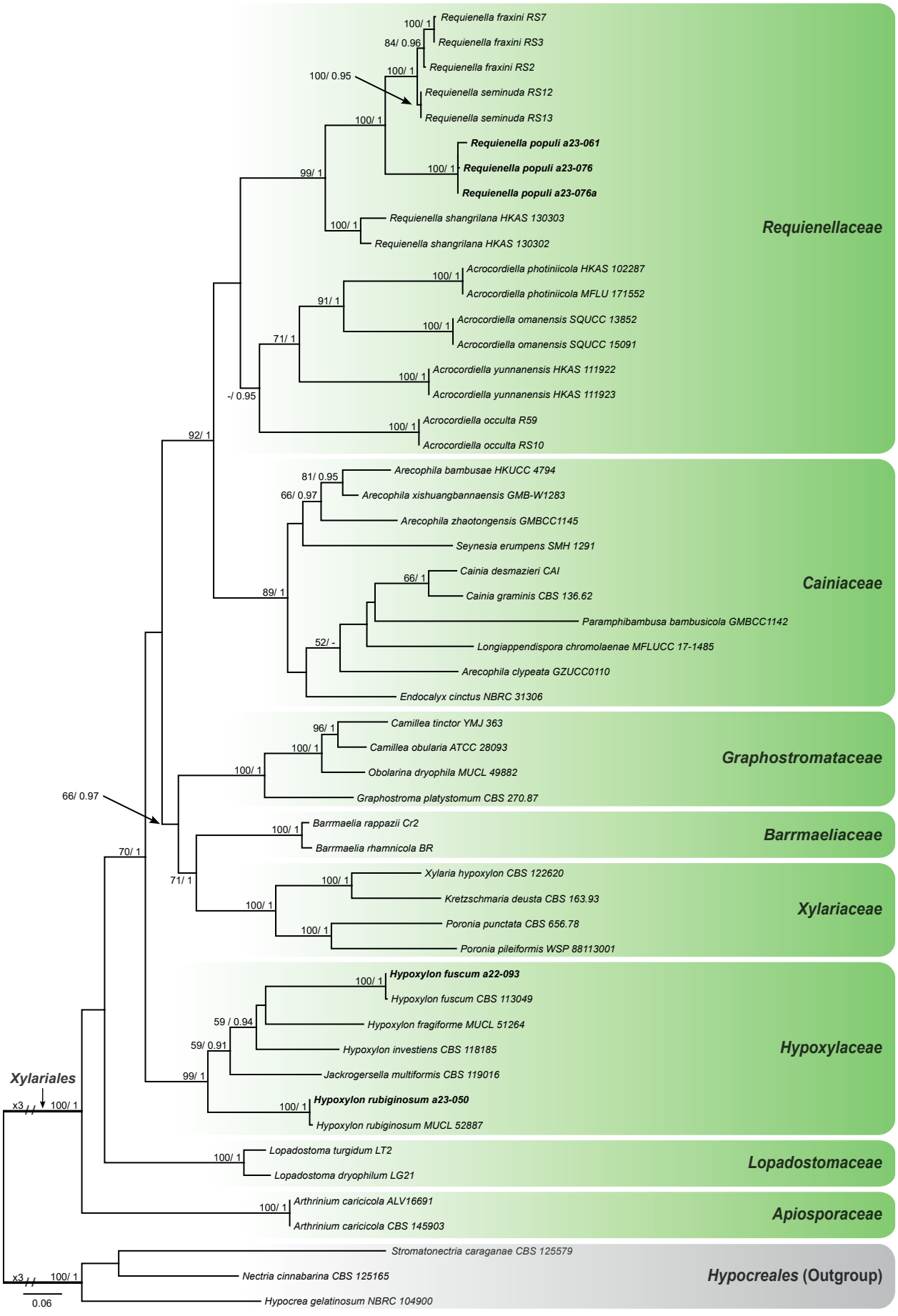

Fig. 1. Phylogeny of representative of *Xylariales* based on ITS, LSU, *RPB2* and *TUB* combined sequence data revealed by RAxML (MLn = - 37393.72). Numbers above branches indicate Maximum likelihood RAxML bootstrap values above 50 % and Bayesian posterior probabilities higher than 0.90 at first and second position, respectively. Newly obtained strains are shown in **bold**. Shortened branches are marked with crossing lines and indications (×2) of how many times the branch has been shortened. Branch length equals substitutions per site.

Table 1. Fungal taxa, strains and GenBank accessions used of *Apiosporaceae*, *Barrmaeliaceae*, *Cainiaceae*, *Graphostromataceae*, *Hypoxylaceae*, *Lopadostomaceae*, *Requienellaceae* and *Xylariaceae,* along with representatives from *Hypocreales* as outgroup. The sequences generated in this current study are indicated in **bold**. Type strains or type specimens are labelled with HT (holotype), ET (epitype), LT

Species	Family	Strain no.	Status	GenBank accession no.				Reference
				ITS	LSU	*RPB2*	*TUB*	
Acrocordiella occulta	*Requienellaceae*	RS9	LT	KT949893	KT949893	n/a	n/a	Jaklitsch *et al.* (2016)
	Requienellaceae	RS10	—	KT949894	KT949894	n/a	n/a	Jaklitsch *et al.* (2016)
Acrocordiella omanensis	*Requienellaceae*	SQUCC 13852	PT	MG584569	MG584571	n/a	n/a	Maharachchikumbura *et al.* (2018)
	Requienellaceae	SQUCC 15091	HT	MG584568	MG584570	n/a	n/a	Maharachchikumbura *et al.* (2018)
Acrocordiella photiniicola	*Requienellaceae*	MFLU 17-1552	HT	MW240627	MW240556	MW658617	MW775583	Samarakoon *et al.* (2022)
	Requienellaceae	HKAS 102287	—	MW240628	MW240557	n/a	MW775584	Samarakoon *et al.* (2022)
Acrocordiella yunnanensis	*Requienellaceae*	HKAS 111922	HT	MW424507	MW424505	n/a	n/a	Dissanayake *et al.* 2021
	Requienellaceae	HKAS 111923	—	MW424497	MW424506	n/a	n/a	Dissanayake *et al.* 2021
Arecophila bambusae	*Cainiaceae*	HKUCC 4794	—	NA	AF452038	NA	NA	Kang *et al.* (1999)
Arecophila clypeata	*Cainiaceae*	GZUCC0110	HT	MT742129	MT742136	MT741732	n/a	Li *et al.* (2022)
Arecophila zhaotongensis	*Cainiaceae*	GMBCC1145	HT	OR995740	OR995747	OR995579	n/a	Han *et al.* (2024)
Arecophila xishuangbannaensis	*Cainiaceae*	GMB-W1283	HT	OR995736	OR995743	n/a	n/a	Han *et al.* (2024)
Arthrinium caricicola	*Apiosporaceae*	ALV16691	—	MK014871	MK014838	n/a	MK017977	Crous *et al.* 2020
	Apiosporaceae	CBS 145903	ET	MN313782	MN317266	n/a	MN313861	Crous *et al.* 2020
Barrmaelia rappazii	*Barrmaeliaceae*	Cr2 = CBS 142771	HT	MF488989	MF488989	MF488998	MF489017	Voglmayr *et al.* (2018)
Barrmaelia rhamnicola	*Barrmaeliaceae*	BR = CBS 142772	ET	MF488990	MF488990	MF488999	MF489018	Voglmayr *et al.* (2018)
Cainia desmazieri	*Cainiaceae*	CAI	—	KT949896	KT949896	n/a	n/a	Jaklitsch *et al.* (2016)
Cainia graminis	*Cainiaceae*	CBS 136.62	—	MH858123	AF431949	n/a	n/a	Vu *et al.* (2019)
Camillea obularia	*Graphostromataceae*	ATCC 28093	—	KY610384	KY610429	KY624238	KX271243	Wendt *et al.* (2018)
Camillea tinctor	*Graphostromataceae*	YMJ 363	—	JX507806	n/a	n/a	JX507795	Mirabolfathy *et al.* (2013)
Endocalyx cinctus	*Cainiaceae*	NBRC 31306	—	MZ313191	MZ313152	n/a	n/a	Delgado *et al.* (2022)
Graphostroma platystomum	*Graphostromataceae*	CBS 270.87	HT	JX658535	AY083827	KY624296	HG934108	Stadler *et al.* (2014)
Hypocrea gelatinosa	*Hypocreales*	NBRC 104900	ET	JN943358	JN941453	n/a	n/a	Schoch *et al.* (2012)
Hypoxylon fragiforme	*Hypoxylaceae*	MUCL51264	ET	KM186294	KM186295	KM186296	KX271282	Daranagama *et al.* (2015)
Hypoxylon fuscum	***Hypoxylaceae***	**α22-093**	**—**	**PV029874**	**n/a**	**n/a**	**n/a**	**This study**
	Hypoxylaceae	CBS 113049	HT	NR172215	n/a	n/a	n/a	Jaklitsch *et al.* (2016)
Hypoxylon investiens	*Hypoxylaceae*	CBS 118185	—	KC968924	KY610451	KY624260	KC977269	Wendt *et al.* (2018)
Hypoxylon rubiginosum	***Hypoxylaceae***	**α23-050**	**—**	**PV029870**	**n/a**	**n/a**	**n/a**	**This study**
	Hypoxylaceae	MUCL 52887	HT	NR155152	NG059785	n/a	n/a	Wendt *et al.* (2018)
Jackrogersella multiformis	*Hypoxylaceae*	CBS 119016	ET	KC477234	KT281893	KY624290	KX271262	Wendt *et al.* (2018)

Table 1. (Continued).

Species	Family	Strain no.	Status	GenBank accession no.				Reference
				ITS	LSU	*RPB2*	*TUB*	
Kretzschmaria deusta	*Xylariaceae*	CBS 163.93	—	KC477237	KY610458	KY624227	KX271251	Stadler *et al.* (2014)
Longiappendispora chromolaenae	*Cainiaceae*	MFLUCC 17-1485	HT	NR169723	NG068714	n/a	n/a	Mapook *et al.* (2020)
Lopadostoma turgidum	*Lopadostomataceae*	LT2	ET	KC774618	KC774618	KC774563	MF489024	Jaklitsch *et al*. 2014
Lopadostoma dryophilum	*Lopadostomataceae*	LG21	ET	KC774570	KC774570	KC774526	MF489023	Jaklitsch *et al*. 2014
Nectria cinnabarina	*Hypocreales*	CBS 125165	—	n/a	HM484562	n/a	n/a	Hirooka *et al*. (2011)
Obolarina dryophila	*Graphostromataceae*	MUCL 49882	—	GQ428316	GQ428316	KY624284	GQ428322	Pažoutová *et al*. (2010)
Paramphibambusa bambusicola	*Cainiaceae*	GMBCC1142	HT	OR995739	OR995746	OR995578	n/a	Han *et al.* (2024)
Poronia pileiformis	*Xylariaceae*	WSP 88113001	ET	GU324760	n/a	GQ853037	GQ502720	Hsieh *et al*. (2010)
Poronia punctata	*Xylariaceae*	CBS 656.78	HT	KT281904	KY610496	KY624278	KX271281	Wendt *et al*. (2018)
Requienella fraxini	*Requienellaceae*	RS2	—	KT949909	KT949909	n/a	n/a	Jaklitsch *et al.* (2016)
	Requienellaceae	RS3	HT	KT949910	KT949910	n/a	n/a	Jaklitsch *et al.* (2016)
	Requienellaceae	RS7	—	KT949911	KT949911	n/a	n/a	Jaklitsch *et al.* (2016)
Requienella populi	***Requienellaceae***	**α23-061**	**PT**	**PV029871**	**n/a**	**n/a**	**n/a**	**This study**
	Requienellaceae	**α23-076**	**HT**	**PV029872**	**PV029875**	**n/a**	**n/a**	**This study**
	Requienellaceae	**α23-076a**	**—**	**PV029873**	**PV029876**	**n/a**	**n/a**	**This study**
Requienella seminuda	*Requienellaceae*	RS12	—	KT949912	KT949912	n/a	n/a	Jaklitsch *et al.* (2016)
	Requienellaceae	RS13	—	KT949913	KT949913	n/a	n/a	Jaklitsch *et al.* (2016)
Requienella shangrilana	*Requienellaceae*	HKAS 130302	HT	PP584755	PP584828	n/a	n/a	Dissanayake *et al.* (2024)
	Requienellaceae	HKAS 130303	—	PP584756	PP584829	n/a	n/a	Dissanayake *et al.* (2024)
Seynesia erumpens	*Cainiaceae*	SMH 1291	—	n/a	AF279410	AY641073	n/a	Bhattacharya *et al.* (2000)
Stromatonectria caraganae	*Hypocreales*	CBS 125579	—	n/a	HQ112288	n/a	n/a	Jaklitsch & Voglmayr (2010)
Xylaria hypoxylon	*Xylariaceae*	CBS 122620	ET	KY610407	KY610495	KY624231	KX271279	Wendt *et al*. (2018)

Fig. 2. *Requienella populi*. A–H, J, M–T, V, Y, X. TRH-F-25081 - α23-076 - holotype. I, K, L, U, V. TRH-F-14022 - α23-061 - paratype. **A–C.** Ascomata in face and lateral views. **D, E.** Ascomata in vertical section. **F.** Hymenium and ascomata in vertical section. **G.** Peridium of basal type (upper) and lateral type (lower) overlapping. **H, I.** Asci with paraphyses. **J, K.** Asci. **L, M.** Ascus apices (in Cotton Blue). **N.** Young asci with paraphyses and free apical ends of paraphyses. **O–R.** Immature ascospores (O–Q in water; R in 5 % KOH). **S–V, X.** Ascospores (S–V in water; X in 5 % KOH). Scale bars: A = 0.6 mm; B = 0.3 mm; C, F = 0.2 mm; D, E = 0.5 mm; G = 20 µm; H, I = 30 µm; J, K, N = 50 µm; L, M, O–V, X = 10 µm.

Table 2. Character matrix for species of *Requienella*.

Species	Ascomata			Papilla	Peridium	Asci	Ascospores			Host	Distribution
	Ascoma position	Neck above host surface	Size	Form and size	Size		Size	Disto-septation	Form		
Requienella fraxini	Erumpent	0.2–0.9 mm	0.45–1 mm diam, 0.5–1.1 mm high	Conical, more or less acute, shiny black, apically 26–160 µm wide papilla	15–30 µm wide at the base, thickened to 150 µm and hard in upper regions	153–206 × 20–30(–33) µm, oblong to narrowly clavate	(23.3–)26.7–31.5(–36) × (8.0–)9.5–12(–14.5) µm	3(–5)	Ellipsoid, oblong to fusiform, yellow, finally brown withpaler ends	*Fraxinus excelsior*	Europe
Requienella populi	Erumpent	0.1–0.3 mm	0.55–1 mm diam, 0.6–1.1 mm high	Conical with rounded base or more or less globose with a prominent, conical, more or less acute, shiny black, apically 25–150 µm wide papilla	(22.5–)21.6–32.5(–36.9) µm diam wide at the base, thickened to (49.2–)56.6–94.3(–99) µm and hard in upper regions	(139–)143.8–160(–175.2) × (17–)20.4–25.9(–27.4) µm, oblong to narrowly clavate, with thick-walled apex	(21–)25.1–31.4(–33.5) × ((9–)10.1–12.4(–13.6) µm (n = 50)	Submuriform, 5 transverse and 1–4 longitudinal	Ellipsoid, oblong to fusiform, brown with paler ends	*Populus tremula*	Norway (Oceanic?)
Requienella seminuda	Erumpent	0.2–0.4 mm	(0.35–)0.45–0.9(–1.1) mm diam, 0.6–1.2 mm high	Papillate to conical, shiny black, apex typically 25–160 µm wide, round, blunt to pointed, sometimes flattened and 90–300 µm diam	15–40 µm wide at the base, thickened to 160 µm in upper regions	(148–)158–178(–182) × (21.7–)23.5–29(–32.5) µm, oblong to subfusiform	(25.3–)28.3–32.7(–37) × (9.8–)10.8–12.7(–13.3) µm	(3–)5(–7)	Ellipsoid, inequilateral, brown	*Olea europae*	Europe (mediterranean)
Requienella shangrilana	Immersed	0 mm	0.48–0.62 mm diam, 0.4–0.52 mm high	N/A	25–40 µm wide, thickened in upper regions	100–160 × 10–18 µm, cyllindrical, unitunicate	20–30 × 9–12 µm	3(–4)	Ellipsoid, inequilaternal, narrowly rounded to nearly acute at the ends, at first hyaline, greyish when young, olivaceous to medium brown when mature	Unknown	China

Ascomata immersed, with upper part erumpent 0.1–0.3 mm (n = 15) above the bark surface, solitary or aggregated in small numbers, 0.6–1.1 mm high, 0.55–1.0 mm diam (n = 15), conical with rounded base or more or less globose with a prominent, conical, more or less acute, shiny black, apically 25–150 µm (n = 15) wide papilla, circular in transverse section, black; often surrounded by white amorphous tissue. *Peridium* (21.5–)22.6–32.5(–36.9) µm diam (n = 8) wide at the base, thickened to (49.2–)56.6–94.3(–99.0) µm (n = 8) and hard in upper regions, dark brown, consisting of 1 × 2–7 µm rectangular pseudoparenchymatous cells. *Hamathecium* consisting of three parts i) 2–4 µm wide, apically free paraphyses containing oil drops when vital, and similarly long as the asci, ii) sparsely branched, 1.5–3 µm wide, apically free 'pseudotrabeculae' nearly reaching the ostiolum, iii) variously curved periphyses within the ostiolar canal, all immersed in a gel matrix. *Asci* (139.0–)143.8–160.0(–175.2) × (17.0–)20.4–25.9(–27.4) µm (n = 15) bitunicate, fissitunicate, oblong to narrowly clavate, with thick-walled apex, wide ocular chamber comprising a slightly refractive, inversely funnel-shaped dome, 8–10 µm long, 5–9 µm wide at the base, turning blue in Cotton Blue and reddish in Congo Red, demarcated by a basal plate, with short simple stipe, containing 8 uni- to biseriately arranged ascospores. *Ascospores* (21.0–)25.1–31.4(–33.5) × (9.0–)10.1–12.4(–13.6) µm (n = 50), ellipsoid, oblong to fusiform, submuriform, first hyaline, 1-celled, with narrow sheath, becoming septate and yellow, finally brown with paler ends, with 5 thick distosepta, lenticular lumina and faintly punctate perispore, often with 1–4 thin longitudinal distosepta, sometimes two in the same compartment/segment, turning darker olivaceous in KOH and lumina becoming smaller, more angular and longitudinal distosepta more evident.

Typus: **Norway**, Møre og Romsdal, Molde, Prestaksla nature reserve, on coarse bark of living *Populus tremula* in mixed forest (*Pinus sylvestris*, *Betula pubescens*, *Corylus avellana*, *Salix caprea*) with low herb vegetation, 21 Sep. 2022, *J.B. Jordal*, α23-076 (**holotype** TRH-F-25081; culture lost, JB22-66); 23 Jun. 2024, *J.B. Jordal, P.G. Larsen & S. Vatne,* **topotype** TRH-F-14021 (JB24-P2); Møre og Romsdal, Jordalsgrenda, on coarse bark of aspen in mixed forest, 21 Sep. 2022, *J.B. Jordal*, **paratype** TRH-F-14022 (α23-061 - culture lost).

Additional materials examined: **Norway**, Møre og Romsdal, Molde, Rislia, On bark of living *Populus tremula* in weak low herb forest, 27 Jun. 2022, *M. Norby Lorentzen & J.B. Jordal,* TRH-F-25063; Vestland (Sogn og Fjordane), Lærdal, Hausen, on bark of living *Populus tremula*, 22 Aug. 2022, *M. Norby Lorentzen,* TRH-F-25067 (MNL202201); Møre og Romsdal, Høystakklia, on coarse bark of living *Populus tremula*, 12 Oct. 2020, *J.B. Jordal,* TRH-F-14023 (JB20-P73); Møre og Romsdal, Molde, Prestaksla nature reserve, on bark of living *Populus tremula* in mixed forest (*Pinus sylvestris, Betula pubescens, Corylus avellana, Salix caprea*), 23 Jun. 2024, *J.B. Jordal* & *P.G. Larsen* & *S. Vatne,* TRH-F-14024 (JB24-P1); Møre og Romsdal, Molde, Prestaksla nature reserve, on bark of living *Populus tremula* in mixed forest (*Pinus sylvestris, Betula pubescens, Corylus avellana, Salix caprea*), 23 Jun. 2024, *J.B. Jordal* & *P.G. Larsen* & *S. Vatne*, TRH-F-14025 (JB24-P3); Møre og Romsdal, Molde, Tjellefonna west, on coarse bark of old living *Populus tremula* in deciduous forest, 1 Jul. 2024, *J.B. Jordal,* TRH-F-14026 (JB24-P4).

Culture characteristics: Ascospores germinated on MEA within 72 h. Growth of cultures reaching 0.5–0.7 cm diam after 4 wk at 20 °C, subcircular, with irregular margins, white, turning slightly yellow, reverse brown. No asexual morph observed.

Ecology: On coarse bark of living trunks of old trees of *Populus tremula.*

Distribution: *Requienella populi* was so far found only in the oceanic parts of Western Norway.

Notes: The presence of 1–4 longitudinal distosepta alongside molecular data and host relations clearly separate *R. populi* from *R. seminuda* and *R. fraxini*. The conical papillae are less markedly protruding than in *R. fraxini*. see also Table 2.

DISCUSSION

The ecology of the three known European species of *Requienella* seem to be defined by host relations. However, the host relations of *R. shangrilana* remains to be studied. Our new species on *Populus tremula* (*Salicaceae*) formed a highly supported sister clade relative to the other two European species of *Requienella*, occurring on *Olea europaea* and *Fraxinus excelsior* (*Oleaceae*), respectively. The association of these species to living hosts may indicate that they have co-evolved with their hosts. They belong to a little-known but species-rich community of *Ascomycota* with unknown nutritional modes, apparently not causing harm to the living tree and possibly being commensals or endophytic symbionts (Bowd *et al.* in press). Other species in this community on rough bark of old aspen trees include *Amphisphaerella dispersella*, *Caesiodiscus populicola, Lasiobelonium corticale, Melaspilea bagliettoana* and *Caliciopsis calicioides*. Pictures of the *Populus* habitat can be seen in Jordal *et al.* (2014).

The finding of the new species *R. populi* and several others during recent years illustrates that much is still unknown about the funga of Northern Europe. One area that appears to be particularly promising for future exploration is Western Norway with its oceanic forests. For instance, *R. populi* sometimes occurs with *Crassistoma norvegicum,* another newly described species on aspen in western Norway (Voglmayr *et al*. 2024). In Norway, the geographical distribution of *R. populi* overlaps with that of *R. fraxini*, which has a broad distribution in northern and southern Europe, but not with R. *seminuda,* which occurs in the mediterranean region. *Requienella populi* was only collected in the Western, oceanic parts of Norway. It was not systematically searched for in other areas. However, as we have not previously encountered it during various field surveys, we suspect that it may in fact have an oceanic distribution.

Our phylogenetic analyses showed relatively low support for the *Requienellaceae* as sister group to the *Cainiaceae* with ML 43 % and BPP 0.7 support. The addition of several strains to the dataset from newly published species of *Acrocordiella* and *Arecophila* resulted in reduced support. Most of these strains are represented only by ITS and LSU genetic markers and we would expect that the addition of further genetic markers for more taxa within the families of *Requienellaceae* and *Cainiaceae* would provide a more stable topology.

ACKNOWLEDGEMENTS

We thank the Norwegian Biodiversity Information Centre for financial support (grant no. 2021/48). We also thank the State Administrator of Møre og Romsdal for funding a species project on the diversity on aspen trees in 2022. We thank the curators of fungarium Trondheim for curation of the material and identification markers. Assoc. Prof. Inger Skrede at Oslo University is thanked for her loan of differential interference contrast microscope.

Conflict of interest: The authors declare that they have no conflict of interest.

REFERENCES

Bhattacharya D, Lutzoni F, Reeb V, *et al.* (2000). Widespread occurrence of spliceosomal introns in the rDNA genes of ascomycetes. *Molecular Biology and Evolution* **12**: 1971–1984.

Boise JR (1986). *Requienellaceae*, a new family of *Loculoascomycetes*. *Mycologia* **78**: 37–41.

Bowd E, Boddy L, Greenwood L, *et al.* (in press). The importance of old and large trees for fungi and fungi for old and large trees. In: *Large and Old Trees - Ecological and Cultural Significance* (Blicharska M, Lindenmayer D, Mikusinski G, eds). Springer.

Crous PW, Schumacher RK, Wood AR, *et al.* (2020). The Genera of Fungi – G5: *Arthrinium*, *Ceratosphaeria*, *Dimerosporiopsis*, *Hormodochis*, *Lecanostictopsis*, *Lembosina*, *Neomelanconium*, *Phragmotrichum*, *Pseudomelanconium*, *Rutola*, and *Trullula*. *Fungal Systematics and Evolution* **5**: 77–98.

Daranagama DA, Camporesi E, Tian Q, *et al*. (2015). *Anthostomella* is polyphyletic comprising several genera in *Xylariaceae*. *Fungal Diversity* **73**: 203–238.

De Hoog GS, Gerrits van den Ende AHG (1998). Molecular diagnostics of clinical strains of filamentous Basidiomycetes. *Mycoses* **41**: 183–189.

Delgado G, Miller A, Hashimoto A, *et al.* (2022). A phylogenetic assessment of *Endocalyx* (*Cainiaceae*, *Xylariales*) with *E. grossus comb. et stat. nov*. *Mycological Progress* **21**: 221–242.

Dissanayake LS, Mahrachchikumbura SSN, Mortimer, *et al.* (2021). *Acrocordiella yunnanensis sp. nov*. (*Requienellaceae*, *Xylariales*) from Yunnan, China. *Phytotaxa* **487**: 103–113.

Dissanayake LS, Samarakoon MC, Maharachchikumbura SSN, *et al.* (2024). Exploring the taxonomy and phylogeny of *Sordariomycetes* taxa emphasizing *Xylariomycetidae* in Southwestern China. *Mycosphere* **15**: 1675–1793.

Han L-S, Wijayawardene NN, Liu C, *et al.* (2024). *Paramphibambusa bambusicola gen. et. sp. nov*., *Arecophila xishuangbannaensis* and *A. zhaotongensis spp. nov*. in *Cainiaceae* from Yunnan, China. *MycoKeys* **104**: 113–132.

Harrington B, Gould T, Hurst N (2003). Inkscape, GNU [WWW Document]. https://www.gimp.org/

Hirooka Y, Rossman AY, Chaverri P (2011). A morphological and phylogenetic revision of the *Nectria cinnabarina* species complex. *Studies in Mycology* **68**: 35–56.

Hsieh HM, Ju YM, Rogers JD (2005). Molecular phylogeny of *Hypoxylon* and closely related genera. *Mycologia* **97**: 914–923.

Hsieh HM, Lin CR, Fang MJ, *et al.* (2010). Phylogenetic status of *Xylaria* subgenus *Pseudoxylaria* among taxa of the subfamily *Xylarioideae* (*Xylariaceae*) and phylogeny of the taxa involved in the subfamily. *Molecular Phylogenetics and Evolution* **54**: 957–969.

Huelsenbeck J, Ronquist F (2001). MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Hyde KD, Norphanphoun C, Maharachchikumbura SSN, *et al.* (2020). Refined families of *Sordariomycetes*. *Mycosphere* **11**: 305–1059.

Jaklitsch WM, Fournier J, Rogers JD, *et al.* (2014). Phylogenetic and taxonomic revision of *Lopadostoma*. *Persoonia* **32**: 52–82.

Jaklitsch W, Gardiennet A, Voglmayr H (2016). Resolution of morphology-based taxonomic delusions: *Acrocordiella*, *Basiseptospora*, *Blogiascospora*, *Clypeosphaeria*, *Hymenopleella*, *Lepteutypa*, *Pseudapiospora*, *Requienella*, *Seiridium* and *Strickeria. Persoonia* **37**: 82–105.

Jaklitsch WM, Voglmayr H (2010). *Stromatonectria gen. nov*. and notes on *Myrmaeciella*. *Mycologia* **103**: 431–440.

Jeewon R, Liew ECY, Hyde KD (2003). Molecular systematics of the *Amphisphaeriaceae* based on cladistic analyses of partial LSU rDNA gene sequences. *Mycological Research* **107**: 1392–1402.

Jordal JB, Nordén B, Gaarder G (2014). *Caliciopsis calicioides*, a corticolous ascomycete on *Populus tremula* new to Norway. *Agarica* **35**: 7–12.

Kang JC, Hyde KD, Kong RYC (1999). Studies on *Amphisphaeriales*: The *Cainiaceae*. *Mycological Research* **103**: 1621–1627.

Katoh K, Standley DM (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.

Kearse M, Moir R, Wilson A (2012). Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**: 1647–1649.

Lanfear R, Frandsen BP, Wright MA, *et al.* (2016). PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* **34**: 772–773.

Li QR, Zhang X, Lin Y, *et al.* (2022). Morpho-molecular characterisation of *Arecophila*, with *A. australis* and *A. clypeata sp. nov.* and *A. miscanthi comb. nov*. *MycoKeys* **88**: 123–149.

Maharachchikumbura SSN, Hyde KD, Perera RH, *et al.* (2018). *Acrocordiella omanensis sp. nov*. (*Requienellaceae*, *Xylariales*) from the Sultanate of Oman. Phytotaxa **338**: 294–300.

Mapook A, Hyde KD, McKenzie EHC, *et al.* (2020). Taxonomic and phylogenetic contributions to fungi associated with the invasive weed *Chromolaena odorata* (Siam weed). *Fungal Diversity* **101**: 1–175.

Mirabolfathy M, Yu-Ming Ju, Hsieh HM, *et al.* (2013). *Obolarina persica sp. nov.*, associated with dying *Quercus* in Iran. *Mycoscience* **54**: 315–320.

Novakova A, Hubka V, Saiz-Jimenez C, *et al*. (2012). *Aspergillus baeticus sp. nov*. and *Aspergillus thesauricus sp. nov.*, two species in section *Usti* from Spanish caves. *International Journal of Systematic and Evolutionary Microbiology* **62**: 2778–2785.

Pažoutová S, Šrůtka P, Holuša J, *et al*. (2010). The phylogenetic position of *Obolarina dryophila* (*Xylariales*). *Mycological Progress* **9**: 501–507.

Phukhamsakda C, Nilsson RH, Bhunjun CS, *et al.* (2022). The numbers of fungi: contributions from traditional taxonomic studies and challenges of metabarcoding. *Fungal Diversity* **114**: 327–386.

Rambaut A, Drummond A, Xie D (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67**: 901–904.

Samarakoon MC, Hyde KD, Maharachchikumbura SSN, *et al.* (2022). Taxonomy, phylogeny, molecular dating and ancestral state reconstruction of *Xylariomycetidae* (*Sordariomycetes*). *Fungal Diversity* **112**: 1–88.

Schoch CL, Seifert KA, Huhndorf S, *et al.* (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. *Proceedings of the National Academy of Sciences of the USA* **109**: 6241–6246.

Senanayake IC, Maharachchikumbura SSN, Hyde KD, *et al.* (2015). Towards unravelling relationships in *Xylariomycetidae* (*Sordariomycetes*). *Fungal Diversity* **73**: 73–144.

Stadler M, Læssøe T, Fournier J, *et al.* (2014). Polyphasic taxonomy of *Daldinia* (*Xylariaceae*). *Studies in Mycology* **77**: 1–143.

Stamatakis A (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.

Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

Voglmayr H, Andreasen M, Nordén B, *et al.* (2024). *Crassistoma norvegicum* (*Crassistomataceae*), a new genus, species and family of *Pleosporales* from Norway. *Sydowia* **77**: 259–269.

Voglmayr H, Friebes G, Gardiennet A, *et al.* (2018). *Barrmaelia*, and *Entosordaria*, in *Barrmaeliaceae* (*fam. nov. Xylariales*) and critical notes on *Anthostomella*-like genera based on multigene phylogenies. *Mycological Progress* **17**: 155–177.

Vu D, Groenewald M, de Vries M, *et al.* (2019). Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. *Studies in Mycology* **92**: 135–154.

Wendt L, Benjamin E, Kuhnert SE, *et al.* (2018). Resurrection and emendation of the *Hypoxylaceae*, recognised from a multigene phylogeny of the *Xylariales*. *Mycological Progress* **17**: 115–154.

White TJ, Bruns T, Lee S, *et al*. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications* (Innis MA, Gelfand DH, Sninsky JJ, *et al*., eds). *Academic Press, Inc*., New York, USA: 315–322.